
NEW ENGLAND
BioLabs® Inc.
be INSPIRED
drive DISCOVERY
stay GENUINE
2023•24

THANK YOU FOR YOUR LEADERSHIP AND SERVICE

DONALD G. COMB

NEW ENGLAND BIOLABS
FOUNDER & CEO
1974–2005

CHAIRMAN OF THE BOARD
1974–2020

Prior to taking a position at Harvard Medical School, Don attended the University of Michigan where he earned a Ph.D. in Biochemistry. Don's commitment to science led to the inception of New England Biolabs® (NEB®) in 1974. His scientific vision shaped NEB as the leader in the discovery and production of recombinant enzymes for molecular biology applications.

Don's passion for science, art and the environment has also led to the creation of the New England Biolabs Foundation, a program that promotes environmental awareness and social change; and the Ocean Genome Legacy Program, which he believed would help to prevent the extinction of endangered sea life. His commitment to the environment is evident on the NEB campus; from the LEED® certified laboratory that was designed to minimize its impact on the surrounding landscape, to the Solar Aquatics System™, designed to treat the campus' wastewater. Don's beliefs are deeply woven into the corporate philosophy.

Don stepped down from his position as CEO in 2005 and continued to serve on the Board of Directors until his passing in 2020. During that time, Don also maintained an active research laboratory at NEB, where he could be found working at the bench, reading scientific journals or chatting about his latest research interests with employees.

JAMES V. ELLARD, JR.

CHIEF EXECUTIVE OFFICER
2005–2022

CHAIRMAN OF THE BOARD
2022–PRESENT

Jim Ellard served as Chief Executive Officer of New England Biolabs from 2005 until his retirement in 2022. He joined NEB as a summer intern in 1983 after his junior year at MIT and was hired the following year to a full-time role as a bench scientist working to purify and optimize enzymes essential for DNA manipulation. In 1990, he created NEB's Marketing Communications Department which he led for 15 years. Even in those early days, Jim recognized that NEB was a unique company that put people and passion above profit.

Jim is incredibly proud of how NEB has grown, persevered and evolved during his tenure as CEO. NEB expanded its product portfolio and technical capabilities to support a wide variety of applications that impact human health, including clinical sequencing, molecular diagnostics and the production of DNA and RNA for nucleic acid vaccines and molecular therapies. In the 17 years that Jim held his leadership role, the company expanded its domestic and international footprint, welcomed hundreds of new employees and remained steadfast in its commitment to basic research, philanthropic pursuits and a family-like culture where everyone has a voice, regardless of role, tenure or department.

As Chairman of the Board, he will help ensure that NEB continues to be guided by a strong sense of purpose to enable research, advance science, value every employee, protect the planet and remain true to the core values of passion, humility and being genuine.

BE INSPIRED.

SINCE OUR ESTABLISHMENT IN 1974, NEW ENGLAND BIOLABS HAS BEEN DIFFERENT.

From our founding principles — placing the advancement of science and stewardship of the environment as our highest priorities — to our unique corporate culture, NEB's philosophy can be distilled down to three core values: passion, humility and being genuine. This was a priority for our Founder, Don Comb, and it continued to guide our decisions as the company expanded under the leadership of Jim Ellard.

As the third CEO in our company's history, I am thrilled to lead NEB into its next phase of growth, while ensuring that we remain true to our core values. The past several years have been challenging from a global health, environmental, and political standpoint. At the same time, they have highlighted the importance of science and innovation, both of which are necessary to overcome many of the toughest issues facing the world today. We are honored that our products and expertise support your scientific creativity to help achieve this goal.

As NEB celebrates its 50th anniversary and looks forward to our future, I am excited to continue to build a sustainable business that is focused on enabling the scientific community, fostering curiosity, and giving back — to those closest to us and the world around us. We truly appreciate your trust and support, and wish you continued success in your research. As always, if there is anything you believe we should be doing differently, please share your thoughts with us.

SALVATORE RUSSELLO
CEO, NEW ENGLAND BIOLABS, INC.

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the NEB Augmented Reality (AR) app for iPhone® or iPad® at the Apple® App Store or for Android™ on Google Play™.

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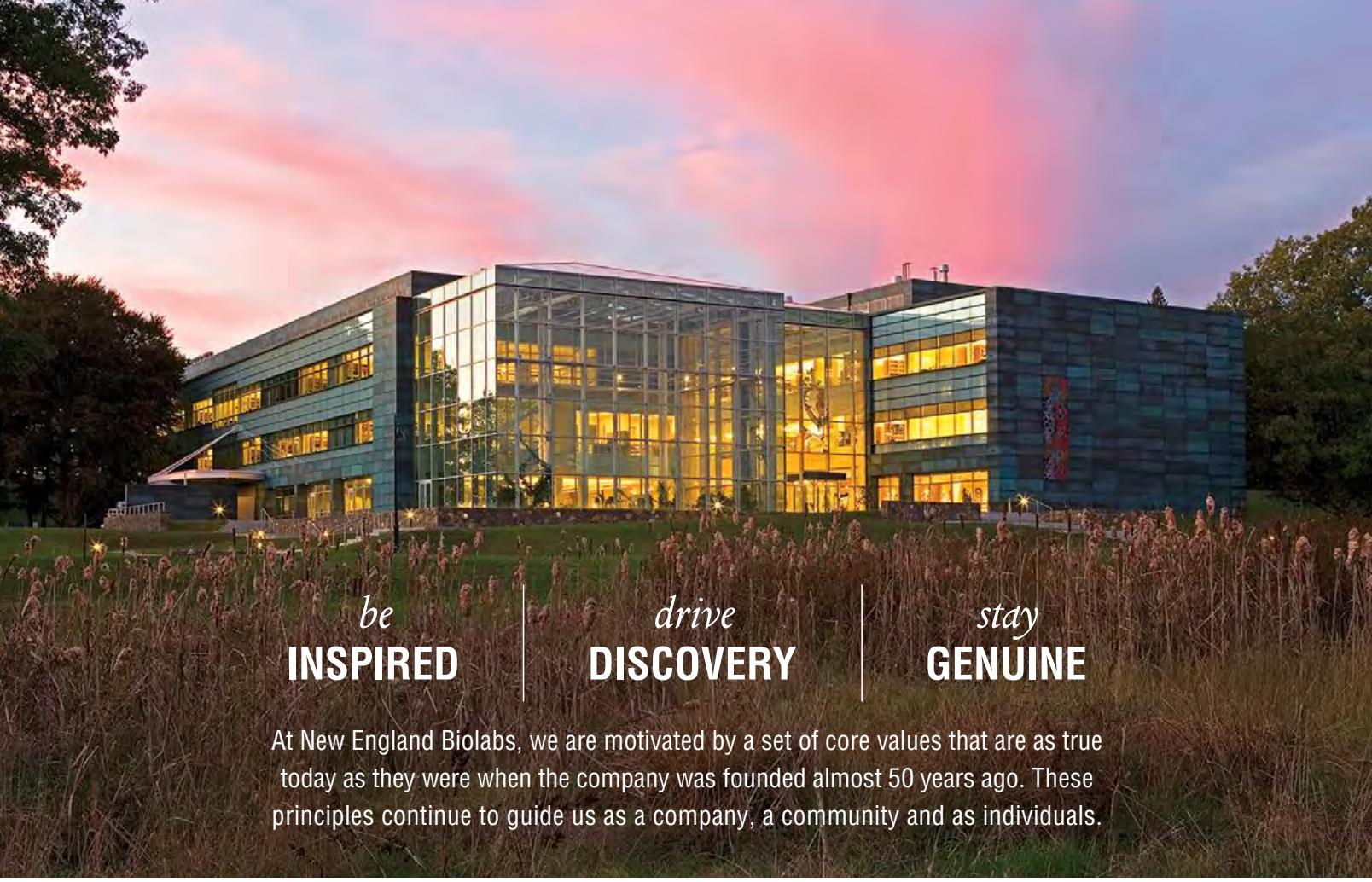
videos, tutorials and immersive experiences that can be accessed by scanning the icon using the AR app.

Explore

the NEB Catalog & Technical Reference and keep an eye out for the augmented reality icon.



be **INSPIRED** | *drive* **DISCOVERY** | *stay* **GENUINE**

At New England Biolabs, we are motivated by a set of core values that are as true today as they were when the company was founded almost 50 years ago. These principles continue to guide us as a company, a community and as individuals.

Advancement of Science

We believe that basic research and the cultivation of scientific knowledge is critical for us to stay connected with our customers and to drive scientific breakthroughs. At NEB, over 30 labs participate in research projects, which are aided by post-doctoral fellows and students in Masters and Ph.D. programs. NEB researchers have authored or co-authored over 1,450 publications to date, the vast majority of which are in peer-reviewed journals. To learn more, visit **www.neb.com/research**.

Environmental Stewardship

We continuously strive to promote ecologically sound practices and environmental sustainability in order to protect our natural resources, both locally and globally. It is our goal to continuously improve our business processes to minimize our impact on the environment. Additionally, NEB supports other organizations that are advancing environmental research and stewardship. In 2021, NEB became a Certified B Corporation™, a recognition awarded to organizations with the highest standards for social and environmental performance, transparency and accountability. To learn more, visit **www.neb.com/environmentalphilosophy**.

Certified B Corporation

Learn more about our commitment to the environment

Social Responsibility

We see opportunities where science can be used to improve lives, and we continue to be guided by our responsibility to each other and our community to work towards a kinder and more just world. This philosophy lies behind NEB's longstanding commitment to its parasitology research program, which contributes to the understanding and treatment of poorly-funded and understudied tropical diseases. NEB also supports several organizations devoted to humanitarian efforts. Further, we recognize that we must work together to build a more equitable society and improve diversity, equity and inclusion in our workplace. To learn more, visit **www.neb.com/corporateresponsibility**.

Delivering the Highest Quality Product

It is our goal to deliver best-in-class product quality and technical support. NEB holds ISO 13485:2016 and ISO 9001:2015 certifications at its manufacturing facilities in Ipswich, Rowley, and Beverly, MA, USA. Our manufacturing facility in Rowley, MA produces GMP-grade* materials for customers requiring an enhanced level of quality documentation and support. Additionally, NEB Lyophilization Sciences™ Ltd., a wholly-owned subsidiary of NEB, is ISO 13485:2016 certified for the contract design, development and manufacture of molecular diagnostic reagents for our customers' *in vitro* diagnostic medical devices.

We are constantly improving the stringency and range of our quality controls to ensure that our products will perform to your expectations, every time.

nqa ISO 13485 Registered Medical Devices

nqa ISO 9001 Registered Quality Management

nqa ISO 14001 Registered Environmental Management

*See page 6 for more details.

NEB Facilities in the U.S.

As our product portfolio and number of employees has grown, so has our infrastructure and facilities. NEB is now spread across the Greater Boston area of MA, USA, with specialized facilities dedicated to ensuring the innovation, availability and quality of our products and services.

NEB headquarters is located in Ipswich, MA, and features a LEED® certified, state-of-the-art research and production facility. Approximately 15 minutes away, our production facility in Rowley, MA, is designed to serve the needs of customers in regulated markets and is used for manufacture of GMP-grade* materials. Also in Rowley, our packaging facility is responsible for kitting and packaging of a selection of NEB products. We also have two locations in Beverly, MA, which is approximately 20 minutes from our main campus. Our Beverly Organic Synthesis Facility is an ISO compliant laboratory responsible for synthesis and manufacture of oligonucleotides, modified nucleotides, and affinity beads/resins. Our R&D facility at Dunham Ridge houses many of our Research and Application & Product Development groups. To learn more, visit **www.neb.com/AboutNEB**.

Take a tour of
New England Biolabs

Diversity, Equity & Inclusion

We recognize that there are areas where we need to grow and we are taking steps to raise our level of consciousness to injustices that have been overlooked and underestimated. Our Diversity, Equity & Inclusion Team works to address these issues and is divided into four subgroups focusing on:

- Social Justice Philanthropy
- STEM Education & Mentorship
- Social Justice Outreach
- Diversity, Equity & Inclusion at NEB

To learn more, visit
www.neb.com/corporateresponsibility.

PARTNERING WITH NEB

NEB has almost 50 years of experience in the discovery, development and manufacture of molecular biology reagents. These are essential components in a vast array of genomic and proteomic technologies that continue to transform our understanding of the world we live in, and ultimately the diagnosis and treatment of disease. With experience in fields as diverse as next generation sequencing, RNA biology, qPCR, and protein engineering, NEB is ready to work with you to develop custom reagent solutions, and to help bring your technologies to market. Further, our global distribution network can help to ensure that your products have worldwide reach.

Customized Solutions

From development to commercialization, NEB provides the technical expertise, consistent scalable manufacturing, quality systems and a global distribution network to enable a successful long-term partnership. Our dedicated team is ready to work with you to develop novel, high performance enzymes tailored to your needs from bench to production scale manufacturing. With our ISO 13485 and ISO 9001 certified manufacturing processes, as well as the ability to manufacture GMP-grade* products, you can be confident in our robust process, documentation, and risk mitigation for the product you need. For more information, contact **custom@neb.com**.

International Business

Our ability to successfully operate as a both a research institute and a commercial enterprise in service of our customers is amplified by the extent of our global reach. The International Business team at NEB operates worldwide to generate sustainable growth through an exceptional network of commercial operations that includes wholly-owned subsidiaries located in Australia, Canada, China, France, Germany, Japan, Singapore and the United Kingdom. Additionally, NEB works with over 60 distribution partners. Together, our subsidiary and distribution network enables us to support customers in more than 90 countries. By leveraging the talents and assets of NEB, including scientific and commercial resources, we ensure that our customers are serviced by a stable, ethical and engaged global network. More information can be found on the inside back cover or contact **globaldev@neb.com**.

NEBnow Freezer Program Network

With NEBnow® on-site freezers, enjoy convenient and affordable access to NEB's high quality reagents, anytime. Our NEBnow Freezer Program Team works closely with your institution to customize inventory best suited to your research program. Save time and avoid shipping fees with consolidated shipments. For more information, contact **freezers@neb.com**.

Enzymes for Innovation

The NEB catalog highlights a wide variety of enzyme functionalities found in nature or engineered for specific purposes. However, in molecular biology, new tools can often lead to new discoveries. Taking advantage of the enzymology expertise at NEB, we now offer a growing selection of novel enzymes with interesting and unique activities for manipulating DNA, RNA, proteins and glycans, even if specific applications for them have yet to be discovered. If you are looking for an enzyme functionality that it is not currently available, visit **www.enzymesforinnovation.com** or contact **enzymesforinnovation@neb.com**.

What are Enzymes for Innovation?

*See page 6 for more details.

> When it comes time to choose a source of enzymes, my first choice is NEB. In addition to having a wide selection of enzymes, I've been impressed with their rigorous test procedures and overall quality of their products. The staff is responsive, knowledgeable, customer-focused and a pleasure to work with.

– Senior Fellow, Analytics & Knowledge Transfer, Molecular Biology Reagents Provider

> The NEBnow freezer program is an amazing addition; having 24/7 access to the NGS reagents has been extremely helpful. NEB allows you to customize the freezer to hold the items you use most, saving us valuable time by cutting out the ordering process!

– Assistant Director, Genomic Sequencing and Analysis Facility, University of Texas, Austin

Practicing Ethical Science

NEB is committed to practicing ethical science – we believe it is our job as researchers to ask the important questions that, when answered, help preserve our quality of life and the world that we live in. However, this research should always be done in safe and ethical manner. Learn more at **www.neb.com/neb-ethics**.

SUPPORTING REGULATED MARKETS

At NEB, we view every challenge as an opportunity. We know that your teams are pushing the boundaries of what is known today to develop innovative solutions to diagnose and treat disease. Whether you are performing your first build or one of many, accessing innovative and critical materials at the scale you need is an important first step in bringing your assay or treatment to market.

With almost 50 years of experience, we can draw upon our expertise in enzymology and reagent manufacturing to find solutions that best fit your needs. As an extension of your team, we will equip you with high-quality enzymes and reagents, whether you are at the stage of validation and verification, or preparing to scale-up production for a commercial launch. Our focus on understanding and supporting your needs means that we can offer you flexibility and customization, from development through to commercial scale. **We are committed to your success.**

GMP-grade Capabilities

NEB is a world leader in the discovery and production of reagents for the life science industry. This expertise effectively positions us to supply reagents for the synthesis of high-quality RNA — from template generation and transcription, to capping, tailing and cleanup after synthesis. These products are designed and manufactured based on decades of molecular biology experience, so that you can be confident they will work for your application.

To better serve the needs of customers in regulated markets, NEB has opened a state-of-the-art, 43,000 sq. ft. production facility in Rowley, MA for the manufacture of GMP-grade* materials. This facility includes Quality Control and Production functions ranging from a shipping/receiving area and dedicated warehouse, to separate inoculation preparation, fermentation, purification and filling suites. To learn more, visit **www.neb.com/GMP**.

Learn about
our GMP-grade
capabilities

**"GMP-grade" is a branding term NEB uses to describe reagents manufactured or finished at NEB's Rowley facility. The Rowley facility was designed to manufacture reagents under more rigorous infrastructure and process controls to achieve more stringent product specifications and customer requirements. Reagents manufactured at NEB's Rowley facility are manufactured in compliance with ISO 9001 and ISO 13485 quality management system standards. However, at this time, NEB does not manufacture or sell products known as Active Pharmaceutical Ingredients (APIs), nor does NEB manufacture its products in compliance with all of the Current Good Manufacturing Practice regulations.*

Supporting Molecular Diagnostics

The COVID-19 pandemic has elevated many of the challenges faced by clinical labs, requiring new and innovative solutions to address them. Technology development is happening faster than ever before, increasing the need for innovation and thinking differently about how diagnostics should be developed, manufactured and deployed.

Many scientists know NEB as a trusted reagent provider to the life science community. What many do not know is that we also offer a portfolio of products that serve as critical components for a wide array of diagnostic products and services. Extensive molecular biology and enzymology experience provide NEB with the unique ability to help customers solve the challenges inherent in technology development and ultimately in scale-up and commercialization. Visit **www.neb.com/MDx** to learn more.

Introducing New England Biolabs Lyophilization Sciences

A wholly-owned subsidiary of NEB, New England Biolabs Lyophilization Sciences™ Ltd. is positioned to offer lyophilized molecular biology reagents to life sciences, including research and applied markets, and the *in vitro* diagnostics (IVD) sector. The NEB Lyophilization Sciences Team are experts in the design, development and manufacture of innovative solutions for ambient storage of products.
Visit **www.neb.com/lyosciences** to learn more.

Ordering Information

Placing an Order

Orders can be placed:
9:00 AM to 8:00 PM EST (Monday – Friday)
Phone: 1-800-632-5227 (1-800-NEB-LABS)
Fax: 1-800-632-7440
Online: www.neb.com

To inquire about an existing order, email customerservice@neb.com or chat with Live Agent on the U.S. Customer and Order Support page at www.neb.com.

NEB supports email ordering in the U.S. For U.S. customers only, email neborders@neb.com. For international orders, please contact the appropriate office.

B2B / E-business Integrations

Please inquire at businessops@neb.com.

Credit Card Purchases

NEB accepts Mastercard, Visa and American Express at the point of sale.

OEM & Customized Products

To inquire about OEM and customized products:
Phone:1-800-NEB-LABS, ext.7275
Email: custom@neb.com
Online: www.neb.com/neb-custom

For more information, see page 4 of this Catalog.

Shipping Rates

NEB offers free shipping to all U.S. available locations for all orders totaling $350 (net) and over.

U.S. orders under $350 (net) are currently charged $34 per order for shipping and handling. Charges are prepaid and added to the invoice.

Please inquire for international shipping charge quotation.

Shipping terms and policies are subject to change at NEB's discretion.

Delivery

Orders received Monday through Thursday by 7:30 PM EST are shipped the same day for next business day delivery (within carrier's overnight delivery area)*. After 7:30 PM, but before 8:00 PM EST, please place orders with Customer Service at 1-800-NEB-LABS, who can confirm if next business day delivery is still possible.

Orders placed by 7:30 PM EST on Friday will be shipped (with extra ice packs, as necessary) to NEB's courier, who stores the products at the appropriate storage temperature, prior to Sunday shipment, for delivery the following Monday (or next business day) nationwide. This service is available for the same cost as NEB's standard shipping service.

PLEASE NOTE: Monday delivery service is not available for products that have storage temperatures colder than -20°C or require shipment on dry ice.

Please contact Customer Service at 1-800-NEB-LABS or customerservice@neb.com with any inquiries regarding NEB holiday deliveries or weather-related delays.

*"Local" orders received Monday through Friday before 10:00 AM EST can be delivered the same day to most of the Boston and Cambridge area. Please contact Customer Service, 1-800-NEB-LABS or customerservice@neb.com to arrange for this special service at no extra charge.

Terms

Net 30 days in U.S. dollars.

Discounts

In the U.S., NEB passes along discounts to its customers based on account history. Other discount opportunities include bulk and new lab discounts. Please note: discounts are not cumulative.

Research Use Only

The products in this Catalog are intended for research purposes only. The products are not intended to be used for therapeutic or diagnostic purposes in humans or animals.

Terms of Sale

The purchase, acceptance, and payment of and for NEB's products is pursuant to NEB's Terms of Sale at www.neb.com/support/terms-of-sale. NEB does not agree to and is not bound by any other terms or conditions, unless those terms and conditions have been expressly agreed to in writing by an authorized representative of NEB.

Legal Information

Products and content in this Catalog are covered by one or more patents, trademarks and/or copyrights owned or controlled by NEB. The use of trademark symbols does not necessarily indicate that the name is trademarked in the country where it is being read; it indicates where the content was originally developed.

While NEB develops and validates its products for various applications, the use of any product may require the buyer to obtain additional third party intellectual property rights for certain applications.

For more information, please email NEB's Global Business Development team at busdev@neb.com.

Notice to Buyer

The information in this Catalog is provided in good faith based on NEB's knowledge as of the publication date. The Catalog is only a guide and may not be up-to-date. For current information, including a current price list, please see NEB's website at www.neb.com. NEB's terms, conditions, policies and product information are subject to change at NEB's discretion.

Supporting Non-Profits and Foundations

New England Biolabs has played a role in the establishment of several organizations that are advancing social responsibility and environmental stewardship worldwide.

New England Biolabs Foundation

The New England Biolabs Foundation is a private independent foundation whose mission is to foster community-based conservation of landscapes and seascapes, and the bio-cultural diversity found in these places. The foundation supports projects in selected countries of Central America, Andean South America, West Africa, and in coastal communities on the North Shore of Massachusetts. Learn more at **NEBF.org**.

creative action INSTITUTE
home of ArtCorps

Creative Action Institute catalyzes community-driven solutions that advance gender equality and build a sustainable planet. Through our experiential trainings, convenings and coaching, we develop leadership, build networks and support grassroots advocacy. Visit **CreativeActionInstitute.org** to learn more.

Northeastern University
The Ocean Genome Legacy Center of New England Biolabs
dedicated to preserving the biological diversity of the sea
www.northeastern.edu/ogl

The Ocean Genome Legacy Center of New England Biolabs is a non-profit research center dedicated to the conservation of marine genome diversity and maintaining a repository of genomic DNA from marine organisms around the world. Learn more at **northeastern.edu/ogl**.


NEW ENGLAND
BioLabs® Inc.

Restriction Endonucleases

AatII
AbaSI
AccI
Acc65I
AciI
AclI
AcuI
AfeI
AflII
AflIII
AgeI-HF
AhdI
AleI-v2
AluI
AlwI
AlwNI
ApaI
ApaLI
ApeKI
ApoI-HF
AscI
AseI
AsiSI
AvaI
AvaII
AvrII
BaeI
BaeGI
BamHI
BamHI-HF
BanI
BanII
BbsI
BbsI-HF
BbvI
BbvCI
BccI
BceAI
BcgI
BciVI
BclI
BclI-HF
BcoDI
BfaI
BfuAI
BglI
BglII
BlpI
BmgBI
BmrI
BmtI-HF
BpmI
Bpu10I
BpuEI
BsaI-HFv2
BsaAI
BsaBI
BsaHI
BsaJI
BsaWI
BsaXI
BseRI
BseYI
BsgI
BsiEI
BsiHKAI
BsiWI
BsiWI-HF
BsmI
BsmAI
BsmBI-v2
BsmFI
BsoBI
Bsp1286I
BspCNI
BspDI
BspEI
BspHI
BspMI
BspQI
BsrI
BsrBI
BsrDI
BsrFI-v2
BsrGI-HF
BssHII
BssSI-v2
BstAPI
BstBI
BstEII-HF
BstNI
BstUI
BstXI
BstYI
BstZ17I-HF
Bsu36I
BtgI
BtgZI
BtsI-v2
BtsIMutI
BtsCI
Cac8I
ClaI
CspCI
CviAII
CviKI-1
CviQI
DdeI
DpnI
DpnII
DraI
DraIII-HF
DrdI
EaeI
EagI-HF
EarI
EciI
Eco53kI
EcoNI
EcoO109I
EcoP15I
EcoRI
EcoRI-HF
EcoRV
EcoRV-HF
Esp3I
FatI
FauI
Fnu4HI
FokI
FseI
FspI
FspEI
HaeII
HaeIII
HgaI
HhaI
HincII
HindIII
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HinfI
HinP1I
HpaI
HpaII
HphI
Hpy99I
Hpy166II
Hpy188I
Hpy188III
HpyAV
HpyCH4III
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HpyCH4V
KasI
KpnI-HF
LpnPI
MboI
MboII
MfeI-HF
MluI-HF
MluCI
MlyI
MmeI
MnlI
MscI
MseI
MslI
MspI
MspA1I
MspJI
MwoI
NaeI
NarI
NciI
NcoI
NcoI-HF
NdeI
NgoMIV
NheI-HF
NlaIII
NlaIV
NmeAIII
NotI
NotI-HF
NruI-HF
NsiI
NsiI-HF
NspI
PacI
PaeR7I
PaqCI
PciI
PflFI
PflMI
PleI
PluTI
PmeI
PmlI
PpuMI
PshAI
PsiI-v2
PspGI
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PspXI
PstI
PstI-HF
PvuI-HF
PvuII
PvuII-HF
RsaI
RsrII
SacI-HF
SacII
SalI
SalI-HF
SapI
Sau3AI
Sau96I
SbfI-HF
ScaI-HF
ScrFI
SexAI
SfaNI
SfcI
SfiI
SfoI
SgrAI
SmaI
SmlI
SnaBI
SpeI-HF
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SwaI
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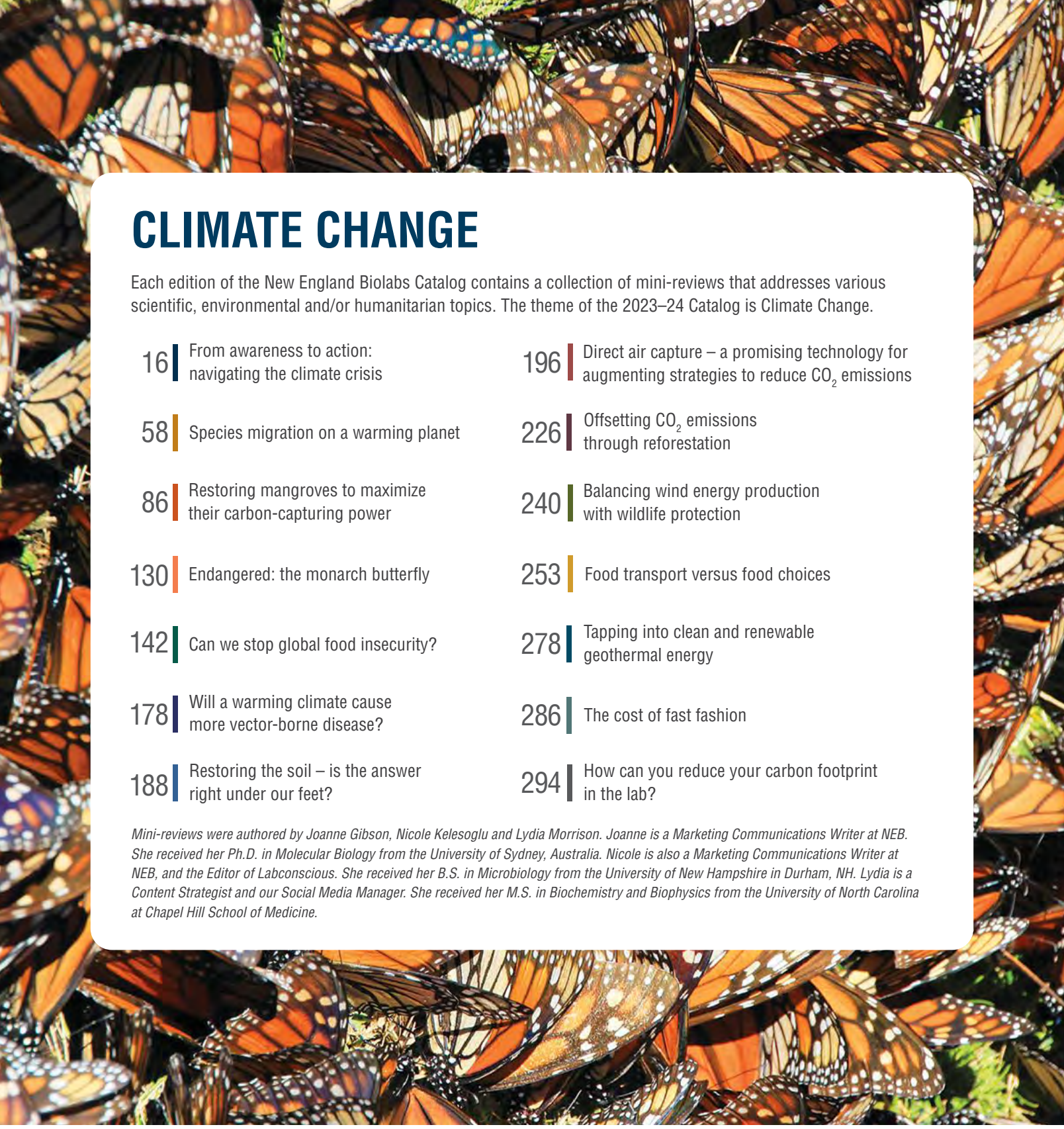

CLIMATE CHANGE

Each edition of the New England Biolabs Catalog contains a collection of mini-reviews that addresses various scientific, environmental and/or humanitarian topics. The theme of the 2023–24 Catalog is Climate Change.

16 From awareness to action: navigating the climate crisis

58 Species migration on a warming planet

86 Restoring mangroves to maximize their carbon-capturing power

130 Endangered: the monarch butterfly

142 Can we stop global food insecurity?

178 Will a warming climate cause more vector-borne disease?

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196 Direct air capture – a promising technology for augmenting strategies to reduce CO_2 emissions

226 Offsetting CO_2 emissions through reforestation

240 Balancing wind energy production with wildlife protection

253 Food transport versus food choices

278 Tapping into clean and renewable geothermal energy

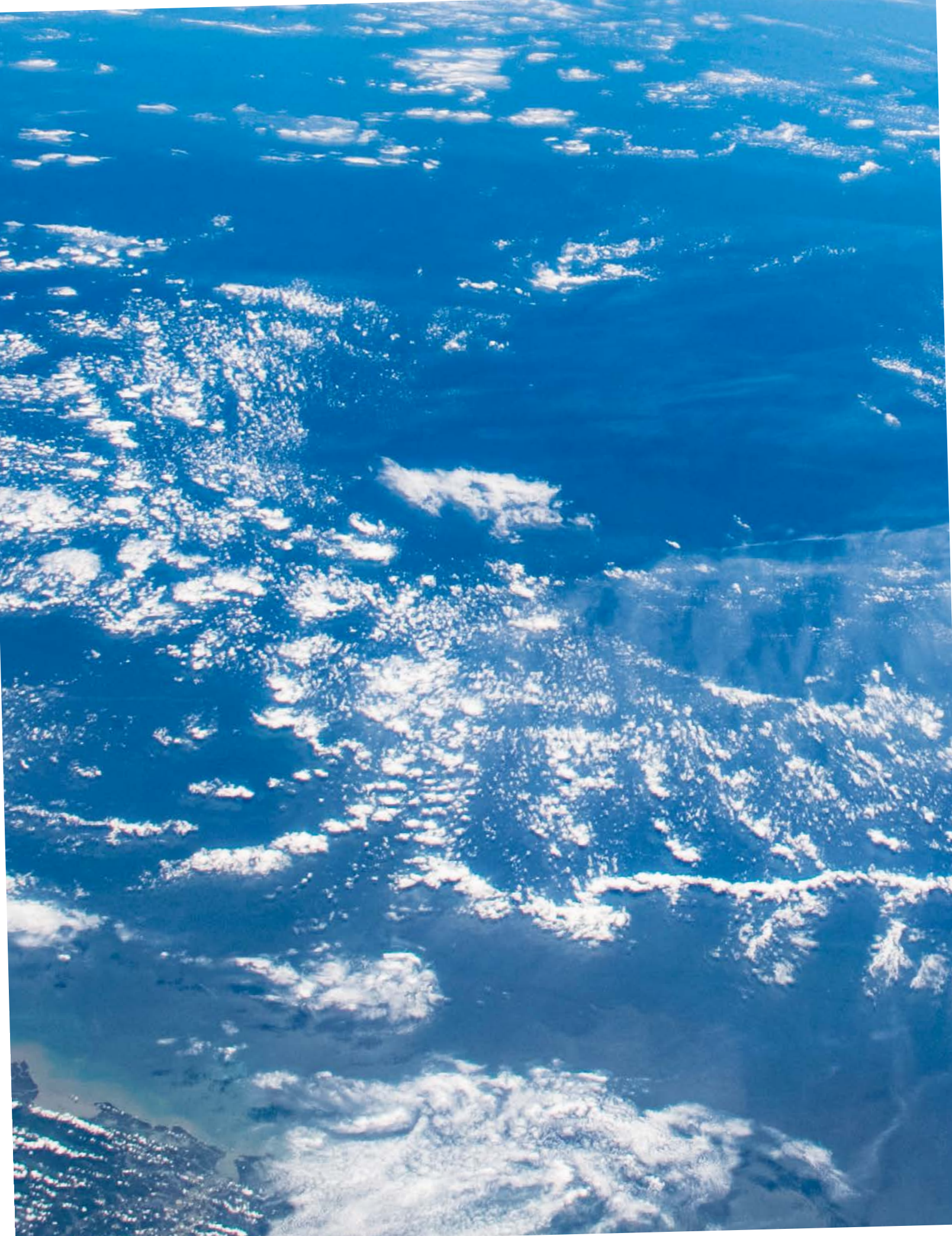
286 The cost of fast fashion

294 How can you reduce your carbon footprint in the lab?

Mini-reviews were authored by Joanne Gibson, Nicole Kelesoglu and Lydia Morrison. Joanne is a Marketing Communications Writer at NEB. She received her Ph.D. in Molecular Biology from the University of Sydney, Australia. Nicole is also a Marketing Communications Writer at NEB, and the Editor of Labconscious. She received her B.S. in Microbiology from the University of New Hampshire in Durham, NH. Lydia is a Content Strategist and our Social Media Manager. She received her M.S. in Biochemistry and Biophysics from the University of North Carolina at Chapel Hill School of Medicine.

labconscious®

Sponsored by New England Biolabs, Labconscious is an open resource and blog for the life science community promoting sustainable practices in the laboratory. Find ways to reduce laboratory waste, tips on recycling and reuse, ideas for conserving water and saving energy, green chemistry and lab supplies, and much more. Join the discussion at **www.labconscious.com**.



From awareness to action: navigating the climate crisis

Scientists realized in the 1970s that the increase in Earth's temperature correlates with the warming effect of greenhouse gases contributed by human activity. In 1988, the Intergovernmental Panel on Climate Change (IPCC) was established to investigate the science behind climate change, and in 1990, the first report on the scientific understanding of climate change was released. This marked the beginning of a more concerted effort to address the problem of global warming.

In 2015, an international treaty on climate change was established called The Paris Agreement, which aims to limit global temperature rise to < 2°C above pre-industrial times. Since the agreement was reached, the world has slowed its greenhouse gas emissions and the rate at which the Earth is warming, but we are still on the brink of several critical thresholds, or "tipping points", beyond which the Earth's climate could cause irreversible damage to the planet.

Implementing the changes needed is challenging due to several factors, including the initial cost of developing infrastructure to support many renewable energy technologies. The global economy is moving towards decarbonization, but changing a system built around burning fossil fuels is a monumental challenge, and conflicting economic interests also impede progress. The transition to renewable energy sources is one of the most promising climate change mitigation strategies, and extensive R&D in this area means it is becoming increasingly cost-competitive with fossil fuels. While there is not one exclusive form of renewable, clean energy that has the potential to harness an equivalent amount of energy to fossil fuels without causing environmental harm, a future of sustainable, clean power will likely consist of many forms of renewable energy sources, each adapted to particular geographies and climates.

Sustainable land use practices are also an essential part of addressing climate change. Knowledge about soil health has dramatically expanded in recent decades, and changes in farming practices are leading to increased crop yields and improved water management. Also, protecting biodiverse ecosystems by integrating crops, trees and livestock leads to climate resiliance. The trees and soils within these ecosystems additionally serve as carbon sinks that can prevent runaway emission effects.

It requires a collective effort and a global commitment to transition to a sustainable future. One of the most promising recent shifts is the increasing commitment of large companies to achieving net-zero carbon status through operational changes and the purchase of managed carbon credits in reforestation projects. At an individual level, we are also making changes in the way we think and act daily that have a significant impact on reducing our carbon footprint. These changes include the clothes we buy, what we eat, and how we conserve resources in the lab, office and home.

Additionally, there is growing support for policies and regulations that implement carbon taxes, cap and trade systems, and regulations on the emissions of certain pollutants. Individuals and communities are becoming involved in inspiring grassroots initiatives and projects to reduce emissions and fight climate change.

There are many positive changes being made to combat this planetary crisis. With the right policies, actions, and growing awareness, we can still avoid the more dire predicted consequences of climate change and create a more prosperous and resilient future.

Scenic view of planet earth, time lapse footage from satellite in orbit. Based on images furnished by NASA.
Credit: fabioderby, Adobe Stock

Exprerience the scenic view of planet earth in motion.

Restriction Endonucleases

The leader in the discovery & production of restriction enzymes.

Having supplied restriction enzymes to the research community for almost 50 years, NEB has earned the reputation of being a leader in enzyme technologies. Working continuously to be worthy of that distinction, NEB strives to develop enzymes of the highest purity and unparalleled quality.

NEB scientists continue to improve our existing portfolio, as well as explore the utility of NEB reagents in new technologies. As a result, NEB scientists continue to publish scientific papers and be awarded grants in this area. With the industry's largest research and development group dedicated to restriction enzymes, we are proud to have been there first: the first to commercialize a recombinant enzyme, the first to introduce a nicking enzyme, and the first to supply a true restriction enzyme master mix. In addition, NEB has a continuing history of innovation by engineering restriction enzymes with altered specificities and improved performance. Through ongoing research in these areas, we are committed to driving the innovations that allow us to offer maximum performance and convenience.

Featured Tools and Resources

300 Tips for Restriction Enzyme Optimization

302 Restriction Enzyme Troubleshooting Guide

303 Performance/Activity Chart for Restriction Enzymes

Visit **NEBrestrictionenzymes.com** to find additional online tools, video tech tips and tutorials to help you in setting up restriction enzyme reactions.

Icon Descriptions

The gene encoding this enzyme was cloned at NEB.

RR This enzyme is purified from a recombinant source.

e This enzyme has been engineered for maximum performance.

Time-Saver qualified enzymes will digest 1 µg of substrate DNA in 5–15 minutes using 1 µl of enzyme under recommended reaction conditions. These enzymes can also be used overnight with no loss of sample.

2*site Indicates that the restriction enzyme requires two or more sites for cleavage.

NEB r1.1 NEB r2.1 NEB r3.1 rCutSmart NEB U
Indicates which reaction buffer is supplied with the enzyme for optimal activity. Enzymes with buffer requirements not met by one of the four standard NEBuffers are supplied with their own unique NEBuffer (NEB U). NEBuffers are color-coded (NEB r1.1 – yellow, NEB r2.1 – blue, NEB r3.1 – red, rCutSmart – green) and supplied as 10X stocks with each enzyme. For more information, consult the Performance Chart found in the Technical Reference section.

Epi This enzyme is EpiMark validated for epigenetics studies.

SAM This enzyme is supplied with a separate tube of S-adenosylmethionine (SAM). To obtain 100% activity, SAM should be added to the 1X reaction mix as indicated. When required, a concentrated stock of SAM is supplied with the enzyme.

rAlbumin This enzyme is supplied with a separate tube of Recombinant Albumin (rAlbumin). To obtain 100% activity, rAlbumin should be added to the 1X reaction mix to a final concentration as indicated.

dam dcm CpG This restriction enzyme is sensitive to dam, dcm, or CpG methylation (note that CpG methylation is applicable to eukaryotic genomic DNA only). For more information, see the methylation sensitivity section of the Technical Reference.

65° 80° No Indicates whether or not the enzyme can be heat inactivated. Enzymes are first tested by incubation at 65°C for 20 minutes; any enzyme not inactivated at 65°C is then tested by incubation at 80°C for 20 minutes. If an enzyme can be heat inactivated, the temperature is indicated in the icon.

25° 37° 50° 55° 60° 65° 75°
Indicates the enzyme's optimal incubation temperature.

dilA dilB dilC Indicates which diluent buffer (A, B or C) is recommended for making dilutions of restriction enzymes. More information can be found in the Technical Reference section.

What are restriction enzymes?

- AatII
- AbaSI
- AccI
- Acc65I
- AciI
- AclI
- AcuI
- AfeI
- AflII
- AflIII
- AgeI-HF
- AhdI
- AleI-v2
- AluI
- AlwI
- AlwNI
- ApaI
- ApaLI
- ApeKI
- ApoI-HF
- AscI
- AseI
- AsiSI
- AvaI
- AvaII
- AvrII
- BaeI
- BaeGI
- BamHI
- BamHI-HF
- BanI
- BanII
- BbsI
- BbsI-HF
- BbvI
- BbvCI
- BccI
- BceAI
- BcgI
- BciVI
- BclI
- BclI-HF
- BcoDI
- BfaI
- BfuAI
- BglI
- BglII
- BlpI
- BmgBI
- BmrI
- BmtI-HF
- BpmI
- Bpu10I
- BpuEI
- BsaI-HFv2
- BsaAI
- BsaBI
- BsaHI
- BsaJI
- BsaWI
- BsaXI
- BseRI
- BseYI
- BsgI
- BsiEI
- BsiHKAI
- BsiWI
- BsiWI-HF
- BslI
- BsmI
- BsmAI
- BsmBI-v2
- BsmFI
- BsoBI
- Bsp1286I
- BspCNI
- BspDI
- BspEI
- BspHI
- BspMI
- BspQI
- BsrI
- BsrBI
- BsrDI
- BsrFI-v2
- BsrGI-HF
- BssHII
- BssSI-v2
- BstAPI
- BstBI
- BstEII-HF
- BstNI
- BstUI
- BstXI
- BstYI
- BstZ17I-HF
- Bsu36I
- BtgI
- BtgZI
- BtsI-v2
- BtsIMutI
- BtsCI
- Cac8I
- ClaI
- CspCI
- CviAII
- CviKI-1
- CviQI
- DdeI
- DpnI
- DpnII
- DraI
- DraIII-HF
- DrdI
- EaeI
- EagI-HF
- EarI
- EciI
- Eco53kI
- EcoNI
- EcoO109I
- EcoP15I
- EcoRI
- EcoRI-HF
- EcoRV
- EcoRV-HF
- Esp3I
- FatI
- FauI
- Fnu4HI
- FokI
- FseI
- FspI
- FspEI
- HaeII
- HaeIII
- HgaI
- HhaI
- HincII
- HindIII
- HindIII-HF
- HinfI
- HinP1I
- HpaI
- HpaII
- HphI
- Hpy99I
- Hpy166II
- Hpy188I
- Hpy188III
- HpyAV
- HpyCH4III
- HpyCH4IV
- HpyCH4V
- KasI
- KpnI-HF
- LpnPI
- MboI
- MboII
- MfeI-HF
- MluI-HF
- MluCI
- MlyI
- MmeI
- MnlI
- MscI
- MseI
- MslI
- MspI
- MspA1I
- MspJI
- MwoI
- NaeI
- NarI
- NciI
- NcoI
- NcoI-HF
- NdeI
- NgoMIV
- NheI-HF
- NlaIII
- NlaIV
- NmeAIII
- NotI
- NotI-HF
- NruI-HF
- NsiI
- NsiI-HF
- NspI
- PacI
- PaeR7I
- PaqCI
- PciI
- PflFI
- PflMI
- PleI
- PluTI
- PmeI
- PmlI
- PpuMI
- PshAI
- PsiI-v2
- PspGI
- PspOMI
- PspXI
- PstI
- PstI-HF
- PvuI-HF
- PvuII
- PvuII-HF
- RsaI
- RsrII
- SacI-HF
- SacII
- SalI
- SalI-HF
- SapI
- Sau3AI
- Sau96I
- SbfI-HF
- ScaI-HF
- ScrFI
- SexAI
- SfaNI
- SfcI
- SfiI
- SfoI
- SgrAI
- SmaI
- SmlI
- SnaBI
- SpeI-HF
- SphI
- SphI-HF
- SrfI
- SspI-HF
- StuI
- StyI-HF
- StyD4I
- SwaI
- TaqI-v2
- TfiI
- TseI
- Tsp45I
- TspMI
- TspRI
- Tth111I
- XbaI
- XcmI
- XhoI
- XmaI
- XmnI
- ZraI

Recombinant Enzyme

Nicking Endonucleases 54
- Nb.BbvCI
- Nb.BsmI
- Nb.BsrDI
- Nb.BssSI
- Nb.BtsI
- Nt.AlwI
- Nt.BbvCI
- Nt.BsmAI
- Nt.BspQI
- Nt.BstNBI
- WarmStart Nt.BstNBI
- Nt.CviPII

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- I-SceI
- PI-PspI
- PI-SceI

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- NEBuffer 2
- NEBuffer 3
- NEBuffer 4
- rCutSmart Buffer
- NEBuffer Set (r1.1, r2.1, r3.1 and rCutSmart)
- NEBuffer Set (EcoRI/SspI, DpnII)
- S-adenosylmethionine (SAM)
- Nuclease-free Water
- NEBuffer r2.1
- NEBuffer r3.1

Diluent Buffers 57
- Diluent A
- Diluent B
- Diluent C

Gel Loading Dyes 57
- Gel Loading Dye, Blue (6X)
- Gel Loading Dye, Orange (6X)
- Gel Loading Dye, Purple (6X)
- Gel Loading Dye, Purple (6X), no SDS

Other 57
- Recombinant Albumin
- Molecular Biology Grade
- NEB Tube Opener

Learn about dam, dcm and CpG methylation.

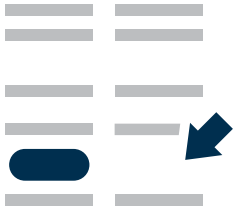
Enzymes in green are supplied with rCutSmart Buffer.

Looking to bring **convenience** to your **workflow**?

Speed up digestions with Time-Saver™ Qualified Restriction Enzymes

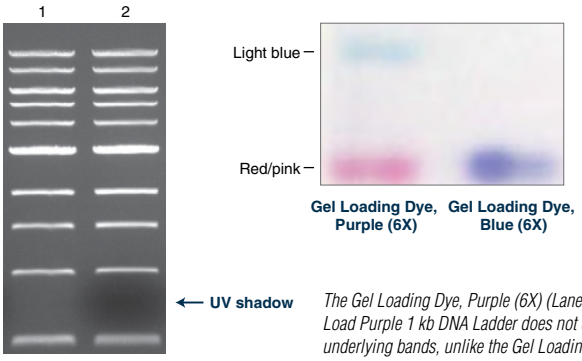
There are > 180 NEB restriction enzymes that can digest DNA in 5–15 minutes; many of which are supplied with rCutSmart Buffer or are High-Fidelity (HF®) Restriction Enzymes. If you prefer, you can also digest overnight with no unwanted star activity. All of our enzymes are rigorously tested for nuclease contamination. Only NEB can offer enzymes with the power to digest in 5–15 minutes, and the flexibility to withstand overnight digestions with no loss of sample.

www.neb.com/timesaver



Improve your analysis with our Purple Gel Loading Dye

Our Gel Loading Dye, Purple (6X), which is supplied with most restriction enzymes and all HF enzymes, sharpens bands and eliminates the UV shadow seen with other dyes. This solution contains SDS, which often results in sharper bands, as some restriction enzymes are known to remain bound to DNA following cleavage.



The Gel Loading Dye, Purple (6X) (Lane 1) included in the Quick-Load Purple 1 kb DNA Ladder does not cast a UV shadow over the underlying bands, unlike the Gel Loading Dye, Blue (6X) (Lane 2).

Simplify reaction setup and double digestion with rCutSmart™ Buffer

Over 210 enzymes are 100% active in a single buffer, rCutSmart Buffer, making it significantly easier to set up double digest reactions. Since rCutSmart Buffer includes Recombinant Albumin, there are fewer tubes and pipetting steps to worry about. Additionally, many DNA modifying enzymes are 100% functionally active in rCutSmart Buffer, eliminating the need for subsequent purification.

www.NEBCutSmart.com

Same high performance, now with BSA-free reaction buffer

To address the increased need for BSA-free reagents, NEB has switched our BSA-containing reaction buffers to Recombinant Albumin (rAlbumin)-containing buffers. We are also in the process of transitioning our enzyme formulations to contain rAlbumin. NEB has rigorously tested these changes and has not seen a difference in performance with these changes.

www.neb.com/BSA-free

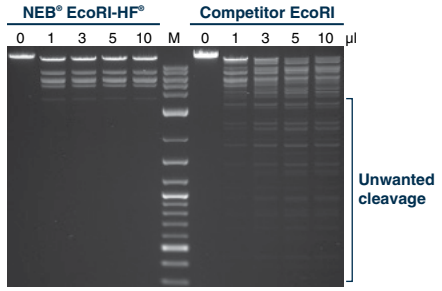
Learn about our switch to Recombinant Albumin.

Looking to **optimize performance** in your **reaction**?

Choose High-Fidelity (HF®) Restriction Enzymes

NEB High-Fidelity (HF) restriction enzymes have the same specificity as native enzymes, with the added benefits of reduced star activity, rapid digestion (5–15 minutes), and 100% activity in rCutSmart Buffer. Enjoy the improved performance of our engineered enzymes at the same price as the native enzymes!

www.neb.com/HF



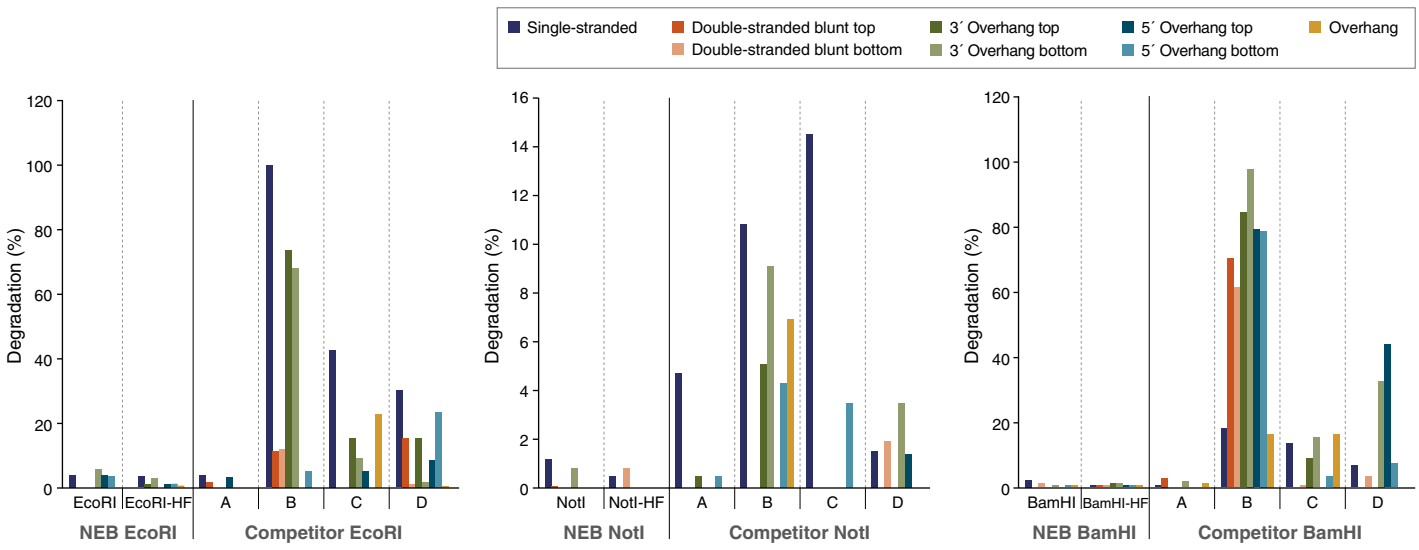
EcoRI-HF (NEB #R3101) shows no star activity in overnight digests, even when used at higher concentrations. *50 µl rxns were set up using 1 µg of Lambda DNA, the indicated amount of enzyme and the recommended reaction buffer. Rxns were incubated overnight at 37°C. Marker M is the 1 kb DNA Ladder (NEB #N3232).*

Benefit from industry-leading quality controls

NEB's reputation as a leader in enzyme technologies stems from the quality and reliability of our restriction enzymes. All of our restriction enzymes undergo stringent quality control testing, ensuring the highest levels of purity and lot-to-lot consistency.

www.neb.com/quality

Restriction Enzyme Competitor Study: Nuclease Contamination



EcoRI, NotI, and BamHI from multiple suppliers were tested in reactions containing a fluorescent labeled single stranded, double stranded blunt, 3´ overhang or 5´ overhang containing oligonucleotides. *The percent degradation is determined by capillary electrophoresis and peak analysis. The resolution is at the single nucleotide level.*

Learn about the benefits of HF enzymes.

AatII

rCutSmart · Cloned at NEB · Recombinant Enzyme · dilB · 37° · 80° · CpG

#R0117S 500 units
#R0117L 2,500 units

5′... G A C G T C ...3′
3′... C T G C A G ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: May exhibit star activity in NEBuffer r2.1.

AciI

rCutSmart · Cloned at NEB · Recombinant Enzyme · dilA · 37° · 65° · CpG

#R0551S 200 units
#R0551L 1,000 units

5′... C C G C ...3′
3′... G G C G ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	25	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

AbaSI

rCutSmart · Cloned at NEB · Recombinant Enzyme · dilC · 25° · 65° · Epi

#R0665S 1,000 units

5′...^{ghm}C $N_{11\text{-}13}$ $N_{9\text{-}10}$ G...3′
3′..... G $N_{9\text{-}10}$ $N_{11\text{-}13}$ ^{x}C...5′

^{x}C = ^{ghm}C, ^{hm}C, ^{m}C or C

Reaction Conditions: rCutSmart Buffer, 25°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	50	100

Activity at 37°C: 0%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

AclI

rCutSmart · Cloned at NEB · Recombinant Enzyme · dilB · 37° · No · CpG

#R0598S 300 units
#R0598L 1,500 units

5′... A A C G T T ... 3′
3′... T T G C A A ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

AccI

rCutSmart · Cloned at NEB · Recombinant Enzyme · dilA · 37° · 80° · CpG

#R0161S 1,000 units
#R0161L 5,000 units

5′... G T M K A C ...3′
3′... C A K M T G ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

AcuI

rCutSmart · Cloned at NEB · Recombinant Enzyme · dilB · 37° · 65°

#R0641S 300 units
#R0641L 1,500 units

5′...C T G A A G $(N)_{16}$ ... 3′
3′...G A C T T C $(N)_{14}$ ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

Acc65I

NEB r3.1 · Cloned at NEB · Recombinant Enzyme · dilA · 37° · 65° · CpG · dcm

#R0599S 2,000 units
#R0599L 10,000 units

5′... G G T A C C ...3′
3′... C C A T G G ...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	75	100	25

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Some Combinations of Overlapping
CpG: Blocked by Some Combinations of Overlapping

Note: May exhibit star activity in NEBuffer 2.1.

AfeI

rCutSmart · Cloned at NEB · Recombinant Enzyme · dilB · 37° · 65° · CpG

#R0652S 200 units
#R0652L 1,000 units

5′... A G C G C T ... 3′
3′... T C G C G A ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

AflII

rCutSmart · 37° · 65°

#R0520S 2,000 units
#R0520L 10,000 units

5′. . . C▼TTAAG . . . 3′
3′. . . GAATT▲C . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

AleI-v2

rCutSmart · dilB · 37° · 65° · CpG

#R0685S 500 units
#R0685L 2,500 units

5′. . .CACNN▼NGTG. . . 3′
3′. . .GTGNNN▲NCAC. . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Overlapping

AflIII

NEB r3.1 · dilB · 37° · 80°

#R0541S 250 units
#R0541L 1,250 units

5′. . . A▼CRYGT . . . 3′
3′. . . TGYRC▲A . . . 5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	100	50

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

AluI

rCutSmart · dilB · 37° · 80°

#R0137S 1,000 units
#R0137L 5,000 units

5′. . . AG▼CT . . . 3′
3′. . . TC▲GA . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

AgeI-HF®

rCutSmart · dilA · 37° · 65° · CpG

#R3552S 300 units
#R3552L 1,500 units

5′. . . A▼CCGGT . . . 3′
3′. . . TGGCC▲A . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

AlwI

rCutSmart · dilA · 37° · NG · dam

#R0513S 500 units
#R0513L 2,500 units

5′. . . GGATC(N)$_4$▼. . . 3′
3′. . . CCTAG(N)$_5$▲. . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	10	100

Methylation Sensitivity:
dam: Blocked
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

AhdI

rCutSmart · dilA · 37° · 65° · CpG

#R0584S 1,000 units
#R0584L 5,000 units

5′. . . GACNNN▼NNGTC . . . 3′
3′. . . CTGNN▲NNNCAG . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	25	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Some Combinations of Overlapping

AlwNI

rCutSmart · dilA · 37° · 80° · dcm

#R0514S 500 units
#R0514L 2,500 units

5′. . .CAGNNN▼CTG. . . 3′
3′. . .GTC▲NNNGAC. . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Not Sensitive

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

ApaI

#R0114S 5,000 units
#R0114L 25,000 units

5´. . . GGGCC▼C . . . 3´
3´. . . C▲CCGGG . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 50,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	25	<10	100

Activity at 25°C: 100%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Blocked by Overlapping

AscI

#R0558S 500 units
#R0558L 2,500 units

5´. . . GG▼CGCGCC . . . 3´
3´. . . CCGCGC▲GG . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	10	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

ApaLI

#R0507S 2,500 units
#R0507L 12,500 units
for high (5X) concentration
#R0507M 12,500 units

5´. . . G▼TGCAC . . . 3´
3´. . . CACGT▲G . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 and 50,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

AseI

#R0526S 2,000 units
#R0526L 10,000 units
for high (5X) concentration
#R0526M 10,000 units

5´. . . AT▼TA AT . . . 3´
3´. . . TA AT▲TA . . . 5´

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 and 50,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	100	10

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in NEBuffer r2.1. Star activity may result from a glycerol concentration of >5%.

ApeKI

#R0643S 250 units
#R0643L 1,250 units

5´. . . G▼CWGC . . . 3´
3´. . . CGWC▲G . . . 5´

Reaction Conditions: NEBuffer r3.1, 75°C

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	100	10

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

AsiSI

#R0630S 500 units
#R0630L 2,500 units

5´. . . GCGAT▼CGC . . . 3´
3´. . . CGC▲TAGCG . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: Star activity may result from extended digestion.

ApoI-HF®

#R3566S 1,000 units
#R3566L 5,000 units

5´. . . R▼AATTY . . . 3´
3´. . . YTTAA▲R . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

AvaI

#R0152S 2,000 units
#R0152L 10,000 units
for high (5X) concentration
#R0152T 2,000 units
#R0152M 10,000 units

5´. . . C▼YCGRG . . . 3´
3´. . . GRGCY▲C . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	100	25	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2+site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

AvaII

rCutSmart · RR · dA · 37° · 80° · CpG · dcm

#R0153S 2,000 units
#R0153L 10,000 units

for high (5X) concentration
#R0153M 10,000 units

5′. . . G▼GWCC . . . 3′
3′. . . CCWG▲G . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	75	10	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Blocked by Overlapping

AvrII

rCutSmart · RR · dB · 37° · No

#R0174S 100 units
#R0174L 500 units

5′. . . C▼CTAGG . . . 3′
3′. . . GGATC▲C . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BaeI

rCutSmart · RR · dA · 37° · 65° · CpG

#R0613S 250 units

5′. .▼10(N) AC (N)4GTAY C (N)12▼. .3′
3′. .▲15(N) TG (N)4CATRG (N)7▲. .5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Activity at 25°C: 100%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

BaeGI

NEB r3.1 · RR · dA · 37° · 80°

#R0708S 500 units

5′. . . G K G C M▼C . . . 3′
3′. . . C▲M C G K G . . . 5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	75	100	25

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BamHI

NEB r3.1 · RR · dA · 37° · No

#R0136S 10,000 units
#R0136L 50,000 units

for high (5X) concentration
#R0136T 10,000 units
#R0136M 50,000 units

5′. . . G▼GATCC . . . 3′
3′. . . CCTAG▲G . . . 5′

Reaction Conditions: NEBuffer r3.1, 37°C

Concentration: 20,000 and 100,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in NEBuffer r1.1, r2.1 and rCutSmart Buffers. Star activity may result from a glycerol concentration of >5%.

BamHI-HF®

rCutSmart · RR · e · dA · 37° · No

#R3136S 10,000 units
#R3136L 50,000 units

for high (5X) concentration
#R3136T 10,000 units
#R3136M 50,000 units

5′. . . G▼GATCC . . . 3′
3′. . . CCTAG▲G . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BanI

rCutSmart · RR · dA · 37° · 65° · CpG · dcm

#R0118S 5,000 units

5′. . . G▼GYRCC . . . 3′
3′. . . CCRY G▲G . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	25	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Some Combinations of Overlapping
CpG: Blocked by Some Combinations of Overlapping

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

BanII

rCutSmart · RR · dA · 37° · 80°

#R0119S 2,000 units

5′. . . G R G C Y▼C . . . 3′
3′. . . C▲Y C G R G . . . 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

BbsI

#R0539S 300 units
#R0539L 1,500 units

5′... G A A G A C $(N)_2$▼... 3′
3′... C T T C T G $(N)_6$▲... 5′

Reaction Conditions: NEBuffer r2.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	25	75

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BbsI-HF®

#R3539S 300 units
#R3539L 1,500 units
for high (2X) concentration
#R3539M 1,000 units

5′... G A A G A C $(N)_2$▼... 3′
3′... C T T C T G $(N)_6$▲... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	10	100

Concentration: 20,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BbvI

#R0173S 300 units

5′... G C A G C $(N)_8$▼... 3′
3′... C G T C G $(N)_{12}$▲... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

BbvCI

#R0601S 100 units
#R0601L 500 units

5′... C C▼T C A G C ... 3′
3′... G G A G T▲C G ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 2,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Overlapping

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	50	100

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

BccI

#R0704S 1,000 units
#R0704L 5,000 units

5′... C C A T C $(N)_4$▼... 3′
3′... G G T A G $(N)_5$▲... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

BceAI

#R0623S 50 units
#R0623L 250 units

5′... A C G G C $(N)_{12}$▼... 3′
3′... T G C C G $(N)_{14}$▲... 5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: May exhibit star activity in NEBuffer r1.1, r2.1 and rCutSmart Buffers. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

BcgI

#R0545S 250 units

5′... $_{10}$▼(N) C G A $(N)_6$ T G C $(N)_{12}$▼... 3′
3′... $_{12}$▲(N) G C T $(N)_6$ A C G $(N)_{10}$▲... 5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	75	100	50

Methylation Sensitivity:
dam: Impaired by Overlapping
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

Note: May exhibit Star Activity in NEBuffer r2.1 and rCutSmart Buffer.

BciVI

#R0596S 200 units
#R0596L 1,000 units

5′... G T A T C C $(N)_6$▼... 3′
3′... C A T A G G $(N)_5$▲... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	25	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

BclI

#R0160S 3,000 units

5′... T▼G A T C A ... 3′
3′... A C T A G▲T ... 5′

Reaction Conditions: NEBuffer r3.1, 50°C

Concentration: 10,000 units/ml

Activity at 37°C: 50%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	75

Methylation Sensitivity:

dam: Blocked

dcm: Not Sensitive

CpG: Not Sensitive

BclI-HF

#R3160S 3,000 units
#R3160L 15,000 units

5′... T▼G A T C A ... 3′
3′... A C T A G▲T ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	10	100

Methylation Sensitivity:

dam: Blocked

dcm: Not Sensitive

CpG: Not Sensitive

BcoDI

#R0542S 1,000 units

5′... G T C T C (N)$_1$▼... 3′
3′... C A G A G (N)$_5$▲... 5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	75	75	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Impaired by Some Combinations of Overlapping

BfaI

#R0568S 500 units
#R0568L 2,500 units

5′... C▼T A G ... 3′
3′... G A T▲C ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	10	<10	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

Note: Star activity may result from extended digestion.

BfuAI

#R0701S 250 units
#R0701L 1,250 units

5′... A C C T G C (N)$_4$▼... 3′
3′... T G G A C G (N)$_8$▲... 5′

Reaction Conditions: NEBuffer r3.1, 50°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Activity at 37°C: 25%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	25	100	10

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Impaired by Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

BglI

#R0143S 2,000 units
#R0143L 10,000 units

5′... G C C N N N N▼N G G C ... 3′
3′... C G G N▲N N N N C C G ... 5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	25	100	10

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked by Some Combinations of Overlapping

BglII

#R0144S 2,000 units
#R0144L 10,000 units

for high (5X) concentration

#R0144M 10,000 units

5′... A▼G A T C T ... 3′
3′... T C T A G▲A ... 5′

Reaction Conditions: NEBuffer r3.1, 37°C

Concentration: 10,000 and 50,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	100	<10

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

BlpI

#R0585S 500 units
#R0585L 2,500 units

5′... G C▼T N A G C ... 3′
3′... C G A N▲T C G ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	10	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

BmgBI

#R0628S 500 units
#R0628L 2,500 units

5′... C A C▼G T C ...3′
3′... G T G▲C A G ...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	10	100	10

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: Star activity may result from a glycerol concentration of >5%.

Bpu10I

#R0649S 200 units

5′... C C▼T N A G C ...3′
3′... G G A N T▲C G ...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	25	100	25

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

BmrI

#R0600S 100 units

5′... A C T G G G (N)$_5$▼... 3′
3′... T G A C C C (N)$_4$▲... 5′

Reaction Conditions: NEBuffer r2.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	75	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in rCutSmart Buffer.

BpuEI

#R0633S 500 units

5′... C T T G A G (N)$_{16}$▼... 3′
3′... G A A C T C (N)$_{14}$▲... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in NEBuffer r1.1, and NEBuffer r3.1.

BmtI-HF®

#R3658S 300 units
#R3658L 1,500 units

5′... G C T A G▼C ...3′
3′... C▲G A T C G ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BsaI-HF®v2

#R3733S 1,000 units
#R3733L 5,000 units

5′... G G T C T C (N)$_1$▼... 3′
3′... C C A G A G (N)$_5$▲... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Impaired by Some Combinations of Overlapping
CpG: Blocked by Some Combinations of Overlapping

BpmI

#R0565S 100 units
#R0565L 500 units

5′... C T G G A G (N)$_{16}$▼... 3′
3′... G A C C T C (N)$_{14}$▲... 5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion.

BsaAI

#R0531S 500 units

5′... Y A C▼G T R ...3′
3′... R T G▲C A Y ...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

BsaBI

rCutSmart · RR · dirB · 60° · 80° · CpG · dam

#R0537S 2,000 units

5′. . . G A T N N ▼ N N A T C . . . 3′
3′. . . C T A N N N N T A G . . . 5′
▲

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	75	100

Reaction Conditions: rCutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 37°C: 25%

Methylation Sensitivity:

dam: Blocked by Overlapping

dcm: Not Sensitive

CpG: Blocked by Some Combinations of Overlapping

Note: Star activity may result from extended digestion.

BsaHI

rCutSmart · RR · dirC · 37° · 80° · CpG · dcm

#R0556S 2,000 units

5′. . . G R ▼ C G Y C . . . 3′
3′. . . C Y G C ▲ R G . . . 5′

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Blocked by Some Combinations of Overlapping

CpG: Blocked

BsaJI

rCutSmart · RR · dirA · 60° · 80°

#R0536S 1,000 units

5′. . . C ▼ C N N G G . . . 3′
3′. . . G G N N C ▲ C . . . 5′

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Reaction Conditions: rCutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 37°C: 25%

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

BsaWI

rCutSmart · RR · dirA · 60° · 80°

#R0567S 250 units

5′. . . W ▼ C C G G W . . . 3′
3′. . . W G G C C ▲ W . . . 5′

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	50	100

Reaction Conditions: rCutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 37°C: 50%

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

BsaXI

rCutSmart · dirC · 37° · No

#R0609S 100 units
#R0609L 500 units

5′. . . ▼ 9(N) A C (N)5 C T C C (N)10 ▼ . . . 3′
3′. . . ▲ 12(N) T G (N)5 G A G G (N)7 ▲ . . . 5′

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	10	100

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 2,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

Note: May exhibit star activity in NEBuffer r1.1, and NEBuffer r2.1.

BseRI

rCutSmart · RR · dirA · 37° · 80°

#R0581S 200 units
#R0581L 1,000 units

5′. . . G A G G A G (N)10 ▼ . . . 3′
3′. . . C T C C T C (N)8 ▲ . . . 5′

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	75	100

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

BseYI

NEB r3.1 · RR · dirB · 37° · 80° · CpG

#R0635S 100 units
#R0635L 500 units

5′. . . C ▼ C C A G C . . . 3′
3′. . . G G G T C ▲ G . . . 5′

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	100	50

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked by Overlapping

BsgI

rCutSmart · RR · 2*site · dirB · 37° · 65°

#R0559S 50 units
#R0559L 250 units

5′. . . G T G C A G (N)16 ▼ . . . 3′
3′. . . C A C G T C (N)14 ▲ . . . 5′

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	25	100

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

BsiEI

rCutSmart · Cloned at NEB · Recombinant · Time-Saver · dilA · 60° · No heat inactivation · CpG

#R0554S 1,000 units

5´. . . C G R Y▼C G . . . 3´
3´. . . G C▲Y R G C . . . 5´

Reaction Conditions: rCutSmart Buffer, 60°C

Concentration: 10,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	<10	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked

BslI

rCutSmart · Cloned at NEB · Recombinant · Time-Saver · dilA · 55° · No heat inactivation · CpG · dcm

#R0555S 1,000 units
#R0555L 5,000 units

5´. . . C C N N N N N▼N N G G . . . 3´
3´. . . G G N N▲N N N N N C C . . . 5´

Reaction Conditions: rCutSmart Buffer, 55°C

Concentration: 10,000 units/ml

Activity at 37°C: 50%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	75	100	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Blocked by Some Combinations of Overlapping

CpG: Blocked by Some Combinations of Overlapping

BsiHKAI

rCutSmart · Cloned at NEB · Recombinant · Time-Saver · dilA · 65° · No heat inactivation

#R0570S 1,000 units

5´. . . G W G C W▼C . . . 3´
3´. . . C▲W C G W G . . . 5´

Reaction Conditions: rCutSmart Buffer, 65°C

Concentration: 10,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

BsmI

rCutSmart · Cloned at NEB · Recombinant · Time-Saver · dilA · 65° · Heat inactivation 80°

#R0134S 500 units
#R0134L 2,500 units

5´. . . G A A T G C N▼. . . 3´
3´. . . C T T A C▲G N . . . 5´

Reaction Conditions: rCutSmart Buffer, 65°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	<10	100

Activity at 37°C: 10%

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Not Sensitive

BsiWI

NEB r3.1 · Cloned at NEB · Recombinant · Time-Saver · dilB · 55° · Heat inactivation 65° · CpG

#R0553S 300 units
#R0553L 1,500 units

5´. . . C▼G T A C G . . . 3´
3´. . . G C A T G▲C . . . 5´

Reaction Conditions: NEBuffer r3.1, 55°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 37°C: 25%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	100	25

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked

Note: May exhibit star activity in NEBuffer r2.1.

BsmAI

rCutSmart · Cloned at NEB · Recombinant · Time-Saver · dilB · 55° · No heat inactivation · CpG

#R0529S 1,000 units
#R0529L 5,000 units

5´. . . G T C T C (N)$_1$▼. . . 3´
3´. . . C A G A G (N)$_5$▲. . . 5´

Reaction Conditions: rCutSmart Buffer, 55°C

Concentration: 5,000 units/ml

Activity at 37°C: 50%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked by Some Combinations of Overlapping

BsiWI-HF®

rCutSmart · Cloned at NEB · Recombinant · Engineered for Performance · Time-Saver · dilB · 37° · No heat inactivation · CpG

#R3553S 300 units
#R3553L 1,500 units

5´. . . C▼G T A C G . . . 3´
3´. . . G C A T G▲C . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	10	100

Methylation Sensitivity:

dam: Not Sensitive

dcm: Not Sensitive

CpG: Blocked

BsmBI-v2

NEB r3.1 · Cloned at NEB · Recombinant · Engineered for Performance · Time-Saver · dilB · 55° · Heat inactivation 80° · CpG

#R0739S 200 units
#R0739L 1,000 units

5´. . . C G T C T C (N)$_1$▼. . . 3´
3´. . . G C A G A G (N)$_5$▲. . . 5´

Reaction Conditions: NEBuffer r3.1, 55°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	100	25

Concentration: 10,000 units/ml

Activity at 37°C: 10%

Methylation Sensitivity:

CpG: Blocked

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2+site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

BsmFI

rCutSmart · RR · dilA · 65° · 80° · CpG · dcm

#R0572S 100 units
#R0572L 500 units

5′...G G G A C $(N)_{10}$▼...3′
3′...C C C T G $(N)_{14}$▲...5′

Reaction Conditions: rCutSmart Buffer, 65°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 2,000 units/ml

Activity at 37°C: 100%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Blocked by Overlapping

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

BsoBI

rCutSmart · RR · dilA · 37° · 80°

#R0586S 10,000 units

5′...C▼Y C G R G...3′
3′...G R G C Y▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Bsp1286I

rCutSmart · RR · dilA · 37° · 65°

#R0120S 500 units

5′...G D G C H▼C...3′
3′...C▲H C G D G...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	25	25	100

Methylation Sensitivity:
dam methylation: Not Sensitive
dcm methylation: Not Sensitive
CpG methylation: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

BspCNI

rCutSmart · RR · dilA · 37° · 80°

#R0624S 100 units

5′...C T C A G $(N)_{10}$▼...3′ and 5′...C T C A G $(N)_{9}$▼...3′
3′...G A G T C $(N)_{8}$▲...5′ 3′...G A G T C $(N)_{7}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	75	10	100

Activity at 25°C: 100%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BspDI

rCutSmart · RR · dilA · 37° · 80° · CpG · dam

#R0557S 2,000 units

5′...A T▼C G A T...3′
3′...T A G C▲T A...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	75	50	100

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Blocked

BspEI

NEB r3.1 · RR · dilB · 37° · 80° · CpG · dam

#R0540S 1,000 units
#R0540L 5,000 units

5′...T▼C C G G A...3′
3′...A G G C C▲T...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	10	100	<10

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Impaired

BspHI

rCutSmart · RR · dilA · 37° · 80° · dam

#R0517S 500 units
#R0517L 2,500 units

5′...T▼C A T G A...3′
3′...A G T A C▲T...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	25	100

Methylation Sensitivity:
dam: Impaired by Overlapping
dcm: Not Sensitive
CpG: Not Sensitive

BspMI

NEB r3.1 · RR · 2*site · dilB · 37° · 65°

#R0502S 100 units

5′...A C C T G C $(N)_{4}$▼...3′
3′...T G G A C G $(N)_{8}$▲...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	100	10

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in NEBuffer r2.1.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

BspQI

#R0712S 500 units
#R0712L 2,500 units

5′...GCTCTTC(N)$_1$▼...3′
3′...CGAGAAG(N)$_4$▲...5′

Reaction Conditions: NEBuffer r3.1, 50°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 37°C: 50%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

BsrFI-v2

#R0682S 1,000 units

5′...R▼CCGGY...3′
3′...YGGCC▲R...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	25	0	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

BsrI

#R0527S 1,000 units
#R0527L 5,000 units

5′...ACTGGN▼...3′
3′...TGAC▲CN...5′

Reaction Conditions: NEBuffer r3.1, 65°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	100	10

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BsrGI-HF®

#R3575S 1,000 units
#R3575L 5,000 units

5′...T▼GTACA...3′
3′...ACATG▲T...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BsrBI

#R0102S 1,000 units
#R0102L 5,000 units

5′...CCG▼CTC...3′
3′...GGC▲GAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

BssHII

#R0199S 500 units
#R0199L 2,500 units

for high (5X) concentration
#R0199M 2,500 units

5′...G▼CGCGC...3′
3′...CGCGC▲G...5′

Reaction Conditions: rCutSmart Buffer, 50°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Concentration: 5,000 and 25,000 units/ml

Activity at 37°C: 100%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

BsrDI

#R0574S 200 units
#R0574L 1,000 units

5′...GCAATGNN▼...3′
3′...CGTTAC▲NN...5′

Reaction Conditions: NEBuffer r2.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

Activity at 65°C: 100%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	75	25

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

BssSI-v2

#R0680S 200 units
#R0680L 1,000 units

5′...C▼ACGAG...3′
3′...GTGCT▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	25	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BstAPI

rCutSmart | 60° | 80° | CpG

#R0654S 200 units
#R0654L 1,000 units

5′...GCANNNNNTGC...3′
3′...CGTNNNNNNACG...5′

Reaction Conditions: rCutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

Activity at 37°C: 25%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

BstBI

rCutSmart | 65° | No | CpG

#R0519S 2,500 units
#R0519L 12,500 units

5′...TTCGAA...3′
3′...AAGCTT...5′

Reaction Conditions: rCutSmart Buffer, 65°C

Concentration: 20,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

BstEII-HF®

rCutSmart | 37° | 80°

#R3162S 2,000 units
#R3162L 10,000 units
for high (5X) concentration
#R3162M 10,000 units

5′...GGTNACC...3′
3′...CCANTGG...5′

Reaction Conditions: rCutSmart Buffer, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	10	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BstNI

r3.1 | 60° | No

#R0168S 3,000 units
#R0168L 15,000 units

5′...CCWGG...3′
3′...GGWCC...5′

Reaction Conditions: NEBuffer r3.1, 60°C

Concentration: 10,000 units/ml

Activity at 37°C: 25%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	100	75

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BstUI

rCutSmart | 60° | No | CpG

#R0518S 1,000 units
#R0518L 5,000 units

5′...CGCG...3′
3′...GCGC...5′

Reaction Conditions: rCutSmart Buffer, 60°C

Concentration: 10,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

BstXI

r3.1 | B | 37° | 80° | dcm

#R0113S 1,000 units
#R0113L 5,000 units

5′...CCANNNNNNTGG...3′
3′...GGTNNNNNNNACC...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	100	25

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Some Combinations of Overlapping
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

BstYI

rCutSmart | 60° | No

#R0523S 2,000 units

5′...RGATCY...3′
3′...YCTAGR...5′

Reaction Conditions: rCutSmart Buffer, 60°C

Concentration: 10,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	75	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BstZ17I-HF®

rCutSmart | 37° | 80° | CpG

#R3594S 1,000 units
#R3594L 5,000 units

5′...GTATAC...3′
3′...CATATG...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

Bsu36I

rCutSmart · Cloned at NEB · Recombinant · Time-Saver · dilC · 37° · 80°

#R0524S 1,000 units
#R0524L 5,000 units

5´. . . C C▼T N A G G . . . 3´
3´. . . G G A N T▲C C . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BtsIMutI

rCutSmart · Cloned at NEB · Recombinant · Engineered · dilA · 55° · 80°

#R0664S 100 units

5´. . . C A G T G N N▼. . . 3´
3´. . . G T C A C▲N N . . . 5´

Reaction Conditions: rCutSmart Buffer, 55°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 1,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Activity at 37°C: 50%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BtgI

rCutSmart · Cloned at NEB · Recombinant · Time-Saver · dilB · 37° · 80°

#R0608S 1,000 units

5´. . . C▼C R Y G G . . . 3´
3´. . . G G Y R C▲C . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BtsCI

rCutSmart · Cloned at NEB · Recombinant · Time-Saver · dilB · 50° · 80°

#R0647S 2,000 units

5´. . . G G A T G N N▼. . . 3´
3´. . . C C T A C▲N N . . . 5´

Reaction Conditions: rCutSmart Buffer, 50°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	25	100

Activity at 37°C: 25%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

BtgZI

rCutSmart · Cloned at NEB · Recombinant · dilA · 60° · 80° · CpG

#R0703S 100 units
#R0703L 500 units

5´. . . G C G A T G (N)$_{10}$▼. . . 3´
3´. . . C G C T A C (N)$_{14}$▲. . . 5´

Reaction Conditions: rCutSmart Buffer, 60°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

Activity at 37°C: 50%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	25	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired

Note: Star activity may result from a glycerol concentration of >5%.

Cac8I

rCutSmart · Time-Saver · dilB · 37° · 65° · CpG

#R0579L 500 units

5´. . . G C N▼N G C . . . 3´
3´. . . C G N▲N C G . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	75	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

BtsI-v2

rCutSmart · Cloned at NEB · Recombinant · Engineered · Time-Saver · dilA · 37° · No

#R0667S 500 units
#R0667L 2,500 units

5´. . . G C A G T G N N▼. . . 3´
3´. . . C G T C A C▲N N . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	25	100

dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

ClaI

rCutSmart · Recombinant · Time-Saver · dilA · 37° · 65° · CpG · dam

#R0197S 1,000 units
#R0197L 5,000 units

5´. . . A T▼C G A T . . . 3´
3´. . . T A G C▲T A . . . 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

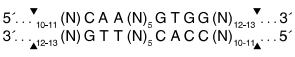
NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	50	100

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Blocked

CspCI

rCutSmart | RR | 2*site | dilA | 37° | 65°

#R0645S 500 units



NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	10	100

dcm: Not Sensitive
CpG: Not Sensitive

Note: The exact positions of cleavage can vary ± one base depending on the sequence of the DNA flanking the recognition site and the digestion conditions

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive

CviAII

rCutSmart | RR | dilC | 25° | 65°

#R0640S 200 units
#R0640L 1,000 units

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	10	100

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Reaction Conditions: rCutSmart Buffer, 25°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

CviKI-1

rCutSmart | RR | dilA | 37° | No

#R0710S 250 units

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

CviQI

NEB r3.1 | RR | dilC | 25° | No

#R0639S 2,000 units
#R0639L 10,000 units

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	75

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in NEBuffer r2.1, and rCutSmart Buffer.

Reaction Conditions: NEBuffer r3.1, 25°C

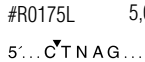
Concentration: 10,000 units/ml

Activity at 37°C: 25%

DdeI

rCutSmart | RR | dilB | 37° | 65°

#R0175S 1,000 units
#R0175L 5,000 units



NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

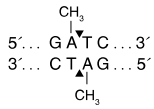
Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

DpnI

rCutSmart | RR | dilB | 37° | 80° | CpG | Epi

#R0176S 1,000 units
#R0176L 5,000 units



NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	75	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

Note: DpnI cleaves only when its recognition site is methylated. DNA purified from a dam$^+$ strain will be a substrate for DpnI.

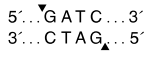
Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 units/ml

DpnII

NEBU | RR | dilB | 37° | 65° | dam | Epi

#R0543S 1,000 units
#R0543L 5,000 units
for high (5X) concentration
#R0543T 1,000 units
#R0543M 5,000 units



NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	25	100	25

Methylation Sensitivity:
dam: Blocked
dcm: Not Sensitive
CpG: Not Sensitive

Note: Will exhibit star activity in NEBuffer 3.1. We recommend the use of NEB DpnII Unique Buffer.

Reaction Conditions:
Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 and 50,000 units/ml

DraI

rCutSmart | RR | dilA | 37° | 65°

#R0129S 2,000 units
#R0129L 10,000 units

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	75	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

DraIII-HF®

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · dilB · 37° · Heat Inactivation · CpG

#R3510S 1,000 units
#R3510L 5,000 units

5′...CACNNN▼GTG...3′
3′...GTG▲NNNCAC...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Overlapping

EarI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · dilB · 37° · Heat Inactivation · CpG

#R0528S 500 units
#R0528L 2,500 units

5′...CTCTTC $(N)_1$▼...3′
3′...GAGAAG $(N)_4$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	10	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Overlapping

DrdI

rCutSmart · Recombinant Enzyme · Time-Saver Qualified · dilA · 37° · Heat Inactivation · CpG

#R0530S 300 units
#R0530L 1,500 units

5′...GACNNNNNNGTC...3′
3′...CTGNNNNNNCAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

EciI

rCutSmart · Cloned at NEB · Recombinant Enzyme · dilA · 37° · Heat Inactivation · CpG

#R0590S 100 units
#R0590L 500 units

5′...GGCGGA $(N)_{11}$▼...3′
3′...CCGCCT $(N)_9$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

Note: Star activity may result from extended digestion.

EaeI

rCutSmart · Cloned at NEB · Recombinant Enzyme · dilA · 37° · Heat Inactivation · CpG · dcm

#R0508S 200 units
#R0508L 1,000 units

5′...Y▼GGCCR...3′
3′...RCCGG▲Y...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Blocked by Overlapping

Eco53kI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · dilA · 37° · Heat Inactivation · CpG

#R0116S 1,000 units

5′...GAG▼CTC...3′
3′...CTC▲GAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 25°C: 50%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

EagI-HF®

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · dilB · 37° · Heat Inactivation · CpG

#R3505S 500 units
#R3505L 2,500 units

for high (5X) concentration
#R3505M 2,500 units

5′...C▼GGCCG...3′
3′...GCCGG▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

EcoNI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · dilA · 37° · Heat Inactivation

#R0521S 1,000 units
#R0521L 5,000 units

5′...CCTNN▼NNNAGG...3′
3′...GGANNN▲NNTCC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	75	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · Recombinant Enzyme · Epi EpiMark Validated · Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

EcoO109I

rCutSmart · dilA · 37° · 65° · dcm

#R0503S 2,000 units

5′...RG▼GNCCY...3′
3′...YCCNG▲GR...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

EcoP15I

NEB r3.1 · 2*site · dilA · 37° · 65°

#R0646S 500 units

5′...CAGCAG(N)$_{25}$▼...3′
3′...GTCGTC(N)$_{27}$▲...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Supplement with 1X ATP. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

EcoRI

NEBU · dilC · 37° · 65° · CpG

#R0101S 10,000 units
#R0101L 50,000 units

for high (5X) concentration
#R0101T 10,000 units
#R0101M 50,000 units

5′...G▼AATTC...3′
3′...CTTAA▲G...5′

Reaction Conditions: NEBuffer EcoRI/SspI, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	50	50

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

Note: May exhibit star activity in NEBuffer r2.1 or rCutSmart Buffer.

EcoRI-HF®

rCutSmart · dilC · 37° · 65° · CpG

#R3101S 10,000 units
#R3101L 50,000 units

for high (5X) concentration
#R3101T 10,000 units
#R3101M 50,000 units

5′...G▼AATTC...3′
3′...CTTAA▲G...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

EcoRV

NEB r3.1 · dilA · 37° · 80° · CpG

#R0195S 4,000 units
#R0195L 20,000 units

for high (5X) concentration
#R0195T 4,000 units.....$67
#R0195M 20,000 units.....$273

5′...GAT▼ATC...3′
3′...CTA▲TAG...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	100	10

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Some Combinations of Overlapping

EcoRV-HF®

rCutSmart · dilB · 37° · 65° · CpG

#R3195S 4,000 units
#R3195L 20,000 units

for high (5X) concentration
#R3195T 4,000 units
#R3195M 20,000 units

5′...GAT▼ATC...3′
3′...CTA▲TAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Some Combinations of Overlapping

Esp3I

rCutSmart · dilB · 37° · 65° · CpG

#R0734S 300 units
#R0734L 1,500 units

5′...CGTCTC(N)$_1$▼...3′
3′...GCAGAG(N)$_5$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

FatI

NEB r2.1 · dilA · 55° · 80°

#R0650S 50 units
#R0650L 250 units

5′...▼CATG...3′
3′...GTAC▲...5′

Reaction Conditions: NEBuffer r2.1, 55°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	50	50

Activity at 37°C: 100%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

FauI

rCutSmart · Cloned at NEB · Recombinant Enzyme · dilA · 55° · 65° · CpG

#R0651S 200 units

5′...CCCGC (N)$_4$▼...3′
3′...GGGCG (N)$_6$▲...5′

Reaction Conditions: rCutSmart Buffer, 55°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Activity at 37°C: 50%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: Star activity may result from a glycerol concentration of >5%.

FspI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · dilC · 37° · No Heat Inactivation · CpG

#R0135S 500 units
#R0135L 2,500 units

5′...TGC▼GCA...3′
3′...ACG▲CGT...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Fnu4HI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · dilA · 37° · No Heat Inactivation · CpG

#R0178S 200 units
#R0178L 1,000 units

5′...GC▼NGC...3′
3′...CGN▲CG...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

FspEI

rCutSmart · Cloned at NEB · Recombinant Enzyme · dilB · 37° · 80° Heat Inactivation · Epi

#R0662S 200 units

5′...C^{m}C (N)$_{12}$▼...3′
3′...G G (N)$_{16}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

FokI

rCutSmart · Cloned at NEB · Recombinant Enzyme · 2*site · dilA · 37° · 65° Heat Inactivation · CpG · dcm

#R0109S 1,000 units
#R0109L 5,000 units

5′...GGATG (N)$_9$▼...3′
3′...CCTAC (N)$_{13}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	75	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Impaired by Overlapping
CpG: Impaired by Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

HaeII

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · dilA · 37° · 80° Heat Inactivation · CpG

#R0107S 2,000 units
#R0107L 10,000 units

5′...RGCGC▼Y...3′
3′...Y▲CGCGR...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

FseI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · dilB · 37° · 65° Heat Inactivation · CpG · dcm

#R0588S 100 units
#R0588L 500 units

5′...GGCCGG▼CC...3′
3′...CC▲GGCCGG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	75	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Impaired by Some Combinations of Overlapping
CpG: Blocked

HaeIII

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · dilA · 37° · 80° Heat Inactivation

#R0108S 3,000 units
#R0108L 15,000 units

for high (5X) concentration
#R0108T 3,000 units
#R0108M 15,000 units

5′...GG▼CC...3′
3′...CC▲GG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	25	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

HgaI

#R0154S 100 units

5′... G A C G C (N)$_5$▼... 3′
3′... C T G C G (N)$_{10}$▲... 5′

Reaction Conditions: NEBuffer r1.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

HhaI

#R0139S 2,000 units
#R0139L 10,000 units

5′... G C G▼C ... 3′
3′... C▲G C G ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

HincII

#R0103S 1,000 units
#R0103L 5,000 units

5′... G T Y▼R A C ... 3′
3′... C A R▲Y T G ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

HindIII

#R0104S 10,000 units
#R0104L 50,000 units

for high (5X) concentration
#R0104T 10,000 units
#R0104M 50,000 units

5′... A▼A G C T T ... 3′
3′... T T C G A▲A ... 5′

Reaction Conditions: NEBuffer r2.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 20,000 and 100,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	50	50

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion.

HindIII-HF®

#R3104S 10,000 units
#R3104L 50,000 units

for high (5X) concentration
#R3104T 10,000 units
#R3104M 50,000 units

5′... A▼A G C T T ... 3′
3′... T T C G A▲A ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

HinfI

#R0155S 5,000 units
#R0155L 25,000 units

for high (5X) concentration
#R0155T 5,000 units.....$69
#R0155M 25,000 units.....$281

5′... G▼A N T C ... 3′
3′... C T N A▲G ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

HinP1I

#R0124S 2,000 units

5′... G▼C G C ... 3′
3′... C G C▲G ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

HpaI

#R0105S 500 units
#R0105L 2,500 units

5′... G T T▼A A C ... 3′
3′... C A A▲T T G ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	75	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

Note: May exhibit star activity in NEBuffer r2.1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

HpaII

rCutSmart · cutA · 37° · 80° · CpG · Epi

#R0171S 2,000 units
#R0171L 10,000 units
for high (5X) concentration
#R0171M 10,000 units

5′...C▼CGG...3′
3′...GGC▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	<10	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

HphI

rCutSmart · cutB · 37° · 65° · dam · dcm

#R0158S 1,000 units
#R0158L 5,000 units

5′...GGTGA(N)$_8$▼...3′
3′...CCACT(N)$_7$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Blocked

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	<10	100

dcm: Blocked
CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5% Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

Hpy99I

rCutSmart · cutA · 37° · 65° · CpG

#R0615S 100 units
#R0615L 500 units

5′...CGWCG▼...3′
3′...▲GCWGC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	10	<10	100

Methylation Sensitivity:
dam methylation: Not Sensitive
dcm methylation: Not Sensitive
CpG methylation: Blocked

Hpy166II

rCutSmart · cutC · 37° · 65° · CpG

#R0616S 1,000 units

5′...GTN▼NAC...3′
3′...CAN▲NTG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

Hpy188I

rCutSmart · cutA · 37° · 65° · dam

#R0617S 1,000 units
#R0617L 5,000 units

5′...TCN▼GA...3′
3′...AGNCT...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	50	100

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

Hpy188III

rCutSmart · cutB · 37° · 65° · CpG · dam

#R0622S 500 units
#R0622L 2,500 units

5′...TCNNGA...3′
3′...AGNNCT...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	10	100

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Blocked by Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

HpyAV

rCutSmart · 37° · 65° · CpG

#R0621S 100 units
#R0621L 500 units

5′...CCTTC(N)$_6$▼...3′
3′...GGAAG(N)$_5$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

HpyCH4III

rCutSmart · cutA · 37° · 65°

#R0618S 250 units
#R0618L 1,250 units

5′...ACN▼GT...3′
3′...TGNCA...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	25	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

HpyCH4IV

rCutSmart · RR · dilA · 37° · 65° · CpG

#R0619S 500 units
#R0619L 2,500 units

5′... A▼CGT ...3′
3′... TGCA▲ ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	25	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

HpyCH4V

rCutSmart · RR · dilA · 37° · 65°

#R0620S 100 units
#R0620L 500 units

5′... TG▼CA ...3′
3′... AC▲GT ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	25	100

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

KasI

rCutSmart · RR · dilB · 37° · 65° · CpG

#R0544S 250 units
#R0544L 1,250 units

5′... G▼GCGCC ...3′
3′... CCGCG▲G ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: Star activity may result from a glycerol concentration of >5%.

KpnI-HF®

rCutSmart · RR · e · dilA · 37° · No

#R3142S 4,000 units
#R3142L 20,000 units
for high (5X) concentration
#R3142M 20,000 units

5′... GGTAC▼C ...3′
3′... C▲CATGG ...5′

Reaction Conditions: rCutSmart Buffer, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	25	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

LpnPI

rCutSmart · RR · dilB · 37° · 65° · Epi

#R0663S 200 units

5′... C^{m}C D G $(N)_{10}$▼...3′
3′... G G H C $(N)_{14}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

MboI

rCutSmart · RR · dilA · 37° · 65° · CpG · dam

#R0147S 500 units
#R0147L 2,500 units
for high (5X) concentration
#R0147M 2,500 units

5′...▼GATC ...3′
3′... CTAG▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 and 25,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Methylation Sensitivity:
dam: Blocked
dcm: Not Sensitive
CpG: Impaired by Overlapping

Note: MboI is blocked by *dam* methylation, however Sau3AI is not sensitive to *dam* methylation.

MboII

rCutSmart · RR · e · 2°site · dilC · 37° · 65° · dam

#R0148S 300 units
#R0148L 1,500 units

5′... GAAGA $(N)_{8}$▼...3′
3′... CTTCT $(N)_{7}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	50	100

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit Star Activity in NEBuffer r1.1.

MfeI-HF®

rCutSmart · RR · e · dilA · 37° · No

#R3589S 500 units
#R3589L 2,500 units

5′... C▼AATTG ...3′
3′... GTTAA▲C ...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	25	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

MluI-HF®

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · 2*site · dilA · 37° · 80° · CpG

#R3198S 1,000 units
#R3198L 5,000 units

5′...A▼CGCGT...3′
3′...TGCGC▲A...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

MluCI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · dilA · 37° · 80°

#R0538S 1,000 units
#R0538L 5,000 units

5′...▼AATT...3′
3′...TTAA▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	10	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

MlyI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · dilA · 37° · 65°

#R0610S 1,000 units
#R0610L 5,000 units

5′...GAGTC(N)$_5$▼...3′
3′...CTCAG(N)$_5$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

MmeI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · 2*site · dilB · 37° · 65° · CpG

#R0637S 100 units
#R0637L 500 units

5′...TCCRAC(N)$_{20}$▼...3′
3′...AGGYTG(N)$_{18}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Concentration: 2,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

MnlI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · dilB · 37° · 65°

#R0163S 500 units
#R0163L 2,500 units

5′...CCTC(N)$_7$▼...3′
3′...GGAG(N)$_6$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	50	100

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

MscI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · dilC · 37° · 80° · dcm

#R0534S 250 units
#R0534L 1,250 units

for high (5X) concentration
#R0534M 1,250 units

5′...TGG▼CCA...3′
3′...ACC▲GGT...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 and 25,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Not Sensitive

Note: The single MscI site in pBR322 overlaps a dcm methylation site; consequently, pBR322 which has been grown in a dcm– host should be used for cloning.

MseI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · dilA · 37° · 65°

#R0525S 500 units
#R0525L 2,500 units

for high (5X) concentration
#R0525M 2,500 units

5′...T▼TAA...3′
3′...AAT▲T...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	75	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

MslI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · dilA · 37° · 80°

#R0571S 500 units
#R0571L 2,500 units

5′...CAYNN▼NNRTG...3′
3′...GTRNN▲NNYAC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

MspI

rCutSmart · RR · dilA · 37° · Epi

#R0106S 5,000 units
#R0106L 25,000 units

for high (5X) concentration
#R0106T 5,000 units
#R0106M 25,000 units

5′...C▼CGG...3′
3′...GGC▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	50	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

NaeI

rCutSmart · RR · 2*site · dilA · 37° · CpG

#R0190S 500 units
#R0190L 2,500 units

5′...GCC▼GGC...3′
3′...CGG▲CCG...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	25	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

MspA1I

rCutSmart · RR · dilB · 37° · 65° · CpG

#R0577S 500 units
#R0577L 2,500 units

5′...CMG▼CKG...3′
3′...GKC▲GMC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

NarI

rCutSmart · RR · 2*site · dilA · 37° · 65° · CpG

#R0191S 500 units
#R0191L 2,500 units

5′...GG▼CGCC...3′
3′...CCGC▲GG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	10	100

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

MspJI

rCutSmart · RR · dilB · 37° · 65° · Epi

#R0661S 200 units
#R0661L 1,000 units

5′...^{m}C NNR $(N)_9$▼...3′
3′... GNNY $(N)_{13}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

NciI

rCutSmart · RR · dilA · 37° · CpG

#R0196S 2,000 units
#R0196L 10,000 units

5′...CC▼SGG...3′
3′...GGS▲CC...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	25	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Overlapping

MwoI

rCutSmart · RR · dilB · 60° · CpG

#R0573S 500 units
#R0573L 2,500 units

5′...GCNNNNN▼NNGC...3′
3′...CGNN▲NNNNNCG...5′

Reaction Conditions: rCutSmart Buffer, 60°C

Concentration: 5,000 units/ml

Activity at 37°C: 25%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

NcoI

NEB r3.1 · RR · dilA · 37° · 80°

#R0193S 1,000 units
#R0193L 5,000 units

for high (5X) concentration
#R0193T 1,000 units
#R0193M 5,000 units

5′...C▼CATGG...3′
3′...GGTAC▲C...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

NcoI-HF®

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · dilB · 37° · 80°

#R3193S 1,000 units
#R3193L 5,000 units
for high (5X) concentration
#R3193M 5,000 units

5′... C▼CATGG...3′
3′... GGTAC▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

NdeI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · dilA · 37° · 65°

#R0111S 4,000 units
#R0111L 20,000 units

5′...CA▼TATG...3′
3′...GTAT▲AC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam methylation: Not Sensitive
dcm methylation: Not Sensitive
CpG methylation: Not Sensitive

NgoMIV

rCutSmart · Recombinant Enzyme · Time-Saver Qualified · 2*site · dilA · 37° · No · CpG

#R0564S 1,000 units
#R0564L 5,000 units

5′...G▼CCGGC...3′
3′...CGGCC▲G...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

NheI-HF®

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · dilC · 37° · 80° · CpG

#R3131S 1,000 units
#R3131L 5,000 units
for high (5X) concentration
#R3131M 5,000 units

5′... G▼CTAGC...3′
3′... CGATC▲G...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	25	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

NlaIII

rCutSmart · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · dilB · 37° · 65°

#R0125S 500 units
#R0125L 2,500 units

5′...CATG▼...3′
3′...▲GTAC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

NlaIV

rCutSmart · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · dilB · 37° · 65° · CpG · dcm

#R0126S 200 units

5′...GGN▼NCC...3′
3′...CCN▲NGG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Blocked by Overlapping

NmeAIII

rCutSmart · Cloned at NEB · Recombinant Enzyme · 2*site · dilB · 37° · 65°

#R0711S 250 units

5′...GCCGAG$(N)_{20\text{-}21}$▼...3′
3′...CGGCTC$(N)_{18\text{-}19}$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: The cleavage point may shift one base pair depending on the DNA sequence context between the recognition site and the position of cleavage. For a given sequence, generally one cut site will predominate.

NotI

NEB r3.1 · Cloned at NEB · Recombinant Enzyme · Time-Saver Qualified · dilC · 37° · 65° · CpG

#R0189S 500 units
#R0189L 2,500 units
for high (5X) concentration
#R0189M 2,500 units

5′... GC▼GGCCGC...3′
3′... CGCCGG▲CG...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	100	25

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

NotI-HF®

rCutSmart · r · RR · e · 37° · dilA · 37° · 65° · CpG

#R3189S 500 units
#R3189L 2,500 units
for high (5X) concentration
#R3189M 2,500 units

5′... GC▼GGCCGC ...3′
3′... CGCCGG▲CG ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	25	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

NspI

rCutSmart · r · RR · dilA · 37° · 65°

#R0602S 250 units
#R0602L 1,250 units

5′... RCATG▼Y ...3′
3′... Y▲GTACR ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

NruI-HF®

rCutSmart · r · RR · e · dilA · 37° · No · CpG · dam

#R3192S 1,000 units
#R3192L 5,000 units

5′... TCG▼CGA ...3′
3′... AGC▲GCT ...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	0	25	50	100

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Blocked

PacI

rCutSmart · r · RR · dilA · 37° · 65°

#R0547S 250 units
#R0547L 1,250 units

5′... TTAAT▼TAA ...3′
3′... AAT▲TAATT ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	75	10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

NsiI

NEB r3.1 · r · RR · dilB · 37° · 65°

#R0127S 1,000 units
#R0127L 5,000 units

5′... ATGCA▼T ...3′
3′... T▲ACGTA ...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	75	100	25

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

PaeR7I

rCutSmart · r · RR · dilA · 37° · No · CpG

#R0177S 2,000 units

5′... C▼TCGAG ...3′
3′... GAGCT▲C ...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

NsiI-HF®

rCutSmart · r · RR · e · dilB · 37° · 80°

#R3127S 1,000 units
#R3127L 5,000 units

5′... ATGCA▼T ...3′
3′... T▲ACGTA ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	20	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

PaqCI®

rCutSmart · r · RR · 2*site · dilB · 37° · 65° · CpG

#R0745S 200 units
#R0745L 1,000 units

5′... CACCTGC(N)$_4$▼ ...3′
3′... GTGGACG(N)$_8$▲ ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Overlapping

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

PciI

#R0655S 200 units
#R0655L 1,000 units

5′...A▼CATGT...3′
3′...TGTAC▲A...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	75	100	50

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit Star Activity in rCutSmart Buffer.

PluTI

#R0713S 500 units

5′...GGCGC▼C...3′
3′...C▲CGCGG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	25	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

PflFI

#R0595S 2,000 units

5′...GACN▼NNGTC...3′
3′...CTGNN▲NCAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

PmeI

#R0560S 500 units
#R0560L 2,500 units

5′...GTTT▼AAAC...3′
3′...CAAA▲TTTG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

PflMI

#R0509S 1,000 units
#R0509L 5,000 units

5′...CCANNNN▼NTGG...3′
3′...GGTNNNN▲NACC...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	0	100	100	50

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

PmlI

#R0532S 2,000 units
#R0532L 10,000 units

5′...CAC▼GTG...3′
3′...GTG▲CAC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

PleI

#R0515S 1,000 units

5′...GAGTC(N)$_4$▼...3′
3′...CTCAG(N)$_5$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

PpuMI

#R0506S 500 units
#R0506L 2,500 units

5′...RG▼GWCCY...3′
3′...YCCWG▲GR...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Not Sensitive

PshAI

rCutSmart · dilA · 37° · 65° · CpG

#R0593S 1,000 units
#R0593L 5,000 units

5′...GACNNN NGTC...3′
3′...CTGNNNNN CAG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

PspXI

rCutSmart · dilB · 37° · No · CpG

#R0656S 200 units
#R0656L 1,000 units

5′...VCTCGAGB...3′
3′...BGAGCTCV...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired

PsiI-v2

rCutSmart · e · dilB · 37° · 65°

#R0744S 400 units
#R0744L 2,000 units

5′...TTATAA...3′
3′...AATATT...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

PstI

NEB r3.1 · dilC · 37° · 80°

#R0140S 10,000 units
#R0140L 50,000 units
for high (5X) concentration
#R0140T 10,000 units
#R0140M 50,000 units

5′...CTGCAG...3′
3′...GACGTC...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	75	100	50

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in rCutSmart Buffer.

PspGI

rCutSmart · dilA · 75° · No · dcm

#R0611S 1,000 units

5′...CCWGG...3′
3′...GGWCC...5′

Reaction Conditions: rCutSmart Buffer, 75°C

Concentration: 10,000 units/ml

Activity at 37°C: 25%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

PstI-HF®

rCutSmart · e · dilC · 37° · No

#R3140S 10,000 units
#R3140L 50,000 units
for high (5X) concentration
#R3140T 10,000 units
#R3140M 50,000 units

5′...CTGCAG...3′
3′...GACGTC...5′

Reaction Conditions: rCutSmart Buffer, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	75	50	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

PspOMI

rCutSmart · dilB · 37° · 65° · CpG · dcm

#R0653S 1,500 units
#R0653L 7,500 units

5′...GGGCCC...3′
3′...CCCGGG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Impaired by Some Combinations of Overlapping
CpG: Blocked by Overlapping

PvuI-HF®

rCutSmart · e · dilB · 37° · No · CpG

#R3150S 500 units
#R3150L 2,500 units

5′...CGATCG...3′
3′...GCTAGC...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

PvuII

#R0151S 5,000 units
#R0151L 25,000 units

for high (5X) concentration
#R0151T 5,000 units.....$67
#R0151M 25,000 units..... $273

5′... C A G▼C T G ...3′
3′... G T C▲G A C ...5′

Reaction Conditions: NEBuffer r3.1, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Concentration: 10,000 and 50,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: May exhibit star activity in rCutSmart Buffer.

PvuII-HF®

#R3151S 5,000 units
#R3151L 25,000 units

for high (5X) concentration
#R3151M 25,000 units

5′... C A G▼C T G ...3′
3′... G T C▲G A C ...5′

Reaction Conditions: rCutSmart Buffer, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

RsaI

#R0167S 1,000 units
#R0167L 5,000 units

5′... G T▼A C ...3′
3′... C A▲T G ...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

RsrII

#R0501S 500 units
#R0501L 2,500 units

5′...C G▼G W C C G...3′
3′...G C C W G▲G C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	75	10	100

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

SacI-HF®

#R3156S 2,000 units
#R3156L 10,000 units

for high (5X) concentration
#R3156M 10,000 units..... $273

5′... G A G C T▼C ...3′
3′... C▲T C G A G ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

SacII

#R0157S 2,000 units
#R0157L 10,000 units

5′...C C G C▼G G...3′
3′...G G▲C G C C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

SalI

#R0138S 2,000 units
#R0138L 10,000 units

for high (5X) concentration
#R0138T 2,000 units
#R0138M 10,000 units

5′... G▼T C G A C ...3′
3′... C A G C T▲G ...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	100	<10

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

SalI-HF®

#R3138S 2,000 units
#R3138L 10,000 units

for high (5X) concentration
#R3138T 2,000 units
#R3138M 10,000 units

5′... G▼T C G A C ...3′
3′... C A G C T▲G ...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	100	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

rCutSmart or NEB r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · Epi EpiMark Validated · e Engineered for Performance · 2*site Multiple Recognition Site · Time-Saver Qualified · dilA Diluent Buffer · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity · rAlbumin Recombinant Albumin

SapI

rCutSmart | dilB | 37° | 65°

#R0569S 250 units
#R0569L 1,250 units

5′...GCTCTTC $(N)_1$▼...3′
3′...CGAGAAG $(N)_4$▲...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	50	<10	100

Concentration: 10,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

ScaI-HF®

rCutSmart | e | dilB | 37° | 80°

#R3122S 1,000 units
#R3122L 5,000 units
for high (5X) concentration
#R3122M 5,000 units

5′...AGT▼ACT...3′
3′...TCA▲TGA...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Sau3AI

r1.1 | dilA | 37° | 65° | CpG

#R0169S 200 units
#R0169L 1,000 units

5′...▼GATC...3′
3′...CTAG▲...5′

Reaction Conditions: NEBuffer r1.1, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Concentration: 5,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Overlapping

ScrFI

rCutSmart | dilC | 37° | 65° | CpG | dcm

#R0110S 1,000 units

5′...CC▼NGG...3′
3′...GGN▲CC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Blocked by Overlapping

Note: Star activity may result from extended digestion.

Sau96I

rCutSmart | dilA | 37° | 65° | CpG | dcm

#R0165S 1,000 units

5′...G▼GNCC...3′
3′...CCNG▲G...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Blocked by Overlapping

SexAI

rCutSmart | dilA | 37° | 65° | dcm

#R0605S 200 units
#R0605L 1,000 units

5′...A▼CCWGGT...3′
3′...TGGWCC▲A...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	75	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

SbfI-HF®

rCutSmart | e | dilB | 37° | 80°

#R3642S 500 units
#R3642L 2,500 units

5′...CCTGCA▼GG...3′
3′...GG▲ACGTCC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	25	<10	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

SfaNI

r3.1 | dilB | 37° | 65° | CpG

#R0172S 300 units

5′...GCATC $(N)_5$▼...3′
3′...CGTAG $(N)_9$▲...5′

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	75	100	25

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired by Some Combinations of Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

SfcI

rCutSmart · RR · diluB · 37° · 65°

#R0561S 200 units
#R0561L 1,000 units

5′...C▼TRYAG...3′
3′...GAYRT▲C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	50	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from a glycerol concentration of >5%.

SfiI

rCutSmart · RR · e · 2*site · diluC · 50° · No · CpG · dcm

#R0123S 3,000 units
#R0123L 15,000 units

5′...GGCCNNNN▼NGGCC...3′
3′...CCGGN▲NNNNCCGG...5′

Reaction Conditions: rCutSmart Buffer, 50°C

Concentration: 20,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Impaired by Overlapping
CpG: Blocked by Some Combinations of Overlapping

SfoI

rCutSmart · RR · e · diluB · 37° · No · CpG · dcm

#R0606S 500 units
#R0606L 2,500 units

5′... GGC▼GCC...3′
3′... CCG▲CGG...5′

Reaction Conditions: rCutSmart Buffer, 37°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Some Combinations of Overlapping
CpG: Blocked

SgrAI

rCutSmart · RR · 2*site · diluA · 37° · 65° · CpG

#R0603S 1,000 units
#R0603L 5,000 units

5′...CR▼CCGGYG...3′
3′...GYGGCC▲RC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

SmaI

rCutSmart · RR · e · diluB · 37° · 65° · CpG

#R0141S 2,000 units
#R0141L 10,000 units

5′...CCC▼GGG...3′
3′...GGG▲CCC...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	<10	<10	100

Activity at 25°C: 100%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: Blocked by CpG methylation.

SmlI

rCutSmart · RR · diluA · 55° · No

#R0597S 500 units
#R0597L 2,500 units

5′...C▼TYRAG...3′
3′...GARY▲TC...5′

Reaction Conditions: rCutSmart Buffer, 55°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	75	25	100

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

SnaBI

rCutSmart · RR · diluA · 37° · 80° · CpG

#R0130S 500 units
#R0130L 2,500 units
for high (5X) concentration
#R0130M 2,500 units

5′...TAC▼GTA...3′
3′...ATG▲CAT...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 and 25,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: May exhibit star activity in NEBuffer r1.1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

SpeI-HF®

rCutSmart · RR · e · e · diluC · 37° · 80°

#R3133S 500 units
#R3133L 2,500 units
for high (5X) concentration
#R3133M 2,500 units

5′...A▼CTAGT...3′
3′...TGATC▲A...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

SphI

#R0182S	500 units
#R0182L	2,500 units

for high (8X) concentration

#R0182M	2,500 units

5′... G C A T G▼C ... 3′
3′... C▲G T A C G ... 5′

Reaction Conditions: NEBuffer r2.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 and 80,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	100	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion.

SphI-HF®

#R3182S	500 units
#R3182L	2,500 units

for high (5X) concentration

#R3182M	2,500 units

5′... G C A T G▼C ... 3′
3′... C▲G T A C G ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	25	10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

SrfI

#R0629S	500 units
#R0629L	2,500 units

5′... G C C C▼G G G C ... 3′
3′... C G G G▲C C C G ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	0	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

SspI-HF®

#R3132S	1,000 units
#R3132L	5,000 units

for high (5X) concentration

#R3132M	5,000 units

5′... A A T▼A T T ... 3′
3′... T T A▲T A A ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	<10	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

StuI

#R0187S	1,000 units
#R0187L	5,000 units

for high (10X) concentration

#R0187M	5,000 units

5′... A G G▼C C T ... 3′
3′... T C C▲G G A ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Concentration: 10,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Not Sensitive

StyI-HF®

#R3500S	3,000 units
#R3500L	15,000 units

5′... C▼C W W G G ... 3′
3′... G G W W C▲C ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	25	100

Concentration: 20,000 units/ml

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

StyD4I

#R0638S	200 units

5′... ▼C C N G G ... 3′
3′... G G N C▲C ... 5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Blocked by Overlapping
CpG: Impaired by Overlapping

SwaI

#R0604S	2,000 units
#R0604L	10,000 units

5′... A T T T▼A A A T ... 3′
3′... T A A A▲T T T A ... 5′

Reaction Conditions: NEBuffer r3.1, 25°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	100	10

Activity at 37°C: 25%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

TaqI-v2

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · Diluent B · 65° Heat Inactivation · dam Methylation Sensitivity

#R0149S 4,000 units
#R0149L 20,000 units

for high (5X) concentration
#R0149T 4,000 units
#R0149M 20,000 units

5′...T▼CGA...3′
3′...AGC▲T...5′

Reaction Conditions: rCutSmart Buffer, 65°C

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	50	100

Concentration: 20,000 and 100,000 units/ml

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Not Sensitive

TfiI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · Diluent C · 65° Heat Inactivation · CpG Methylation Sensitivity

#R0546S 500 units

5′...G▼AWTC...3′
3′...CTWA▲G...5′

Reaction Conditions: rCutSmart Buffer, 65°C

Concentration: 10,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

TseI

rCutSmart · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · Diluent B · 65° Heat Inactivation · CpG Methylation Sensitivity

#R0591L 500 units

5′...G▼CWGC...3′
3′...CGWC▲G...5′

Reaction Conditions: rCutSmart Buffer, 65°C

Concentration: 10,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

Note: Star activity may result from a glycerol concentration of >5%.

Tsp45I

rCutSmart · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · Diluent A · 65° Heat Inactivation

#R0583S 400 units
#R0583L 2,000 units

5′...▼GTSAC...3′
3′...CAST▲G...5′

Reaction Conditions: rCutSmart Buffer, 65°C

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	<10	100

Activity at 37°C: 10%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

TspMI

rCutSmart · Time-Saver Qualified · Diluent B · 75° Heat Inactivation · CpG Methylation Sensitivity

#R0709S 200 units

5′...C▼CCGGG...3′
3′...GGGCC▲C...5′

Reaction Conditions: rCutSmart Buffer, 75°C

Concentration: 5,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	75	50	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Note: May exhibit star activity in NEBuffer r1.1, NEBuffer r2.1 or NEBuffer r 3.1.

TspRI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · Diluent B · 65° Heat Inactivation

#R0582S 1,000 units

5′...NNCASTGNN▼...3′
3′...▲NNGTSACNN...5′

Reaction Conditions: rCutSmart Buffer, 65°C

Concentration: 10,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Tth111I

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · Diluent B · 65° Heat Inactivation

#R0185S 400 units

5′...GACNNNGTC...3′
3′...CTGNN▲NCAG...5′

Reaction Conditions: rCutSmart Buffer, 65°C

Concentration: 5,000 units/ml

Activity at 37°C: 10%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

XbaI

rCutSmart · Cloned at NEB · Recombinant Enzyme · Engineered for Performance · Time-Saver Qualified · Diluent A · 37° Incubation Temperature · 65° Heat Inactivation · dam Methylation Sensitivity

#R0145S 3,000 units
#R0145L 15,000 units

for high (5X) concentration
#R0145T 3,000 units
#R0145M 15,000 units

5′...T▼CTAGA...3′
3′...AGATC▲T...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	100	75	100

Concentration: 20,000 and 100,000 units/ml

Methylation Sensitivity:
dam: Blocked by Overlapping
dcm: Not Sensitive
CpG: Not Sensitive

XcmI

#R0533S 1,000 units
#R0533L 5,000 units

5′...C C A N N N N N N N N N T G G...3′
3′...G G T N N N N N N N N N A C C...5′

Reaction Conditions: NEBuffer r2.1, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Note: Star activity may result from extended digestion.

XhoI

#R0146S 5,000 units
#R0146L 25,000 units
for high (5X) concentration
#R0146M 25,000 units

5′...C T C G A G...3′
3′...G A G C T C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 and 100,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired

XmaI

#R0180S 500 units
#R0180L 2,500 units
for high (5X) concentration
#R0180M 2,500 units

5′...C C C G G G...3′
3′...G G G C C C...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 and 50,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	50	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Impaired

Note: Star activity may result from a glycerol concentration of >5%.

XmnI

#R0194S 1,000 units
#R0194L 5,000 units

5′...G A A N N N N T T C...3′
3′...C T T N N N N A A G...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 20,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	75	<10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

ZraI

#R0659S 200 units
#R0659L 1,000 units

5′...G A C G T C...3′
3′...C T G C A G...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	25	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

Nicking Endonucleases

As a rule, when restriction endonucleases bind to their recognition sequences in DNA, they cleave both strands of the duplex at the same time. Two independent hydrolytic reactions proceed in parallel, most often driven by the presence of two catalytic sites within each enzyme, one for hydrolyzing each strand. One of the focuses of our research program is to engineer restriction enzymes so that they hydrolyze only one strand of the duplex, generating DNA molecules that are "nicked", rather than cleaved. These conventional nicks (3´-hydroxyl, 5´-phosphate) can serve as initiation points for a variety of further enzymatic reactions such as replacement DNA synthesis, strand-displacement amplification (1), exonucleolytic degradation or the creation of small gaps (2).

Nicking endonucleases (NEases) are as simple to use as restriction endonucleases. Since the nicks generated by 6- or 7-base nicking endonucleases do not fragment DNA, their activities are monitored by conversion of supercoiled plasmids to open circles. Alternatively, substrates with nicking sites close enough on opposite strands to create a double-stranded cut can be used instead.

The uses of nicking endonucleases are still being explored. NEases can generate nicked or gapped duplex DNA for DNA mismatch repair studies and for diagnostic applications. The long overhangs that nicking enzymes make can be used in DNA fragment assembly. Nt.BbvCI has been used to generate long and non-complementary overhangs when used with XbaI in the USER® cloning protocol from NEB. Nicking endonucleases are also useful for isothermal DNA amplification, which relies on the production of site-specific nicks. For example, isothermal DNA amplification using Nt.BstNBI in concert with Vent® (exo⁻) DNA Polymerase (NEB #M0257) (EXPAR) has been reported for detection of a specific DNA sequence in a sample (3). Another isothermal DNA amplification technique [Nicking Endonuclease Mediated- DNA Amplification (NEMDA)] (4) has been described using the 3-base cutter Nt.CviPII and *Bst* DNA Polymerase I. Frequent cutting NEases can generate short partial duplex DNA fragments from genomic DNA. These fragments can be used for cloning or used as probes for hybridization-based applications. Nicking enzymes have also been used for genome mapping.

NEB continues to engineer more nicking enzymes, particularly in response to specific customer needs and applications.

References:

(1) Walker, G.T. et al. (1992) *Proc. Natl. Acad. Sci. USA*, 89, 392–396.

(2) Wang, H. and Hays, J.B. (2000) *Mol. Biotechnol.*, 15, 97–104.

(3) Van Ness, J. et al. (2003) *Proc. Natl. Acad. Sci. USA*, 89, 4504–4509.

(4) Chan, S.H. et al. (2004) *Nucl. Acids Res.*, 32, 6187–6199.

Nb.BbvCI

rCutSmart · RR · dirA · 37° · 80°

#R0631S 1,000 units
#R0631L 5,000 units

5´... C C T C A G C ... 3´
3´... G G A G T C G ... 5´

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Nb.BsrDI

rCutSmart · RR · dirA · 65° · 80°

#R0648S 1,000 units
#R0648L 5,000 units

5´... G C A A T G N N ... 3´
3´... C G T T A C N N ... 5´

Reaction Conditions: rCutSmart Buffer, 65°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	25	100	100	100

Activity at 37°C: 50%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Nb.BsmI

r3.1 · RR · dirA · 65° · 80°

#R0706S 1,000 units

5´... G A A T G C N ... 3´
3´... C T T A C G N ... 5´

Reaction Conditions: NEBuffer r3.1, 65°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 37°C: 100%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	50	100	10

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Nb.BssSI

r3.1 · RR · dirB · 37° · No

#R0681S 1,000 units
#R0681T 5,000 units

5´... C A C G A G ... 3´
3´... G T G C T C ... 5´

Reaction Conditions: NEBuffer r3.1, 37°C

Concentration: 20,000 and 100,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	100	25

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Learn about nicking enzymes, including WarmStart Nt.BstNBI.

Nb.BtsI

rCutSmart · RR · dilA · 37° · 80°

#R0707S 1,000 units

5′...GCAGTGNN...3′
3′...CGTCACNN...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	75	100	75	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Nt.BspQI

NEB r3.1 · RR · dilB · 50° · 80°

#R0644S 1,000 units

5′...GCTCTTCN...3′
3′...CGAGAAGN...5′

Reaction Conditions: NEBuffer r3.1, 50°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

Activity at 37°C: 50%

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	<10	25	100	10

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Nt.AlwI

rCutSmart · RR · dilA · 37° · 80° · dam

#R0627S 500 units

5′...GGATCNNNNN...3′
3′...CCTAGNNNNN...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	100	100

Methylation Sensitivity:
dam: Blocked
dcm: Not Sensitive
CpG: Not Sensitive

Nt.BstNBI

NEB r3.1 · RR · dilA · 55° · 80°

#R0607S 1,000 units
#R0607L 5,000 units

5′...GAGTCNNNNN...3′
3′...CTCAGNNNNN...5′

Reaction Conditions: NEBuffer r3.1, 55°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	0	10	100	10

Activity at 37°C: 50%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Nt.BbvCI

rCutSmart · RR · dilA · 37° · 80° · CpG

#R0632S 1,000 units
#R0632L 5,000 units

5′...CCTCAGC...3′
3′...GGAGTCG...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	50	100	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked by Some Combinations of Overlapping

NEW

WarmStart® Nt.BstNBI

NEB r3.1 · RR · dilA · 55° · 80°

#R0725S 1,000 units

5′...GAGTCNNNNN...3′
3′...CTCAGNNNNN...5′

Reaction Conditions: NEBuffer r3.1, 55°C. Heat inactivation: 80°C for 20 minutes.

Concentration: 10,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	0	10	100	25

Activity at 37°C: 0%

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Not Sensitive

Nt.BsmAI

rCutSmart · RR · dilA · 37° · 65° · CpG

#R0121S 500 units

5′...GTCTCNN...3′
3′...CAGAGNN...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	100	50	10	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Nt.CviPII

rCutSmart · RR · dilA · 37° · 65° · CpG

#R0626S 40 units

5′...CCD...3′
3′...GGH...5′

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Concentration: 2,000 units/ml

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	100	25	100

Methylation Sensitivity:
dam: Not Sensitive
dcm: Not Sensitive
CpG: Blocked

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

Homing Endonucleases

Homing endonucleases are double stranded DNases that have large, asymmetric recognition sites (12–40 base pairs) and coding sequences that are usually embedded in either introns or inteins (1). Introns are spliced out of precursor RNAs, while inteins are spliced out of precursor proteins (2,3). Homing endonucleases are named using conventions similar to those of restriction endonucleases with intron-encoded endonucleases containing the prefix, "I-" and intein endonucleases containing the prefix, "PI-"(1,7).

Homing endonuclease recognition sites are extremely rare. For example, an 18 base pair recognition sequence will occur only once in every 7 x 10^{10} base pairs of random sequence. This is equivalent to only one site in 20 mammalian-sized genomes (4). However, unlike standard restriction endonucleases, homing endonucleases tolerate some sequence degeneracy within their recognition sequence (5,6). As a result, their observed sequence specificity is typically in the range of 10–12 base pairs.

Homing endonucleases do not have stringently-defined recognition sequences in the way that restriction enzymes do. That is, single base changes do not abolish cleavage but reduce its efficiency to variable extents. The precise boundary of required bases is generally not known. The recognition sequence listed is one site that is known to be recognized and cleaved.

References:

(1) Belfort, M. and Roberts, R.J. (1997) *Nucleic Acids Res.*, 25, 3379–3388.
(2) Dujon, B. et al. (1989) *Gene*, 82, 115–118.
(3) Perler, F.B. et al. (1994) *Nucleic Acids Res.*, 22, 1125–1127.
(4) Jasin, M. (1996) *Trends in Genetics*, 12, 224–228.
(5) Gimble, F.S. and Wang, J. (1996) *J. Mol. Biol.*, 263, 163–180.
(6) Argast, M.G. et al. (1998) *J. Mol. Biol.*, 280, 345–353.
(7) Roberts, R.J. et al. (2003) *Nucleic Acids Res.*, 31, 1805–1812.

I-CeuI

rCutSmart · RR · dilB · 37° · 65°

#R0699S 500 units
#R0699L 2,500 units
(Supplied with 5 µg of plasmid DNA)

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	10	100

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Specificity: The homing or recognition site for this endonuclease is shown below:

5′...TAACTATAACGGTCCTAAGGTAGCGAA...3′
3′...ATTGATATTGCCAGGATTCCATCGCTT...5′

Concentration: 5,000 units/ml

Note: Homing endonucleases do not have stringently-defined recognition sequences in the way that restriction enzymes do. That is, single base changes do not abolish cleavage but reduce its efficiency to variable extents. The precise boundary of required bases is generally not known. The recognition sequence listed is one site that is known to be recognized and cleaved. Note that this enzyme requires a 3-hour incubation time.

I-SceI

rCutSmart · RR · dilB · 37° · 65°

#R0694S 500 units
#R0694L 2,500 units
(Supplied with 5 µg of plasmid DNA)

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	50	25	100

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Specificity: The homing or recognition site for this endonuclease is shown below:

5′...TAGGGATAACAGGGTAAT...3′
3′...ATCCCTATTGTCCCATTA...5′

Concentration: 5,000 units/ml

Note: Homing endonucleases do not have stringently-defined recognition sequences in the way that restriction enzymes do. That is, single base changes do not abolish cleavage but reduce its efficiency to variable extents. The precise boundary of required bases is generally not known. The recognition sequence listed is one site that is known to be recognized and cleaved.

PI-PspI

NEBU · RR · dilB · 65° · No · rAlbumin

#R0695S 500 units
(Supplied with 5 µg of plasmid DNA)

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	10	10

Reaction Conditions: NEBuffer PI-PspI, 65°C. Supplement with Recombinant Albumin, Molecular Biology Grade.

Specificity: The homing or recognition site for this endonuclease is shown below:

5′...TGGCAAACAGCTATTATGGGTATTATGGGT...3′
3′...ACCGTTTGTCGATAATACCCATAATACCCA...5′

Concentration: 5,000 units/ml

Activity at 37°C: 10%

Note: Homing endonucleases do not have stringently-defined recognition sequences in the way that restriction enzymes do. That is, single base changes do not abolish cleavage but reduce its efficiency to variable extents. The precise boundary of required bases is generally not known. The recognition sequence listed is one site that is known to be recognized and cleaved. Note that this enzyme requires a 3-hour incubation time.

PI-SceI

NEBU · RR · dilB · 37° · 65° · rAlbumin

#R0696S 250 units
(Supplied with 5 µg of plasmid DNA)

NEBuffer	r1.1	r2.1	r3.1	rCutSmart
% Activity	10	10	10	10

Reaction Conditions: NEBuffer PI-SceI, 37°C. Supplement with Recombinant Albumin, Molecular Biology Grade. Heat inactivation: 65°C for 20 minutes.

Specificity: The homing or recognition site for this endonuclease is shown below:

5′...ATCTATGTCGGGTGCGGAGAAAGAGGTAATGAAATGG...3′
3′...TAGATACAGCCCACGCCTCTTTCTCCATTACTTTACC...5′

Concentration: 5,000 units/ml

Note: Homing endonucleases do not have stringently-defined recognition sequences in the way that restriction enzymes do. That is, single base changes do not abolish cleavage but reduce its efficiency to variable extents. The precise boundary of required bases is generally not known. The recognition sequence listed is one site that is known to be recognized and cleaved.

Recombinant Albumin, Molecular Biology Grade

#B9200S 12 mg

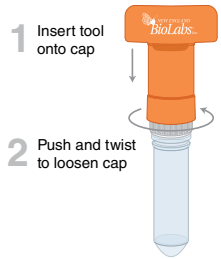
Recombinant Albumin, Molecular Biology Grade, is a non-bovine derived albumin that can serve as an alternative to Bovine Serum Albumin (BSA). Like BSA, it has been shown to prevent adhesion of enzymes to reaction tubes and pipette surfaces. It also stabilizes some proteins during incubation. Choose Recombinant Albumin, when there is a need to avoid BSA.

NEB Tube Opener

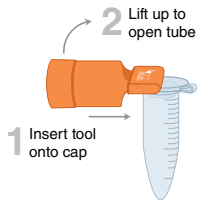
#C1008S 2 Each

Description: Use to open a variety of microcentrifuge tubes. Can be used for snap-on caps or screw-on caps.

TO OPEN SCREW-CAP TUBES:



TO OPEN SNAP-CAP TUBES:



Reaction Buffers

NEBuffer 1
#B7001S 5 ml

NEBuffer 2
#B7002S 5 ml

NEBuffer 3
#B7003S 5 ml

NEBuffer 4
#B7004S 5 ml

NEW
rCutSmart Buffer
#B6004S 5 ml

NEW
NEBuffer Set (r1.1, r2.1, r3.1 and rCutSmart)
#B7030S 1.25 ml each

NEBuffer Set (EcoRI/SspI, DpnII)
#B7006S 1.25 ml each

S-adenosylmethionine (SAM)
#B9003S 0.5 ml

Nuclease-free Water
#B1500S 25 ml
#B1500L 100 ml

NEW
NEBuffer r2.1
#B6002S 5 ml

NEW
NEBuffer r3.1
#B6003S 5 ml

Description: New England Biolabs provides a color-coded 10X NEBuffer with each restriction endonuclease to ensure optimal (100%) activity. Most of our enzymes are supplied with one of four standard NEBuffers. Occasionally, an enzyme has specific buffer requirements not met by one of the four standard NEBuffers, in which case the enzyme is supplied with its own unique NEBuffer.

The NEBuffer Sets contain multiple vials of buffers, which are indicated in the product name. NEBuffer Set (r1.1, r2.1, r3.1 and rCutSmart) is formulated with Recombinant Albumin.

Nuclease-free Water is ideal for the preparation of reagents and for use in enzymatic reactions. No toxic agents, such as DEPC, are used in the production of this water, so as to avoid inhibition in enzymatic reactions.

Reaction Buffer Compositions: See Performance Chart for Restriction Enzymes or visit www.neb.com for details.

Diluent Buffers

Diluent A
#B8001S 5 ml

Diluent B
#B8002S 5 ml

Diluent C
#B8003S 5 ml

Description: Diluent Buffers are recommended for making dilutions of restriction endonucleases. When necessary, we recommend diluting enzymes just prior to use and suggest that the final concentration of diluted enzymes be at least 1,000 units/ml. Diluent preference for each restriction endonuclease is listed with its catalog entry.

Storage Conditions: Store at –20°C.

Diluent Buffer Compositions: See Performance Chart for Restriction Enzymes or visit www.neb.com for details.

Gel Loading Dyes

Gel Loading Dye, Blue (6X)
#B7021S 4 ml

Gel Loading Dye, Orange (6X)
#B7022S 4 ml

Gel Loading Dye, Purple (6X)
#B7024S 4 ml

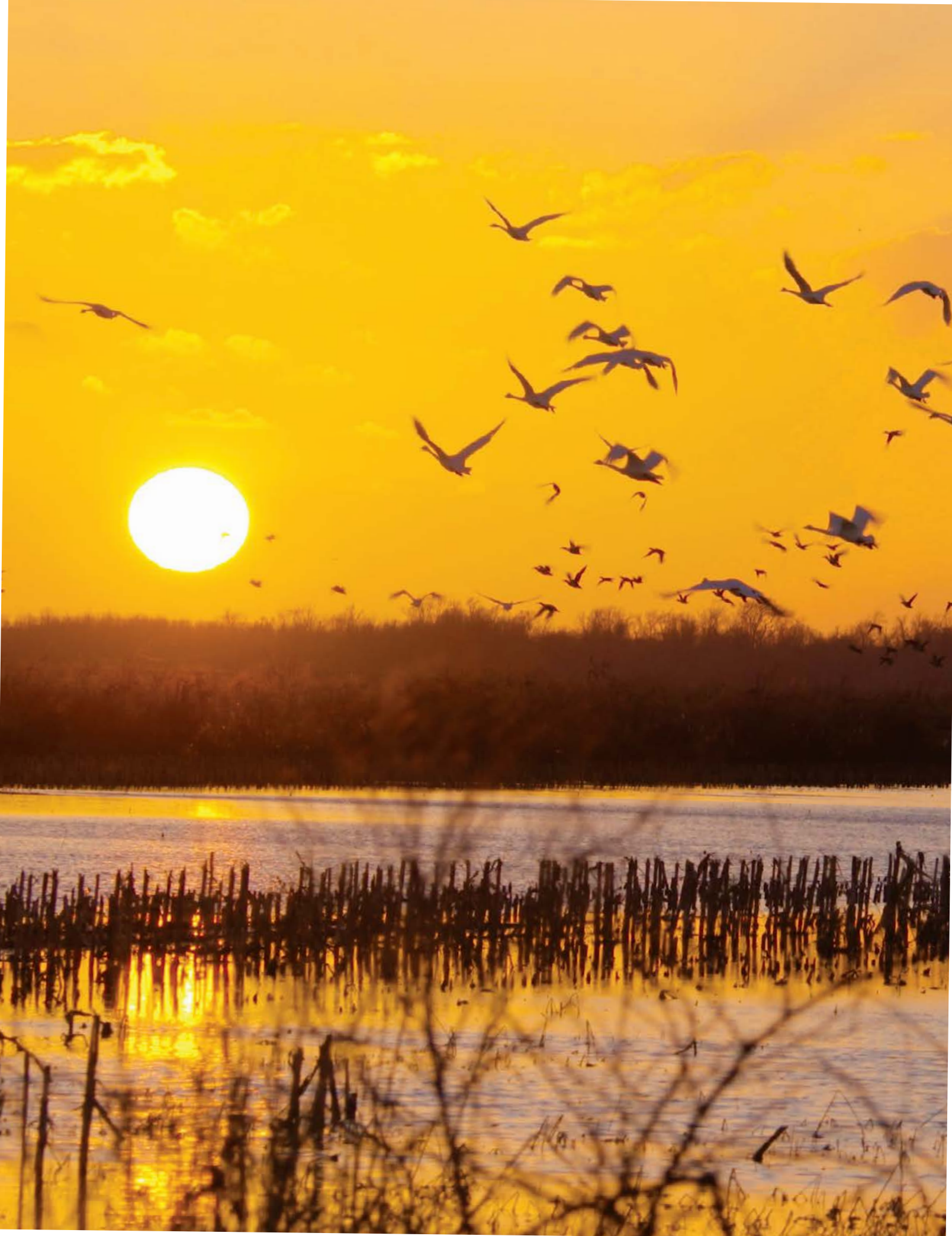
Gel Loading Dye, Purple (6X), no SDS
#B7025S 4 ml

Description: Pre-mixed loading dye solutions are available with a choice of tracking dyes, for agarose and non-denaturing polyacrylamide gel electrophoresis. Three of the solutions contain SDS, which often results in sharper bands, as some restriction enzymes remain bound to their DNA substrates following cleavage. Some interference may be observed when using SYBR® or GelRed® as precast dyes in the presence of increased concentrations of SDS. When using these dyes as precast dyes, NEB recommends using our Gel Loading Dye, Purple (6X), no SDS (NEB #B7025). Gel Loading Dyes contain EDTA to chelate up to 10 mM magnesium, thereby stopping the reaction. Bromophenol blue is the standard tracking dye for electrophoresis. It migrates at approximately 300 bp on a standard 1% TBE agarose gel. Orange G will not appear in gel photographs, and runs ahead of all but the smallest restriction fragments, migrating at approximately 50 bp on a standard 1% TBE agarose gel. NEB also offers a unique purple dye which migrates similarly to Bromophenol blue. Specifically chosen, this dye does not leave a shadow under UV light.

Gel Loading Dye Compositions: Visit www.neb.com for details.

Note: Use 5 µl of Gel Loading Dye per 25 µl reaction, or 10 µl per 50 µl reaction. Mix well before loading gel. Store at room temperature.

Single Letter Code: **R** = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T



Species migration on a warming planet

Global flora and fauna are responding differently to ecosystem disruptions associated with climate change. Stressed species must adapt, migrate, or often tackle both at once, in order to survive. Organism mobility is a delineating factor in these endeavors. Physical barriers to species redistribution can be naturally occurring or manmade. Internal ecosystem pressures temper the potential of any species population to adapt. Fundamentally, the resilience of life on Earth is being rapidly tested during the largest global climate change in 65 million years.

It's been estimated that roughly half of the species on Earth today are on the move to new geographical regions. From the tropics to the poles, physiological tolerances have been provoked by higher temperatures, and changes in rainfall, sunlight, ice and snow cover. Sensitivity to changing conditions varies broadly among organisms. For example, the warming of only 1°C for four weeks triggers coral bleaching – which involves corals expelling symbiotic algae and beginning to starve. In contrast, insect species and some terrestrial vertebrate ectotherms living at higher latitudes exhibit greater thermal tolerance and may even benefit from global warming, compared to their tropical cousins. No matter what tolerance organisms have for climate change, there is an evolutionary race against time to grab space in optimal habitats.

Mobility is an advantage. Migratory species can move more readily to find new, cooler habitats. For example, North American birds encountering increasing winter temperatures have shifted northward and away from coasts to take advantage of food sources. Scientists have also observed changes in the timing of seasonal migrations. It's been shown that brown-veined white butterflies migrate from South Africa to Mozambique earlier in warmer summers with less rainfall.

Less mobile organisms, such as plants and trees, migrate slowly through reproduction and seed distribution. Important phenotypic traits that improve fitness under climate change conditions tend to cluster together in the genomes of tree species. Species with multiple adaptive traits are better able to expand potential habitats. Arctic tundra shrubs have grown in size and expanded territories based on longer summers with changes in temperature and precipitation. Flowering plants adapt breeding seasons and flower production to local nutrient availability, temperatures, and symbiotic pollinators to optimize their re-distribution potential. This phenomenon is known as climate tracking.

Whether rapid or slow, colonization of new habitats presents new challenges. Migrating species begin with unique ecological relationships, growth rates, phenotypic plasticity, and levels of genetic diversity for survival. Movement that generates new predatory relationships can be overwhelming. This is the case for Antarctic seafloor echinoderms and mollusks that previously evolved in isolation, but now face a rapid invasion of crabs. Human-made obstacles can also act as barriers that reduce genetic connectivity and thus, evolutionary potential in both plant and animal species. Climate tracking by plants is hindered by the loss of mammalian wildlife and birds to transport seeds over landscapes. Such mutualistic ecosystem relationships are often threatened, demonstrating that migration on a warming planet involves complex tradeoffs.

Ecologists are very concerned whether there will be enough time for plant and animal migrations and adaptations to occur. How will gene flows of migrating species affect the Earth's biodiversity? The survival or extinction of multiple species and ecosystems lies in the balance.

Tundra swans in the marsh lands of eastern North Carolina.
Credit: blackboxguild, Adobe Stock

Experience tundra swans in flight.

DNA Polymerases & Amplification Technologies

NEB has pursued the discovery & development of DNA polymerases for over 35 years.

As the first company to sell *Taq* DNA polymerase and the first to discover a PCR-stable high-fidelity DNA polymerase, NEB established a foundation in developing innovative, high quality tools for the research and diagnostic community.

PCR & qPCR

NEB's product portfolio features a large selection of polymerases for PCR. Q5® High-Fidelity DNA Polymerase offers fidelity 280 times higher than *Taq*, along with superior performance with minimal optimization. One*Taq* is ideal for robust amplification in routine PCR applications. Both are available in hot start formulations, utilizing a novel aptamer-based approach that does not require an activation step.

Fluorescence-based quantitative real-time PCR (qPCR) is the gold standard for detection and quantitation of nucleic acids due to its specificity and sensitivity. Luna qPCR & RT-qPCR products feature an inert blue tracking dye for easy reaction setup and are available for intercalating dye or fluorescent probe-based detection methods.

Isothermal Amplification

Sequence specific isothermal amplification approaches eliminate the need for temperature cycling, providing advantages for certain point-of-care diagnostic needs. NEB's broad suite of reagents continue to enable advancement in isothermal amplification. Nicking enzymes, WarmStart enzymes, strand-displacing DNA polymerases, and RNA polymerases offer flexibility to assemble and design an isothermal amplification platform.

Introducing LyoPrime™ Lyophilized Products

With the point-of-care market becoming more focused on the development of robust, accurate and cost-effective diagnostic tests for use outside of traditional hospital and laboratory settings, there is a growing need for reagents that can withstand ambient shipping and storage. Lyophilization is the preferred solution and is a well-established technology across a number of industries.

Bringing together expertise in enzyme development, manufacturing and lyophilization, NEB Lyophilization Sciences™ has created shelf-stable, lyophilized products that do not sacrifice the high-performance qualities of their liquid counterparts. The first of these products includes a mixture of enzymes and inhibitors to enable robust detection of RNA via hydrolysis-probe-based RT-qPCR (page 72). The ability to develop complex, yet simple to use lyophilized products enables us to provide a more complete solution for our customers, particularly those in the molecular diagnostics space.

Find an overview of PCR.

Featured Products

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RI Recombinant Enzyme

Amplification-based Molecular Diagnostics

Our extensive expertise in amplification, including PCR, qPCR, RT-qPCR and isothermal amplification has allowed us to develop optimized enzymes for a variety of applications, including incorporation into diagnostics. Learn more at **www.neb.com/MDx**.

Application		Products	Product Notes	Custom Formulations
PCR Applications	qPCR/ RT-qPCR	**DNA, Dye** • Luna Universal qPCR Master Mix (NEB #M3003) **DNA, Probe** • Luna Universal Probe qPCR Master Mix (NEB #M3004)	• Compatible with automated liquid handling and reaction miniaturization • Room temperature stable for ≥ 24 hours	• Blue-dye-free • Lyo-compatible
		RNA (1-step), Dye • Luna Universal One-Step RT-qPCR Kit (NEB #E3005) **RNA (1-step), Probe** • Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) • Luna Probe One-Step RT-qPCR 4X Mix with UDG (No ROX) (NEB #M3029) • Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006) • Luna Probe One-Step RT-qPCR Kit (No ROX) (NEB #E3007) • Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit (NEB #E3019) • LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG (NEB #L4001)	• Luna WarmStart RT paired with Hot Start *Taq* increases reaction specificity and robustness • Compatible with automation and reaction miniaturization • Room temperature stable for ≥ 24 hours • Lyophilized format (NEB #L4001)	• Blue-dye-free • Lyo-compatible • Add primers and probes (NEB #L4001)
		RNA (2-step) • LunaScript® RT SuperMix (NEB #E3010/#M3010)	• Novel thermostable RT • Single-tube format • 13-minute cDNA synthesis protocol	• Blue-dye-free
	PCR/ RT-PCR	**Master Mixes** • Q5 Hot Start High-Fidelity 2X Master Mix (NEB #M0494) • Q5 High-Fidelity 2X Master Mix (NEB #M0492) **Standalone Enzyme & Buffer** • Q5 Hot Start High-Fidelity DNA Polymerase (NEB #M0493) • Q5 High-Fidelity DNA Polymerase (NEB #M0491)	• ~280X fidelity of *Taq* • Consistent, fast, reliable performance • Compatible with automation and reaction miniaturization • Room temperature stable for ≥ 24 hours	• High conc. • Glycerol-free • Custom mixes
		• Q5 Blood Direct 2X Master Mix (NEB #M0500)	• Amplification direct from blood	
		• Hot Start *Taq* DNA Polymerase (NEB #M0495) • Hot Start *Taq* 2X Master Mix (NEB #M0496)	• Unique aptamer-based enzyme control supports fast protocols	• High conc. • Glycerol-free
Isothermal Applications	LAMP	• WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA) (NEB #M1800) • WarmStart Colorimetric LAMP 2X Master Mix with UDG (NEB #M1804)	• Fast, clear pink-to-yellow visible detection of amplification • Results in approximately 30 minutes • Automation-compatible when coupled with absorbance plate reader	
		• SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit (NEB #E2019)	• Simple, colorimetric detection of amplification of SARS-CoV-2 nucleic acid	
		• WarmStart LAMP Kit (DNA & RNA) (NEB #E1700) • WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG) (NEB #M1708) • WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG) (NEB #E1708)	• Master mix for LAMP and RT-LAMP workflows • Supports multiple detection methods, including fluorescence and turbidity	• Lyo-compatible • High conc.
		• *Tte* UvrD Helicase (NEB #M1202)	• Improves specificity of fluorescent LAMP reactions	• High conc.
		• *Bst* 2.0 WarmStart DNA Polymerase (NEB #M0538) • *Bst* 2.0 DNA Polymerase (NEB #M0537)	• Improved reaction properties compared to wild-type *Bst* DNA Polymerase • Increased dUTP tolerance enables carryover prevention	• Glycerol-free • High conc.
		• *Bst* 3.0 DNA Polymerase (NEB #M0374)	• DNA binding domain fusion supports robust performance • Significantly increased RT activity up to 72°C enables single enzyme RT-LAMP	• Glycerol-free • High conc.
		• WarmStart RTx Reverse Transcriptase (NEB #M0380)	• *In-silico* designed RT for RT-LAMP with reversibly-bound aptamer that inhibits activity below 40°C	• Glycerol-free • High conc.
	Strand Displacement	• Nt.BstNBI (NEB #R0607) • WarmStart Nt.BstNBI (NEB #R0725)	• High purity, high quality nicking endonuclease	• Glycerol-free • High conc.
	Helicase-dependent Amplification	• IsoAmp® II Universal tHDA Kit (NEB #H0110)	• Requires only two primers • Produces short, discrete DNA products	
	Other	• *Bsu* DNA Polymerase, Large Fragment (NEB #M0330)	• Enables low temperature isothermal applications	• High conc.
		• T4 Gene 32 Protein (NEB #M0300)	• Can increase yield and efficiency of amplification reactions	• Glycerol-free
		• Deoxynucleotide (dNTP) Solution Mix (NEB #N0447)		• Custom conc.
		• Nuclease-free Water (NEB #B1500)		
		• Antarctic Thermolabile UDG (NEB #M0372)	• Unique thermolabile version is completely inactivated in typical isothermal and RT-qPCR workflows	• High conc.
		• Proteinase K, Molecular Biology Grade (NEB #P8107)		• Custom conc.
		• Thermolabile Proteinase K (NEB #P8111)	• Unique thermolabile version is completely inactivated in typical isothermal and RT-qPCR workflows	• Custom conc.

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/ WarmStart · Recombinant Albumin · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

PCR Polymerase Selection Chart

For almost 50 years, New England Biolabs, Inc. has been a world leader in the discovery and production of reagents for the life science industry. NEB offers a wide range of DNA polymerases, and through our commitment to research, ensures the development of innovative and high quality tools for PCR and related applications. The following table simplifies the selection of a polymerase that best suits your experiment.

★ indicates recommended choice for application	Standard PCR		High-Fidelity PCR			Specialty PCR				
			Highest Fidelity		Moderate Fidelity	Long Amplicons	dU Tolerance		Blood Direct	
	One*Taq*®/ One*Taq* Hot Start	*Taq*/ Hot Start *Taq*	Q5®/Q5 Hot Start	Phusion®(4)/ Phusion (4) Flex	Vent®/ Deep Vent	LongAmp®/ LongAmp Hot Start *Taq*	Q5U®	EpiMark® Hot Start *Taq*	Q5 Blood	Hemo Klen*Taq*®
Properties										
Fidelity vs. Taq	2X	1X	~280X (2)	> 50X	5–6X	2X	ND	1X	ND	ND
Amplicon Size	< 6 kb	≤ 5 kb	≤ 20 kb	≤ 20 kb	≤ 6 kb	≤ 30 kb	App-specific	≤ 1 kb	≤ 7.5 kb	≤ 2 kb
Extension Time	1 kb/min	1 kb/min	6 kb/min	4 kb/min	1 kb/min	1.2 kb/min	2 kb/min	1 kb/min	2-4 kb/min	0.5 kb/min
Resulting Ends	3´ A/Blunt	3´ A	Blunt	Blunt	Blunt	3´ A/Blunt	Blunt	3´ A	Blunt	3´ A
3´→5´ exo	Yes	No	Yes	Yes	Yes	Yes	Yes	No	Yes	No
5´→3´ exo	Yes	Yes	No	No	No	Yes	No	Yes	No	No
Units/50 µl Reaction	1.25	1.25	1.0	1.0	0.5–1.0	5.0	1.0	1.25	N/A	N/A
Annealing Temperature	Tm–5	Tm–5	Tm+3	Tm+3	Tm–5	Tm–5	Tm+3	Tm–5	Tm+3	Tm–5
Applications										
Routine PCR	★	•	•	•	•	•				
Colony PCR	★	•								
Enhanced Fidelity	•		★	•	•	•			•	
High Fidelity			★	•					•	
High Yield	★	•	★	•					•	
Fast			★	•					•	
Long Amplicon			★	•		★				
GC-rich Targets	★		★		•	•			•	
AT-rich Targets	★	•	★	•		•	★	•		
High Throughput	•	•	•	•			★	•		
Multiplex PCR	•	★(1)	•	•					•	
Extraction-free PCR									★	•
DNA Labeling		★								
Site-directed Mutagenesis			★	•						
Carryover Prevention							★	•		
USER® Cloning							★	•		
NGS Applications										
NGS Library Amplification			★(3)	•				★(5)		
Formats										
Hot Start Available	•	•	•	•		•	•	•	•	
Kit		•	•	•		•	•			
Master Mix Available	•	•	•	•		•	•		•	
Direct Gel Loading	•	•								

(1) Use Multiplex PCR 5X Master Mix.

(2) Due to the very low frequency of misincorporation events being measured, the error rate of high-fidelity enzymes like Q5 is challenging to measure in a statistically significant manner. We continue to investigate improved assays to characterize Q5's very low error rate to ensure that we present the most robust accurate fidelity data possible (Popatov, V. and Ong, J.L. (2017) *PLoS One*, 12(1):e0169774. doi 10.1371/journal. pone. 0169774).

(3) Use NEBNext High-Fidelity 2X PCR Master Mix.

(4) Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific.

(5) Use NEBNext Enzymatic Methyl-seq Kit (EM-seq™) for Illumina.

Tm Calculator

High-Fidelity polymerases benefit from a Tm+3 annealing temp. Use the **NEB Tm Calculator** to ensure successful PCR at **TmCalculator.neb.com**

Why choose Q5 for your PCR?

Q5® High-Fidelity DNA Polymerases

Q5® Hot Start High-Fidelity DNA Polymerase

Q5 High-Fidelity DNA Polymerase
#M0491S 100 units
#M0491L 500 units

Q5 High-Fidelity 2X Master Mix
#M0492S 100 reactions
#M0492L 500 reactions

Q5 Hot Start High-Fidelity DNA Polymerase
#M0493S 100 units
#M0493L 500 units

Q5 Hot Start High-Fidelity 2X Master Mix
#M0494S 100 reactions
#M0494L 500 reactions
#M0494X 500 reactions

Q5U Hot Start High-Fidelity DNA Polymerase
#M0515S 100 units
#M0515L 500 units

Q5 High-Fidelity PCR Kit
#E0555S 50 reactions
#E0555L 200 reactions

NEW
Q5 Blood Direct 2X Master Mix
#M0500S 100 reactions
#M0500L 500 reactions

Q5 POLYMERASE DETAILS

Extension Rate	6 kb/min
Amplicon Size	≤ 20 kb
Fidelity	~ 280X *Taq*
Units / 50 µl rxn	1 unit
Resulting Ends	Blunt
3´→5´ Exonuclease Activity	Yes
5´→3´ Exonuclease Activity	No
Supplied Buffer	Q5 Reaction Buffer
Supplied Enhancer	Q5 High GC Enhancer
Extraction-free PCR	Yes

PRODUCT FORMATS

Hot Start Available	Yes
Activation Required	No
Master Mix Available	Yes
PCR Kit Available	Yes
NGS Version Available	Yes

APPLICATIONS

High-Fidelity PCR	Yes
Difficult PCR	Yes
High GC PCR	Yes
T/A, U/A Cloning	No
Colony PCR	No
Blunt Cloning	Yes
Multiplex PCR	Yes
USER Cloning	Yes (Q5U)
Carryover Prevention	Yes (Q5U)

Description: Q5 High-Fidelity DNA Polymerase sets the standard for performance, ultra-low error rates and fidelity (~ 280 times higher than *Taq*). Q5 DNA Polymerase is composed of a novel polymerase that is fused to the processivity-enhancing Sso7d DNA binding domain, improving speed, fidelity and reliability.

The Q5 buffer system provides superior performance with minimal optimization across a broad range of amplicons, regardless of GC content. For routine or complex amplicons up to ~65% GC content, Q5 Reaction Buffer provides reliable and robust amplification. For amplicons with high GC content (> 65% GC), addition of the Q5 High GC Enhancer ensures continued maximum performance.

Q5 Hot Start DNA Polymerase: In contrast to chemically-modified or antibody-based hot start polymerases, NEB's Q5 Hot Start utilizes a unique synthetic aptamer. This structure binds to the polymerase through non-covalent interactions, blocking activity during the reaction setup. The polymerase is activated during normal cycling conditions, allowing reactions to be set up at room temperature. Q5 Hot Start does not require a separate high temperature activation step, reducing the potential for sample degredation, shortening reaction times and increasing ease-of-use. Q5 Hot Start is an ideal choice for high specificity amplification and provides robust amplification of a wide variety of amplicons, regardless of GC content.

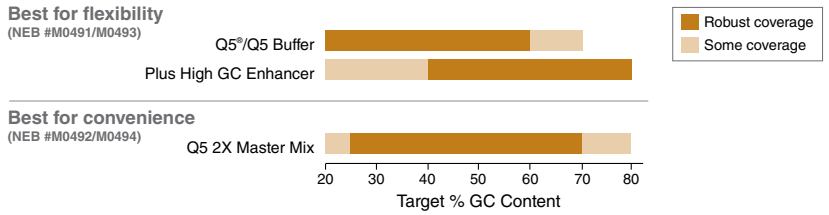
Q5U Hot Start High-Fidelity DNA Polymerase: A modified version of Q5 High-Fidelity DNA Polymerase capable of incorporating dUTP for carryover prevention. Q5U is also compatible with USER cloning methods and enables the amplification of bisulfite treated/ deaminated DNA.

Q5 Blood Direct 2X Master Mix: Amplify a wide variety of targets direct from dried blood spots or up to 30% whole human blood with this unique master mix.

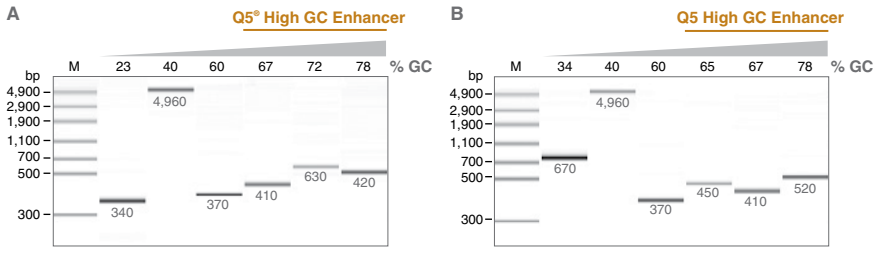
Additional Formats: For added convenience, Q5 DNA Polymerase is available in master mix format or as a kit. Master mix formulations include dNTPs, Mg^{++} and all necessary buffer components. The Q5 High-Fidelity PCR Kit contains the Q5 High-Fidelity 2X Master Mix, nuclease-free water and the Quick-Load Purple 1 kb Plus DNA Ladder. The Q5 Site-Directed Mutagenesis Kit, with or without competent cells, is also available.

Concentration: 2,000 units/ml

Visit **Q5PCR.com** for more information.



The Q5 buffer system provides superior performance for a wide range of targets. *The stand-alone enzyme comes with a reaction buffer that supports robust amplification of high AT to routine targets. Addition of the High GC Enhancer allows amplification of GC rich and difficult targets. For added convenience, the master mix formats allow robust amplification of a broad range of targets with a single formulation.*



Robust amplification with Q5 (A) and Q5 Hot Start (B) High-Fidelity DNA Polymerases, regardless of GC content. *Amplification of a variety of human genomic amplicons from low-to-high GC content using either Q5 or Q5 Hot Start High-Fidelity DNA Polymerase. Reactions using Q5 Hot Start were set up at room temperature. All reactions were conducted using 30 cycles of amplification. and visualized by microfluidic LabChip® analysis.*

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/ WarmStart · Recombinant Albumin · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

Phusion® High-Fidelity DNA Polymerase

Phusion® Hot Start Flex DNA Polymerase

Phusion High-Fidelity DNA Polymerase
#M0530S 100 units
#M0530L 500 units

Phusion High-Fidelity PCR Master Mix with HF Buffer
#M0531S 100 reactions
#M0531L 500 reactions

Phusion High-Fidelity PCR Master Mix with GC Buffer
#M0532S 100 reactions
#M0532L 500 reactions

Phusion Hot Start Flex DNA Polymerase
#M0535S 100 units
#M0535L 500 units

Phusion Hot Start Flex 2X Master Mix
#M0536S 100 reactions
#M0536L 500 reactions

Phusion High-Fidelity PCR Kit
#E0553S 50 reactions
#E0553L 200 reactions

Description: DNA polymerases with high fidelity are important for applications in which the DNA sequence needs to be correct after amplification. Manufactured and quality controlled at New England Biolabs, Thermo Scientific® Phusion High-Fidelity DNA Polymerase offers both high fidelity and robust performance, and thus can be used for all PCR applications. Its unique structure, a novel Pyrococcus-like enzyme fused with a processivity-enhancing domain, increases fidelity and speed. Phusion Hot Start Flex DNA Polymerase is available as a standalone enzyme or in a master mix format, and enables high specificity amplification. Phusion DNA Polymerase is an ideal choice for cloning and can be used for long amplicons.

Additional Formats: Phusion and Phusion Hot Start Flex DNA Polymerases are also available in master mix format. Phusion master mixes are available with HF or GC Buffer. The Phusion PCR Kit contains Phusion Polymerase, Phusion HF and GC buffers, deoxynucleotides, $MgCl_2$, DMSO and DNA size standards.

Concentration: 2,000 units/ml

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

PHUSION POLYMERASE DETAILS

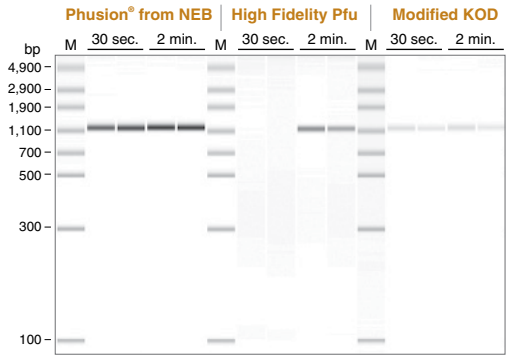
Extension Rate	4 kb/min
Amplicon Size	≤ 20 kb
Fidelity	> 50X *Taq*
Units / 50 µl rxn	1 unit
Resulting Ends	Blunt
3´→5´ Exonuclease Activity	Yes
5´→3´ Exonuclease Activity	No
Supplied Buffers	- 5X Phusion HF Buffer - 5X Phusion GC Buffer
Supplied Enhancer	100% DMSO

PRODUCT FORMATS

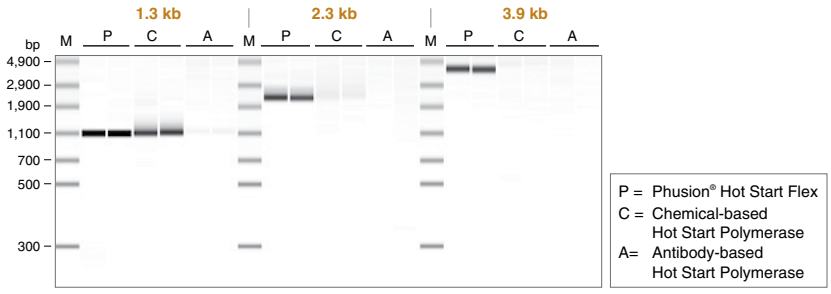
Hot Start Available Activation Required	Yes No
Master Mix Available	Yes
PCR Kit Available	Yes

APPLICATIONS

High-Fidelity PCR	Yes
T/A, U/A Cloning	No
Colony PCR	No
Blunt Cloning	Yes



Phusion DNA Polymerase generates robust amplification even with short extension times. *A 1.2 kb C. elegans genomic amplicon was analyzed using different polymerases. Reactions were set up in duplicate and visualized by microfluidic LabChip analysis. All reactions were conducted according to manufacturer's instructions using 30 cycles and extension times of either 30 seconds or 2 minutes as indicated.*



Phusion Hot Start Flex DNA Polymerase delivers robust amplification. *All amplicons are from human Jurkat template except for the 1.3 kb C. elegans amplicon. Amplicon sizes are indicated above gel. All reactions were conducted in duplicate according to manufacturer's instructions using 30 cycles and visualized after microfluidic LabChip analysis.*

Tm Calculator

High-Fidelity polymerases benefit from a Tm+3 annealing temp. Use the **NEB Tm Calculator** to ensure successful PCR at **TmCalculator.neb.com**

One*Taq*® DNA Polymerase

One*Taq*® Hot Start DNA Polymerase

One*Taq* DNA Polymerase
#M0480S 200 units
#M0480L 1,000 units
#M0480X 5,000 units

One*Taq* Hot Start DNA Polymerase
#M0481S 200 units
#M0481L 1,000 units
#M0481X 5,000 units

One*Taq* 2X Master Mix with Standard Buffer
#M0482S 100 reactions
#M0482L 500 reactions

One*Taq*® Hot Start 2X Master Mix with Standard Buffer
#M0484S 100 reactions
#M0484L 500 reactions

One*Taq*® Hot Start 2X Master Mix with GC Buffer
#M0485S 100 reactions
#M0485L 500 reactions

One*Taq* Quick-Load 2X Master Mix with Standard Buffer
#M0486S 100 reactions
#M0486L 500 reactions

One*Taq* Hot Start Quick-Load 2X Master Mix with Standard Buffer
#M0488S 100 reactions
#M0488L 500 reactions

One*Taq* Hot Start Quick-Load 2X Master Mix with GC Buffer
#M0489S 100 reactions
#M0489L 500 reactions

One*Taq* Quick-Load DNA Polymerase
#M0509L 500 units
#M0509X 2,500 units

One*Taq* RT-PCR Kit
#E5310S 30 reactions

One*Taq* One-Step RT-PCR Kit
#E5315S 30 reactions

Description: One*Taq* DNA Polymerase is an optimized blend of *Taq* and Deep Vent® DNA polymerases for use with routine and difficult PCR experiments. The 3´→5´ exonuclease activity of Deep Vent DNA Polymerase increases the fidelity and robust amplification of *Taq* DNA Polymerase. The One*Taq* reaction buffers and High GC Enhancer have been formulated for robust yields with minimal optimization, regardless of a template's GC content.

One*Taq* Hot Start DNA Polymerase: One*Taq* Hot Start DNA Polymerase utilizes an aptamer-based inhibitor. The hot start formulation combines convenience with decreased interference from primer-dimers and secondary products.

The aptamer-based inhibitor binds reversibly, blocking polymerase activity at temperatures below 45°C, allowing reactions to be set up at room temperature. OneTaq Hot Start DNA Polymerase is activated during normal cycling conditions, eliminating the need for a separate high temperature incubation step to activate the enzyme. One*Taq* Hot Start DNA Polymerase can therefore be substituted into typical or existing *Taq*-based protocols.

One*Taq* and One*Taq* Hot Start are provided with Standard Reaction Buffer, GC Reaction Buffer and High GC Enhancer. Recommendations for buffer selection, based on % GC content, are shown below.

Quick-Load formats offer direct loading of PCR products onto gels, eliminating the need for a PCR clean-up step.

Additional Formats: For added convenience, One*Taq* and One*Taq* Hot Start DNA Polymerases are available in master mix format. Master mixes are available with Standard or GC Buffer. High GC Enhancer is also provided with master mixes containing GC Buffer. For direct gel loading, Quick-Load versions of master mixes are also available. One*Taq*® RT-PCR Kit combines two powerful mixes, M-MuLV Enzyme Mix and One*Taq* Hot Start 2X Master Mix with Standard Buffer for 2-step RT-PCR applications. The One*Taq* One-Step RT-PCR Kit offers sensitive and robust end-point detection of RNA templates. cDNA synthesis and PCR amplification steps are performed in a single reaction using gene-specific primers, resulting in a streamlined RT-PCR protocol.

Concentration: 5,000 units/ml

ONE*Taq* POLYMERASE DETAILS

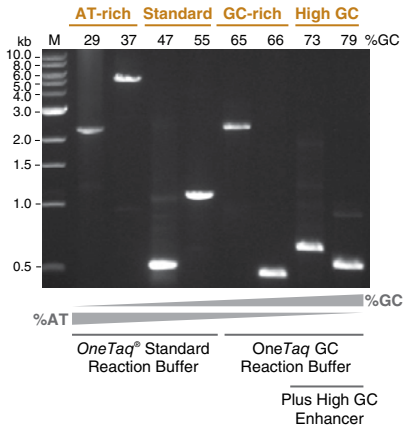
Extension Rate	1 kb/min
Amplicon Size	≤ 6 kb
Fidelity	> 2X *Taq*
Units / 50 µl rxn	1.25 units
Resulting Ends	3´ A/Blunt
3´→5´ Exonuclease Activity	Yes
5´→3´ Exonuclease Activity	Yes
Supplied Buffers	- One*Taq* Std Rxn Buffer - OneTaq GC Rxn Buffer
Supplied Enhancer	One*Taq* High GC Enhancer

PRODUCT FORMATS

Hot Start Available Activation Required	Yes No
Master Mix Available	Yes
Direct Gel-loading Available	Yes
PCR Kit Available	Yes

APPLICATIONS

Routine PCR	Yes
T/A, U/A Cloning	Yes
Colony PCR	Yes



Amplification of a selection of sequences with varying GC content from human and C. elegans genomic DNA using OneTaq DNA Polymerase. *GC content is indicated above gel. Marker M is the 1 kb DNA Ladder (NEB #N3232).*

One*Taq* Buffer Recommendations

Amplicon % GC Content	Recommended Default Buffer	Optimization Notes
< 50% GC	One*Taq* Standard Reaction Buffer	Adjust annealing temperature, primer/template concentration, etc., if needed.
50–65% GC	One*Taq* Standard Reaction Buffer	One*Taq* GC Reaction Buffer can be used to enhance performance of difficult amplicons.
> 65% GC	One*Taq* GC Reaction Buffer	One*Taq* GC Reaction Buffer with 10-20% One*Taq* High GC Enhancer can be used to enhance performance of difficult amplicons.

Taq DNA Polymerase

Hot Start *Taq* DNA Polymerase

Taq DNA Polymerase with ThermoPol Buffer
#M0267S 400 units
#M0267L 2,000 units
#M0267X 4,000 units
#M0267E 20,000 units

Taq DNA Polymerase with Standard *Taq* Buffer
#M0273S 400 units
#M0273L 2,000 units
#M0273X 4,000 units
#M0273E 20,000 units

Taq DNA Polymerase with Standard *Taq* (Mg-free) Buffer
#M0320S 400 units
#M0320L 2,000 units

Taq PCR Kit
#E5000S 200 reactions

Taq 2X Master Mix
#M0270L 500 reactions

Quick-Load *Taq* 2X Master Mix
#M0271L 500 reactions

Taq 5X Master Mix
#M0285L 500 reactions

Multiplex PCR 5X Master Mix
#M0284S 100 reactions

Hot Start *Taq* DNA Polymerase
#M0495S 200 units
#M0495L 1,000 units

Hot Start *Taq* 2X Master Mix
#M0496S 100 reactions
#M0496L 500 reactions

Description: *Taq* DNA Polymerase is a thermostable DNA polymerase that possesses a 5´→ 3´ polymerase activity and a 5´ flap endonuclease activity. It is the most widely used enzyme for PCR. To accomodate a variety of PCR applications, *Taq* is available with different reaction buffers. Standard *Taq* Reaction Buffer is designed to support existing PCR platforms, and is an ideal choice for DHPLC and high-throughput applications. ThermoPol Reaction Buffer was designed at NEB, and is formulated to promote high product yields, even under demanding conditions. For additional convenience, *Taq* DNA Polymerase is also available in kit and master mix formats. For direct gel loading, a Quick-Load version of the *Taq* 2X Master Mix is also available.

Hot Start Taq DNA Polymerase: With value pricing and attractive commercial terms, Hot Start *Taq* from NEB is an ideal choice for molecular diagnostics and other applications. In contrast to chemically modified or antibody-based hot start polymerases, NEB's Hot Start *Taq* utilizes an aptamer-based technology. The unique aptamer binds to the polymerase through non-covalent interactions, inhibiting polymerization at non-permissive temperatures. This novel method eliminates the need for an activation step, reducing the potential for sample degradation and decreasing overall reaction time.

Additional Formats: For added convenience, *Taq* and Hot Start *Taq* DNA Polymerase are available in master mix format. For direct gel loading, a Quick-Load version of the *Taq* 2X Master Mix is also available. The *Taq* PCR Kit contains *Taq* DNA Polymerase, dNTP Mix, Buffer, MgCl2 and the Quick-Load Purple 1 kb Plus DNA Ladder. The Multiplex PCR 5X Master Mix formulation has been specifically optimized for enhanced performance in multiplex PCR reactions.

Concentration: 5,000 units/ml

NEB also offers a selection of Nucleotides and Deoxynucleotides, as well as Reaction Buffers sold separately.

***Taq* DNA POLYMERASE DETAILS**

Extension Rate	1 kb/min
Amplicon Size	≤ 5 kb
Units / 50 µl rxn	1.25 units
Resulting Ends	3´ A
3´→5´ Exonuclease Activity	No
5´→3´ Exonuclease Activity	Yes
Supplied Buffer (product dependent)	- Std *Taq* Rxn Buffer or - ThermoPol Rxn Buffer

PRODUCT FORMATS

Hot Start Available Activation Required	Yes No
Master Mix Available	Yes
Direct Gel-loading Available	Yes
PCR Kit Available	Yes
APPLICATIONS	
Routine PCR	Yes
SNP Detection	Yes
T/A, U/A Cloning	Yes
Colony PCR	Yes

***Taq* Buffer Selection Chart**

CHOICE OF BUFFER	AVAILABLE PRODUCTS
ThermoPol Reaction Buffer: Designed for optimal yield and specificity	*Taq* DNA Polymerase with ThermoPol Buffer (NEB #M0267)
Standard *Taq* Reaction Buffer: Detergent-free and designed to be compatible with existing assay systems	*Taq* DNA Polymerase with Standard Taq Buffer (NEB #M0273) *Taq* DNA Polymerase with Standard Taq (Mg-free) Buffer (NEB #M0320)

Kelly and Jennifer are both members of the Research Department. Kelly joined NEB in 2014 and is leading a new research lab in the Molecular Enzymology Division that studies archaeal nucleic acid maintenance mechanisms. Jennifer joined NEB in 2006 and is the Scientific Director of the Nucleic Acid Replication Division. Jennifer leads a research lab that creates new enzymes by directed evolution and protein engineering.

LongAmp® *Taq* DNA Polymerase

LongAmp® Hot Start *Taq* DNA Polymerase

LongAmp *Taq* DNA Polymerase
#M0323S 500 units
#M0323L 2,500 units

LongAmp Hot Start *Taq* DNA Polymerase
#M0534S 500 units
#M0534L 2,500 units

LongAmp *Taq* 2X Master Mix
#M0287S 100 reactions
#M0287L 500 reactions

LongAmp Hot Start *Taq* 2X Master Mix
#M0533S 100 reactions
#M0533L 500 reactions

LongAmp *Taq* PCR Kit
#E5200S 100 reactions

LONGAMP *Taq* POLYMERASE DETAILS

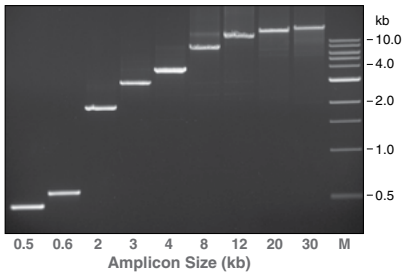
Extension Rate	1.2 kb/min
Amplicon Size	≤ 30 kb
Fidelity	2X *Taq* DNA Polymerase
Units / 50 µl rxn	5 units
Resulting Ends	3´ A/Blunt
3´→5´ Exonuclease Activity	Yes
5´→3´ Exonuclease Activity	Yes
Supplied Buffer (product dependent)	LongAmp *Taq* Rxn Buffer

PRODUCT FORMATS

Hot Start Available	Yes
Activation Required	No
Master Mix Available	Yes
Direct Gel-loading Available	No
PCR Kit Available	Yes

APPLICATIONS

Long Amplicons	Yes
Routine PCR	Yes
T/A, U/A Cloning	Yes
Colony PCR	Yes



Amplification of longer templates with LongAmp Taq.
Amplification of specific sequences from human genomic DNA using LongAmp Taq DNA Polymerase. Amplicon sizes are indicated below gel. Ladder (M) is NEB 1 kb DNA Ladder (NEB #N3232).

Description: An optimized blend of *Taq* and Deep Vent DNA Polymerases, LongAmp *Taq* DNA Polymerase enables amplification of longer PCR products with higher fidelity than *Taq* DNA Polymerase alone.

LongAmp Hot Start *Taq* DNA Polymerase: LongAmp Hot Start *Taq* DNA Polymerase utilizes a unique synthetic aptamer. This structure binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 45°C, but releases the enzyme during normal PCR cycling conditions.

Additional Formats: For added convenience, LongAmp *Taq* and LongAmp Hot Start *Taq* are available in master mix format. The LongAmp *Taq* PCR Kit includes LongAmp *Taq* DNA Polymerase (2,500 units/ml), dNTP Mix (10 mM), LongAmp *Taq* Reaction Buffer Pack (5X), MgSO4 (100 mM) and nuclease-free water.

Concentration: 2,500 units/ml

Hemo KlenTaq®

#M0332S 200 reactions
#M0332L 1,000 reactions

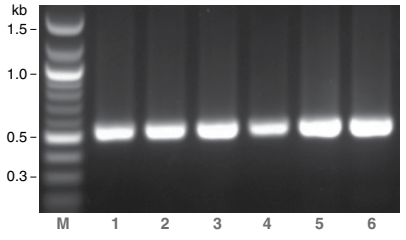
HEMO KLEN*Taq* DETAILS

Extension Rate	0.5 kb/min
Amplicon Size	≤ 2 kb
Units / 50 µl rxn	4 units
Resulting Ends	3´ A
3´→5´ Exonuclease Activity	No
5´→3´ Exonuclease Activity	No
Supplied Buffer	Hemo Klen*Taq* Rxn Buffer

APPLICATIONS

Extraction-free PCR	Yes
T/A, U/A Cloning	Yes
Colony PCR	Yes

Description: Hemo KlenTaq is a truncated version of *Taq* DNA Polymerase, lacking the first 280 amino acids. Hemo KlenTaq also contains mutations that make it resistant to inhibitors present in whole blood. It can amplify DNA from whole blood samples from humans and mice, without the need for DNA extraction. Hemo KlenTaq tolerates up to 20% whole blood in a 25 µl reaction (30% in a 50 µl reaction). Hemo KlenTaq works well with most common anticoagulants, including heparin, citrate and EDTA.



Amplification of human whole blood with Hemo KlenTaq.
Lane 1: 5% blood + Na-EDTA; Lane 2: 5% blood + K-EDTA; Lane 3: 5% blood + Na-Heparin; Lane 4: 5% blood + Na-Citrate; Lane 5: 1.2 mm² FTA Guthrie Card containing dried human blood + Na-Heparin; Lane 6: 1.2 mm² PTA Guthrie Card containing dried human blood + Na-Heparin (washed with 50 µl H2O at 50°C for 5 min.). Ladder (M) is the 1 kb Plus DNA Ladder (NEB #N3200).

Source: An *E. coli* strain that carries a mutant *Taq* DNA polymerase gene. The protein lacks the N-terminal 5´→3´ exonuclease domain and the gene has three internal point mutations.

Heat Inactivation: Not Heat Inactivated

EpiMark® Hot Start *Taq* DNA Polymerase

#M0490S 100 reactions
#M0490L 500 reactions

EPIMARK POLYMERASE DETAILS

Extension Rate	1 kb/min
Amplicon Size	≤ 1 kb
Units / 50 µl rxn	1.25 units
Resulting Ends	3´A
3´→5´ Exonuclease Activity	No
5´→3´ Exonuclease Activity	Yes
Supplied Buffer	EpiMark Hot Start *Taq* Rxn Buffer

APPLICATIONS

AT-rich Targets	Yes
Bisulfite-converted DNA	Yes
Routine PCR	Yes
T/A, U/A Cloning	Yes

Description: EpiMark Hot Start *Taq* DNA Polymerase is an excellent choice for use on bisulfite-converted DNA. It is a mixture of *Taq* DNA Polymerase and a temperature-sensitive, aptamer-based inhibitor. The inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 45°C, but releases the enzyme during normal PCR cycling conditions. This permits room temperature reaction assembly with no separate high-temperature incubation step to activate the enzyme.

Source: An *E. coli* strain that carries the *Taq* DNA Polymerase gene from *Thermus aquaticus* YT-1

Concentration: 5,000 units/ml

Heat Inactivation: Not Heat Inactivated

Vent® & Deep Vent® DNA Polymerases

Vent® DNA Polymerase
#M0254S 200 units
#M0254L 1,000 units

Vent (exo-) DNA Polymerase
#M0257S 200 units
#M0257L 1,000 units

Deep Vent DNA Polymerase
#M0258S 200 units
#M0258L 1,000 units

Deep Vent (exo-) DNA Polymerase
#M0259S 200 units
#M0259L 1,000 units

VENT/DEEP VENT POLYMERASES DETAILS

Extension Rate	1 kb/min
Amplicon Size	≤ 6 kb
Fidelity	5-6X *Taq*
Resulting Ends	Blunt
3´→5´ Exonuclease Activity	Yes (M0254, M0258)
5´→3´ Exonuclease Activity	No
Supplied Buffer	ThermoPol Rxn Buffer

Description: Vent DNA Polymerase was the first high fidelity thermophilic DNA polymerase available for PCR. The fidelity is 5-fold higher than that observed for *Taq* DNA Polymerase, and is derived in part from an integral 3´→ 5´ proofreading exonuclease activity. Greater than 90% activity remains following a 1 hour incubation at 95°C.

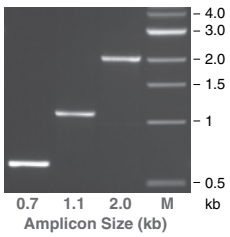
Deep Vent DNA Polymerase is a modified version of Vent DNA Polymerase. Deep Vent has similar fidelity with even more stability than Vent at temperatures of 95 to 100°C, with a half-life of 8 hours at 100°C.

Vent (exo–) DNA Polymerase has been genetically engineered to eliminate the 3´→ 5´ proofreading exonuclease activity associated with Vent DNA Polymerase. The fidelity of polymerization by this form is reduced to a level about 2-fold higher than that of *Taq* DNA Polymerase. Likewise, Deep Vent (exo–) DNA Polymerase has been genetically engineered to eliminate the 3´→ 5´ proofreading exonuclease activity associated with Deep Vent DNA Polymerase.

Source: Vent DNA Polymerase is purified from a strain of E. coli that carries the Vent DNA Polymerase gene from the archaea Thermococcus litoralis. Vent (exo–) is purified from an E. coli strain that carries the Vent (D141A/E143A) DNA Polymerase gene, a genetically engineered form of the native DNA polymerase.

Deep Vent DNA Polymerase is purified from a strain of E. coli that carries the Deep Vent DNA Polymerase gene from Pyrococcus species GB-D. Deep Vent (exo–) is purified from an E. coli strain that carries the Deep Vent (D141A/E143A) DNA Polymerase gene, a genetically engineered form of the native polymerase.

Concentration: 2,000 units/ml



Amplification of Jurkat genomic DNA with Vent DNA Polymerase. *Amplicon Sizes are indicated below gel. Marker (M) is the 1 kb DNA Ladder (NEB #N3232).*

Luna® qPCR and RT-qPCR

- *Convenient master mix and supermix formats with user-friendly protocols simplify reaction setup*
- *Non-interfering, visible tracking dye helps to eliminate pipetting errors*
- *Novel, thermostable reverse transcriptase (RT) improves performance*
- *One-Step RT-qPCR kits feature Luna WarmStart RT paired with Hot Start Taq for increased reaction specificity and robustness*

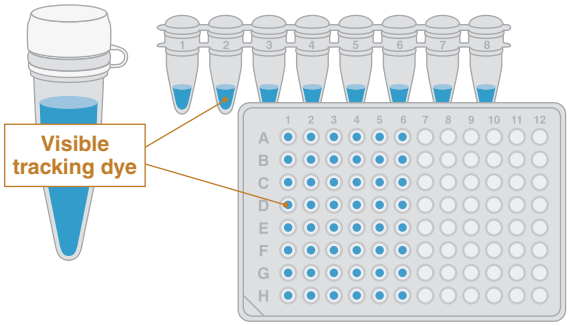
Fluorescence-based quantitative real-time PCR (qPCR) is the gold standard for the detection and quantification of nucleic acids due to its sensitivity and specificity. Luna products from NEB are optimized for qPCR or RT-qPCR, and are available for either intercalating dye or probe-based detection methods. Each Hot Start *Taq*-based Luna qPCR master mix has been formulated with a unique passive reference dye that is compatible across a wide variety of thermal cyclers, regardless of ROX requirements. No additional components are required to ensure compatibility. For two-step RT-qPCR, the LunaScript® RT SuperMix Kit offers a fast (less than 15 minute), robust, and sensitive option for cDNA synthesis upstream of your Luna qPCR experiment.

The Luna Probe One-Step RT-qPCR Mix with UDG is supplied at a 4X concentration and enables higher amounts of sample input, which is relevant for applications where increased sensitivity is needed, such as molecular diagnostics. Performance in multiplexing applications has been optimized, with linear detection achieved for up to 5 targets across a range of inputs.

Find the right Luna product for your application

1 Select your target	2 Select your detection method: Dye-based	Probe-based
Genomic DNA or cDNA	Luna® Universal qPCR Master Mix (NEB #M3003)	Luna Universal Probe qPCR Master Mix (NEB #M3004)*
Purified RNA One-Step RT-qPCR	Luna Universal One-Step RT-qPCR Kit (NEB #E3005)	Luna Universal Probe One-Step RT-qPCR: • Kit (NEB #E3006) • Kit (No ROX, NEB #E3007) • 4X Mix with UDG (NEB #M3019) • 4X Mix with UDG (No ROX, NEB #M3029) LyoPrime Luna™ Probe One-Step RT-qPCR Mix with UDG (NEB #L4001)
Two-Step RT-qPCR	LunaScript® RT SuperMix (NEB #E3010/M3010) + Luna Universal qPCR Master Mix (NEB #M3003)	LunaScript RT SuperMix (NEB #E3010/M3010) + Luna Universal Probe qPCR Master Mix (NEB #M3004)
RNA from cell lysate	Luna Cell Ready One-Step RT-qPCR Kit (NEB #E3030)	Luna Cell Ready Probe One-Step RT-qPCR Kit (NEB #E3031)

* No ROX version available (OEM)
For bulk, lyophilized or custom options, contact us at www.neb.com/CustomContactForm



Learn about "Dots in Boxes" visualization of qPCR data.

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/WarmStart · Recombinant Albumin · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

Luna Universal qPCR & Probe qPCR Master Mixes

Luna Universal qPCR Master Mix

#M3003S	200 reactions
#M3003L	500 reactions
#M3003X	1,000 reactions
#M3003E	2,500 reactions

Luna Universal Probe qPCR Master Mix

#M3004S	200 reactions
#M3004L	500 reactions
#M3004X	1,000 reactions
#M3004E	2,500 reactions

Companion Product:

Antarctic Thermolabile UDG

#M0372S	100 units
#M0372L	500 units

- *Convenient master mix formats and user-friendly protocols simplify reaction setup*
- *Non-interfering, visible tracking dye helps to eliminate pipetting errors*
- *Rigorously tested to optimize specificity, sensitivity, accuracy and reproducibility*
- *Unique passive reference dye for compatibility across wide range of instruments*

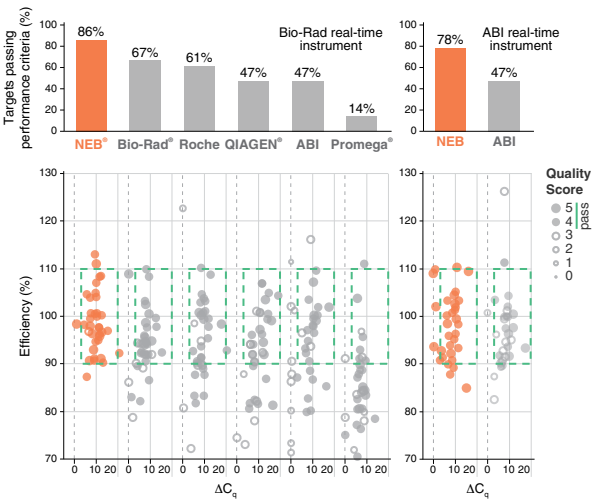
Learn more about our comprehensive qPCR/RT-qPCR testing and **"dots in boxes"** data visualization at **LUNAqPCR.com**.

Description: The Luna Universal qPCR Master Mix is an optimized 2X reaction mix for real-time qPCR detection and quantitation of target DNA sequences using the SYBR®/FAM channel of most real-time qPCR instruments.

The Luna Universal Probe qPCR Master Mix is a 2X reaction mix optimized for real-time qPCR detection and quantitation of target DNA sequences using hydrolysis probes.

Each Hot Start *Taq*-based Luna qPCR master mix has been formulated with a unique passive reference dye that is compatible across a wide variety of instrument platforms, including those that require a ROX reference signal. This means that no additional components are required to ensure machine compatibility. The mixes also contain dUTP, enabling carryover prevention when reactions are treated with Antarctic Thermolabile UDG. A blue visible dye assists in tracking the reagents when pipetting into clear, multi-welled PCR plates. These features, combined with rapid, sensitive and precise real-time qPCR performance, make Luna the universal choice for all your qPCR experiments.

SYBR® is a registered trademark of Thermo Fisher Scientific.



Extensive performance evaluation of commercially available dye-based qPCR reagents demonstrates the robustness and specificity of Luna. *qPCR reagents from NEB and other manufacturers were tested across 16–18 qPCR targets varying in abundance, length and %GC, using either Jurkat genomic DNA or Jurkat-derived cDNA as input (10 genomic DNA targets and 8 cDNA targets on Bio-Rad real-time instrument, 9 genomic and 7 cDNA targets on ABI instrument). For each testing condition, data was collected by 2 users and according to manufacturer's specifications. Results were evaluated for efficiency, low input detection and lack of non-template amplification (where ? C_q = average C_q of non-template control – average C_q of lowest input). In addition, consistency, reproducibility and overall curve quality were assessed (Quality Score). Bar graph indicates % of targets that met acceptable performance criteria (indicated by green box on dot plot and Quality Score > 3). NEB's Luna Universal qPCR Master Mix outperformed all other reagents tested.*

LunaScript® RT SuperMix, SuperMix Kit & Master Mix Kit (Primer-free)

NEW

LunaScript RT SuperMix

#M3010L	100 reactions
#M3010X	500 reactions
#M3010E	2,500 reactions

LunaScript RT SuperMix Kit

#E3010S	25 reactions
#E3010L	100 reactions

LunaScript RT Master Mix Kit (Primer-free)

#E3025S	25 reactions
#E3025L	100 reactions

Description: The LunaScript RT SuperMix Kit is an optimized master mix containing all the necessary components for first strand cDNA synthesis in the context of a two-step RT-qPCR workflow. It features the thermostable Luna Reverse Transcriptase, which supports cDNA synthesis at elevated temperatures. Murine RNase inhibitor is also included to protect template RNA from degradation. LunaScript RT SuperMix Kit contains random hexamer and poly-dT primers, which allow for even coverage across the length of the RNA targets. LunaScript RT SuperMix Kit also includes a No-RT Control Mix and Nuclease-free Water.

LunaScript RT Master Mix Kit (Primer-free) is the same formulation as the LunaScript RT SuperMix Kit but does not contain primers. It includes all components needed for carrying out first strand cDNA synthesis except for RNA template and user-supplied primers. It provides flexible options for using different primers (dT primers, random primers, gene-specific primers, modified primers, etc.) for first strand cDNA synthesis.

The LunaScript RT SuperMix Kit Includes:

- LunaScript RT SuperMix
- No-RT Control Mix
- Nuclease-free Water

The LunaScript RT Master Mix Kit (Primer-free) Includes:

- LunaScript RT Master Mix (Primer-free)
- No-RT Control Mix (Primer-free)
- Nuclease-free Water

Luna One-Step RT-qPCR Products

Luna Probe One-Step RT-qPCR 4X Mix with UDG
#M3019S 200 reactions
#M3019L 500 reactions
#M3019X 1,000 reactions
#M3019E 2,000 reactions

NEW
Luna Probe One-Step RT-qPCR 4X Mix with UDG (No ROX)
#M3029S 200 reactions
#M3029L 500 reactions
#M3029E 2,000 reactions

NEW
LyoPrime Luna Probe One-Step RT-qPCR Mix with UDG
#L4001S 120 reactions

Luna Universal One-Step RT-qPCR Kit
#E3005S 200 reactions
#E3005L 500 reactions
#E3005X 1,000 reactions
#E3005E 2,000 reactions

Luna Universal Probe One-Step RT-qPCR Kit
#E3006S 200 reactions
#E3006L 500 reactions
#E3006X 1,000 reactions
#E3006E 2,000 reactions

Luna Probe One-Step RT-qPCR Kit (No ROX)
#E3007E 2,500 reactions

Companion Product:

Antarctic Thermolabile UDG
#M0372S 100 units
#M0372L 500 units

- *Novel, thermostable RT improves performance*
- *Luna WarmStart paired with Hot Start Taq increases reaction specificity and robustness*
- *Non-interfering, visible tracking dye helps to eliminate pipetting errors*
- *Products perform consistently across a wide variety of sample sources*
- *Lyophilized format (NEB #L4001) removes cold chain shipping requirements, enables room temperature storage, and can be quickly rehydrated*

NEB also offers a selection of Nucleotides and Deoxynucleotides, as well as Reaction Buffers sold separately.

Learn more about our new LyoPrime portfolio of lyophilized reagents.

Description: The Luna RT-qPCR kits contain a novel, *in silico*-designed reverse transcriptase (RT) engineered for improved performance. Both the Luna WarmStart Reverse Transcriptase and Hot Start *Taq* DNA Polymerase, included in these kits, utilize a temperature-sensitive, reversible aptamer, which inhibits activity below 45°C. This enables room temperature reaction setup and prevents undesired non-specific activity. Furthermore, the WarmStart RT has increased thermostability, improving performance at higher reaction temperatures.

The Luna Probe One-Step RT-qPCR 4X Mix with UDG (NEB #M3019) supports robust, sensitive detection and quantitation of up to 5 targets in a multiplexed reaction. It is supplied at a 4X concentration and enables higher amounts of sample input, which is relevant for applications where RNA present in low abundance is of interest, such as pathogen detection. For simplified reaction setup, the single tube master mix format consolidates components for the one-step RT-qPCR reaction. It also includes dUTP and UDG in the mix for reduced risk of carryover contamination. This mix is also available without ROX (NEB #M3029) for instruments that do not require the ROX passive reference dye.

The LyoPrime™ Luna Probe One-Step RT-qPCR Mix with UDG is supplied in a lyophilized format, which can be rehydrated by simply adding nuclease-free water prior to use.

The Luna Universal One-Step RT-qPCR Kit is optimized for dye-based real-time quantitation of target RNA sequences via the SYBR/FAM fluorescence channel of most real-time instruments.

The Luna Universal Probe One-Step RT-qPCR Kit is optimized for real-time quantitation of target RNA sequences using hydrolysis probes.

For instruments that do not utilize ROX normalization, the Luna Probe One-Step RT-qPCR Kit (No ROX) (NEB #E3007) contains no reference dye. If ROX normalization is desired, ROX can be added; this is only necessary with the E3007 product.

The other Luna products contain dUTP and enable carryover prevention when reactions are treated with Antarctic Thermolabile UDG (NEB #M0372). A blue visible dye assists in tracking the reagents when pipetting into clear, multi-welled PCR plates. The reverse transcriptase, featured in the Luna One-Step RT-qPCR products is a novel, engineered WarmStart enzyme developed for robust performance and increased thermostability. These features, combined with rapid, sensitive and precise real-time qPCR performance, make Luna the universal choice for all your qPCR and RT-qPCR experiments.

The Luna Probe One-Step RT-qPCR Kit (No ROX) Includes:

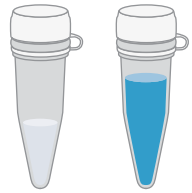
- Luna Universal Probe One-Step Reaction Mix (No ROX)
- Luna WarmStart RT Enzyme Mix
- Nuclease-free Water

The Luna Universal One-Step RT-qPCR Kit Includes:

- Luna Universal One-Step Reaction Mix
- Luna WarmStart RT Enzyme Mix
- Nuclease-free Water

The Luna Universal Probe One-Step RT-qPCR Kit Includes:

- Luna Universal Probe One-Step Reaction Mix
- Luna WarmStart RT Enzyme Mix
- Nuclease-free Water

	LyoPrime Luna® Probe One-Step RT-qPCR Mix with UDG	Luna® Probe One-Step RT-qPCR 4X Mix with UDG	Luna Universal Probe One-Step RT-qPCR Kit
	NEB #L4001	NEB #M3019	NEB #E3006
	Lyophilized Master Mix	4X Master Mix	20X RT Enzyme Mix / 2X Reaction Mix
			
Number of tubes	1	1	2
Sample-limiting concentration	2–4X	4X	2X
dUTP included	✓	✓	✓
UDG included	✓	✓	✗
Universal ROX included	✓	✓	✓
Storage temperature	Room temperature	–20°C	–20°C

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/WarmStart · Recombinant Albumin · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

Luna Cell Ready One-Step RT-qPCR Kit

Luna Cell Ready Probe One-Step RT-qPCR Kit

Luna Cell Ready One-Step RT-qPCR Kit
#E3030S 100 reactions

Luna Cell Ready Probe One-Step RT-qPCR Kit
#E3031S 100 reactions

Luna Cell Ready Lysis Module
#E3032S 100 reactions

Companion Product:

Antarctic Thermolabile UDG
#M0372S 100 units
#M0372L 500 units

- *Go direct from cells to RNA quantitation without purification*
- *Coordinated cell lysis, RNA release, and genomic DNA removal in a fast, 15-minute protocol*
- *Effective cell lysis preparation from 10-10,000 cells across numerous cell lines*
- *Features Luna WartStart RT paired with HotStart Taq for increased thermostability and room temperature setup*

Description: The Luna Cell Ready One-Step RT-qPCR Kit provides all the necessary components for direct, dye-based RNA detection and quantitation, bypassing the need for RNA extraction and purification.

The Luna Cell Ready Probe One-Step RT-qPCR Kit provides all the necessary components for direct, probe-based RNA detection and quantitation, bypassing the need for RNA extraction and purification.

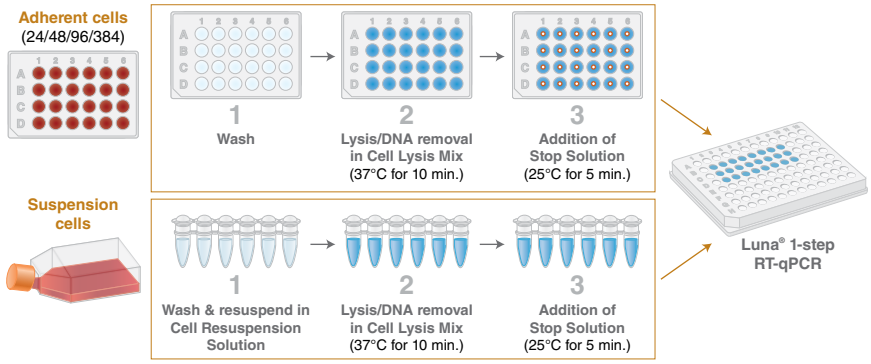
Cell cultures are often analyzed for gene expression or treatment responses as a proxy for a living organism. Traditionally, RNA is extracted and purified from treated cells via column-based or chemical methods. Coordinating the actions of DNase I and the Luna Cell Ready Protease, the Luna Cell Ready Lysis Module offers a simple alternative workflow resulting in effective cell lysis, RNA release, and genomic DNA removal simultaneously in a 15-minute protocol. The Lysis Module includes a unique Luna Cell Ready RNA Protection Reagent that maintains RNA integrity during cell lysis. The lysis capacity spans 10–100,000 cells in a 50 µl lysis reaction. Up to 2 µl of lysate (equivalent to RNA from 0.2–4,000 cells) can be transferred into 20 µl downstream RT-qPCR reactions. Similar to other Luna products, the lysis buffer includes an inert blue tracking dye for visual assistance throughout the workflow.

The Luna Cell Ready One-Step RT-qPCR Kit Includes:

- Luna Cell Ready Lysis Module
- Luna Universal One-Step RT-qPCR Kit (NEB #E3005)

The Luna Cell Ready Probe One-Step RT-qPCR Kit Includes:

- Luna Cell Ready Lysis Module
- Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006)



The Luna Cell Ready One-Step RT-qPCR Kit provides all the necessary components for direct RNA detection and quantitation from cultured cells (up to 100,000 cells per 50 µl lysis reaction). *By coordinating the actions of DNase I and the Luna Cell Ready Protease, the Luna Cell Ready Lysis Module offers a simple workflow resulting in effective cell lysis, RNA release and genomic DNA removal simultaneously in a 15-minute protocol. Up to 2 µl lysate (equivalent to RNA from 0.2 - 4,000 cells) can be transferred into 20 µl downstream RT-qPCR reactions.*

Hannah joined NEB in 2021 as an Analyst and Search Specialist in the Marketing Technologies group. Learn more about Hannah's role at NEB in her video reel.

#NEBiographies

SARS-CoV-2 Detection

Virus detection often utilizes nucleic acid amplification to identify the presence of specific sequences in SARS-CoV-2 viral RNA. These methods include RT-qPCR for real time quantitation and loop-mediated isothermal amplification (LAMP) for robust amplification performed at a single reaction temperature.

SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit

#E2019S 96 reactions

Companion Products:

SARS-CoV-2 Positive Control (N gene)
#N2117S 0.05 ml

Control LAMP Primer Mix (rActin)
#S0164S 50 reactions

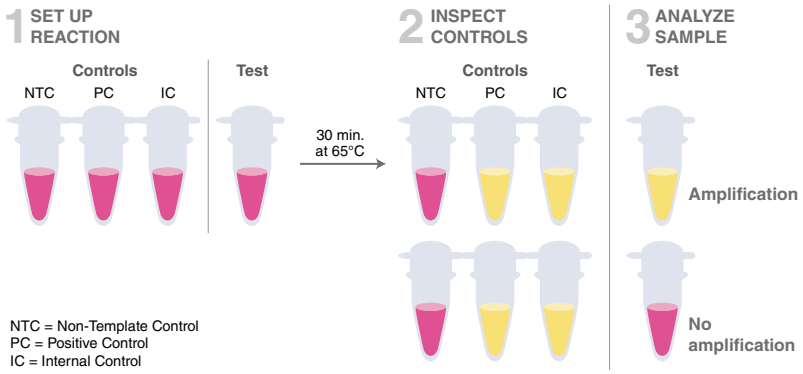
SARS-CoV-2 LAMP Primer Mix (N/E)
#S1883S 85 reactions

- *Colorimetric LAMP enables simple, visual detection (pink-to-yellow) of amplification of SARS-CoV-2 nucleic acid*
- *Set up reactions quickly and easily, using a simple heat source and unique WarmStart technology*
- *Reduce risk of carryover contamination, with UDG and dUTP included in the master mix*
- *Assay targets N and E regions of the SARS-CoV-2 genome, for optimized sensitivity and specificity*
- *Bring confidence to your results using the provided controls*

Description: The SARS-CoV-2 Rapid Colorimetric LAMP Assay Kit utilizes Loop-Mediated Isothermal Amplification (LAMP) to detect SARS-CoV-2 nucleic acid. The kit is available for research use only and includes WarmStart Colorimetric LAMP 2X Master Mix with UDG and a primer mix targeting the N and E regions of the viral genome. Controls are provided to verify assay performance, and include an internal control primer set and a positive control template. Guanidine hydrochloride has been found to increase the speed and sensitivity of the RT-LAMP reaction and is also included.

Kit Includes:

- WarmStart Colorimetric LAMP 2X Master Mix with UDG
- SARS-CoV-2 Positive Control (N gene)
- Control LAMP Primer Mix (rActin)
- SARS-CoV-2 LAMP Primer Mix (N/E)
- Nuclease-free Water
- Guanidine Hydrochloride



In the SARS-CoV-2 Colorimetric LAMP Assay Kit, three control reactions are run with each test sample. *All reactions should be pink prior to incubation. The NTC reaction will contain all materials of the test sample (master mix, primers, etc.) except for the test input nucleic acid and serves as a measure of reaction contamination and primer-based mis-amplification. The NTC should stay pink throughout the experiment. The PC will contain master mix, a plasmid that contains the SARS-CoV-2 N-gene (GenBank: MN908947.3) and primers that will amplify this sequence. Amplification should be observed and the PC should become yellow after incubation. The IC will contain master mix, test input nucleic acid, and LAMP primers for rActin, an endogenous housekeeping gene. If reagents are active and samples have been handled appropriately, the IC should become yellow after incubation.*

Luna® SARS-CoV-2 RT-qPCR Multiplex Assay Kit

#E3019S 96 reactions
#E3019L 480 reactions

Companion Products:

SARS-CoV-2 Positive Control (N gene)
#N2117S 0.05 ml

Tte UvrD Helicase
#M1202S 0.5 µg

- *Multiplex detection of N1 and N2 targets*
- *Enables sample pooling of purified RNA*
- *Internal controls include a redesigned RNase P reverse primer for reduced background amplification*

View our loop mediated isothermal amplification tutorial.

Description: The Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit is optimized for real-time qualitative detection of SARS-CoV-2 nucleic acid using hydrolysis probes. It features the Luna Probe One-Step RT-qPCR 4X Mix with UDG, an optimized SARS-CoV-2 Primer/Probe mix containing primers and probes specific to two regions of the SARS-CoV-2 virus N-gene, and a positive control template. The probes have been modified to contain different fluorophores (N1, HEX; N2, FAM) to enable simultaneous observation on two different channels of a real-time instrument. To ensure the integrity of the input material and absence of inhibition, an internal control (IC) primer and probe set, designed to amplify the human RNase P gene, is also provided in the primer mix. The reverse primer of this target has been modified from the CDC design to target an exon/exon boundary to reduce background amplification from possible contaminating genomic DNA. Amplification of the IC is observed in the Cy5 channel.

Kit Includes:

- Luna Probe One-Step RT-qPCR 4X Mix with UDG
- SARS-CoV-2 Positive Control (N gene)
- SARS-CoV-2 Primer/Probe Mix (N1/N2/RP) (10X)
- Nuclease-free Water

Cloned at NEB · Recombinant Enzyme · High-Fidelity · PCR Enzyme · Hot Start/WarmStart · Recombinant Albumin · Heat Inactivation · Annealing Temperature · EpiMark · Extraction Free

WarmStart Fluorescent LAMP/RT-LAMP Kit (with or without UDG)

NEW
WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG)
#E1708S 100 reactions
#E1708L 500 reactions

WarmStart LAMP Kit (DNA & RNA)
#E1700S 100 reactions
#E1700L 500 reactions

Companion Products:

WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)
#M1708S 100 reactions
#M1708L 500 reactions

LAMP Fluorescent Dye
#B1700S 0.25 ml

Control LAMP Primer Mix (rActin)
#S0164S 50 reactions

- *Reduce risk of carryover contamination with UDG and dUTP included in master mix*
- *Improve LAMP specificity and sensitivity with optimized master mixes*
- *Set up reactions at room temperature with our unique dual WarmStart formulation*
- *WarmStart Multi-Purpose LAMP/ RT LAMP 2X Master Mix (with UDG) supports a variety of detection methods, including turbidity, visual detection and electrophoresis*

WarmStart Fluorescent LAMP/RT-LAMP kits are designed to provide a simple, one-step solution for Loop Mediated Isothermal Amplification (LAMP) of DNA or RNA (RT-LAMP) targets. LAMP and RT-LAMP are commonly used isothermal techniques that provide rapid detection of a target nucleic acid using LAMP-specific primers (supplied by the user) and a strand-displacing DNA polymerase.

The supplied master mixes contain an optimized blend of *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx Reverse Both enzymes have been engineered for improved performance in LAMP and RT-LAMP reactions. The inclusion of dUTP and UDG in the master mix (as noted in the product name) reduces the possibility of carryover contamination between reactions. A fluorescent dye is also supplied to enable real-time fluorescence measurement of LAMP. All WarmStart LAMP/RT-LAMP kits are compatible with multiple detection methods, including turbidity detection, real-time fluorescence detection (when used with LAMP fluorescent dye) and end-point visualization.

The WarmStart Fluorescent LAMP/RT-LAMP Kit (with UDG) includes:

- WarmStart Multi-Purpose LAMP/RT-LAMP 2X Master Mix (with UDG)
- LAMP Fluorescent Dye

The WarmStart Fluorescent LAMP/RT-LAMP Kit includes:

- WarmStart LAMP 2X Master Mix
- LAMP Fluorescent Dye (50X)

WarmStart® Colorimetric LAMP/RT-LAMP 2X Master Mix

WarmStart® Colorimetric LAMP/RT-LAMP 2X Master Mix (with UDG)

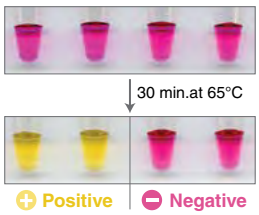
WarmStart Colorimetric LAMP 2X Master Mix (DNA & RNA)
#M1800S 100 reactions
#M1800L 500 reactions

WarmStart Colorimetric LAMP 2X Master Mix with UDG
#M1804S 100 reactions
#M1804L 500 reactions

- *Simple, one-step solution for Loop-Mediated Isothermal Amplification (LAMP) of DNA or RNA (RT-LAMP) targets*
- *Fast, clear, pink-to-yellow visible detection of amplification*
- *WarmStart feature inhibits enzyme activity at room temperature allowing for flexible reaction setup*
- *Reduce risk of carryover contamination with UDG and dUTP included in the master mix (NEB #M1804)*

Description: The WarmStart Colorimetric LAMP/ RT-LAMP 2X Master Mix is an optimized formulation of *Bst* 2.0 WarmStart DNA Polymerase and WarmStart RTx in a special low-buffer reaction solution containing a visitble pH indicator for rapid and easy detection of Loop Mediated Isothermal Amplification (LAMP) and RT-LAMP reactions. WarmStart Colorimetric LAMP/ RT-LAMP 2X Master Mix (with UDG) contains dUTP and UDG in the master mix, which reduces the possibility of carryover contamination between reactions.

This system is designed to provide a fast, clear visual detection of amplification based on the production of protons and subsequent drop in pH that occurs from the extensive DNA polymerase activity in a LAMP reaction, producing a change in solution color from pink to yellow (an overview of LAMP and primer design can be found in the Featured Videos section). This mix can be used for any LAMP or RT-LAMP reaction and requires only a heated chamber and samples, with readout of positive amplification judged by eye in 15–40 minutes.



How is colorimetric LAMP used in point of care diagnostics?

Bst DNA Polymerase-based Products for Isothermal DNA Amplification

Product	5´→ 3´ Exo Activity	Amplification Speed	Room Temp. Setup	Reverse Transcriptase Actvity	Inhibitor Tolerance	Applications
***Bst* DNA Polymerase, Full Length**	★★	N/A	N/A	N/A	★	• Nick translation reactions at elevated temperatures • Primer extension
***Bst* DNA Polymerase, Large Fragment**	N/A	★	N/A	★	★	• General strand-displacement reactions
***Bst* 2.0 DNA Polymerase**	N/A	★★	N/A	★★	★	• Improved LAMP, SDA, and other amplification reactions • Minimal effect of substitution of dTTP with dUTP
***Bst* 2.0 WarmStart DNA Polymerase**	N/A	★★	★★★	★★	★★	• Consistent, room-temperature, and high-throughput amplification assays • Minimal effect of substitution of dTTP with dUTP
***Bst* 3.0 DNA Polymerase**	N/A	★★★	★★	★★★	★★★	• Fused to novel nucleic acid binding domain for enhanced performance • Fastest, most robust LAMP and RT-LAMP reactions • High reverse transcriptase activity up to 72°C • Strand displacement DNA synthesis

★★★ Optimal, recommended product for selected application
★★ Works well for selected application
★ Will perform selected application, but is not recommended
N/A Not applicable to this application

Bst DNA Polymerases

Bst DNA Polymerase, Large Fragment
#M0275S 1,600 units
#M0275L 8,000 units
#M0275M 8,000 units

Bst DNA Polymerase, Full Length
#M0328S 500 units

Bst 2.0 DNA Polymerase
#M0537S 1,600 units
#M0537L 8,000 units
#M0537M 8,000 units

Bst 2.0 WarmStart DNA Polymerase
#M0538S 1,600 units
#M0538L 8,000 units
#M0538M 8,000 units

Bst 3.0 DNA Polymerase
#M0374S 1,600 units
#M0374L 8,000 units
#M0374M 8,000 units

Companion Products:

WarmStart RTx Reverse Transcriptase
#M0380S 50 reactions
#M0380L 250 reactions

Tte UvrD Helicase
#M1202S 0.5 µg

NEB also offers a selection of Nucleotides and Deoxynucleotides, as well as Reaction Buffers sold separately.

Description: *Bst* DNA Polymerase, Large Fragment, is the portion of the Bacillus *stearothermophilus* DNA Polymerase protein that contains the 5´→ 3´ polymerase activity, but lacks 5´→ 3´ exonuclease activity.

Bst DNA Polymerase, Full Length is the full length polymerase from *Bacillus stearothermophilus*. It has 5´→ 3´ polymerase and double-strand specific 5´→ 3´ exonuclease activities, but lacks 3´→ 5´ exonuclease activity.

Bst 2.0 DNA Polymerase is an *in silico* designed homologue of *Bst* DNA Polymerase I, Large Fragment. It contains 5´→ 3´ DNA polymerase activity and strong strand displacement activity but lacks 5´→ 3´ exonuclease activity. It has improved amplification speed, yield, salt tolerance and thermostability compared to wild-type *Bst* DNA Polymerase, Large Fragment.

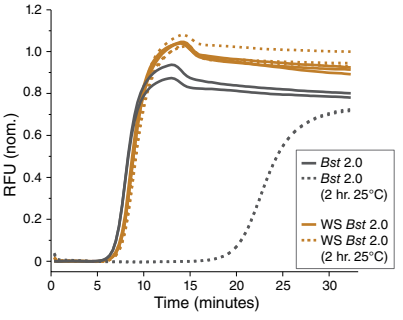
Bst 2.0 WarmStart DNA Polymerase utilizes aptamer technology to inhibit activity at non-permissive temperatures (< 50°C). Like "Hot Start" PCR polymerases, the WarmStart feature enables room temperature set up and prevents non-templated addition of nucleotides, increasing reaction efficiencies. Additionally, no separate activation step is required to release the aptamer from the enzyme. *Bst* 2.0 WarmStart DNA Polymerase permits reaction temperatures from 60–72°C.

Bst 3.0 DNA Polymerase is a similarly designed *in silico* homologue engineered and fused to a novel nucleic acid binding domain for improved isothermal amplification performance and increased reverse transcription activity. Bst 3.0 DNA Polymerase contains 5´→ 3´ DNA polymerase activity with either DNA or RNA templates but lacks 5´→ 3´ and 3´→ 5´ exonuclease activity. It demonstrates robust performance in the presence of inhibitors and significantly increased reverse transcriptase activity compared to *Bst* DNA Polymerase.

Concentration: *Bst* DNA Polymerase, Full Length: 5,000 units/ml. All others: 8,000 and 120,000 units/ml

Heat Inactivation: 80°C for 20 minutes

Usage Notes: No *Bst* DNA Polymerase-based products can be used for thermal cycle sequencing or PCR. *Bst* 2.0 WarmStart DNA Polymerase permits reaction temperatures from 60–72°C. Generally, reaction temperatures above 72°C are not recommended for any *Bst* DNA Polymerase-based product.



Benefits of Bst 2.0 WarmStart: *Identical LAMP reactions were run either immediately after setup (solid line) or after a 2 hour incubation at 25°C. Without the protection from Bst 2.0 WarmStart, this room temperature incubation results in variable LAMP performance. Bst 2.0 WarmStart provides more consistent amplification reaction and enables room-temperature and high-throughput setup.*

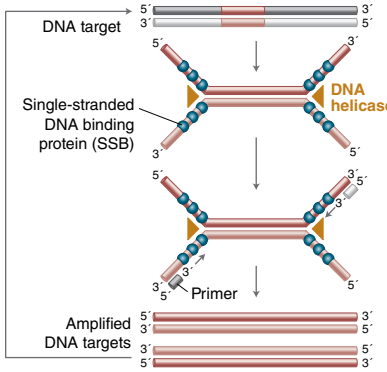
IsoAmp® II Universal tHDA Kit

#H0110S 50 reactions

- *Easy-to-use for assay development*
- *Helicase eliminates need for thermocycler*
- *Reactions performed at constant temp*
- *Amplify & detect short DNA sequences (70–120 bp)*
- *Use with a variety of templates (microbial genomic DNA, viral DNA, plasmid DNA and cDNA)*
- *Amplify a single copy of target DNA by tHDA when optimized primers and buffer are used*

Description: Thermophilic Helicase-Dependent Amplification (tHDA) is a novel method for isothermal amplification of nucleic acids. Like PCR, the tHDA reaction selectively amplifies a target sequence defined by two primers. However, unlike PCR, tHDA uses an enzyme called a helicase to separate DNA, rather than heat. This allows DNA amplification without the need for thermocycling. The tHDA reaction can also be coupled with reverse transcription for RNA analysis.

IsoAmp II Universal tHDA Kit is based on a second-generation thermophilic Helicase-Dependent Amplification platform. The reactions supported by IsoAmp II Universal tHDA Kit include tHDA, reverse transcription HDA (RT-HDA), real-time quantitative HDA (qHDA) and real-time quantitative RT-HDA (qRT-HDA), from a single reaction buffer.



HDA technology. *Helicase Dependent Amplification: Step 1: Helicase unwinding and primer binding. Step 2: DNA polymerization. Step 3: DNA amplification.*

Developed by BioHelix Corporation a NEB-affiliated company, now part of Quidel Corporation.
ISOAMP® is a registered trademark of BioHelix Corporation

NEW

phi29-XT RCA Kit

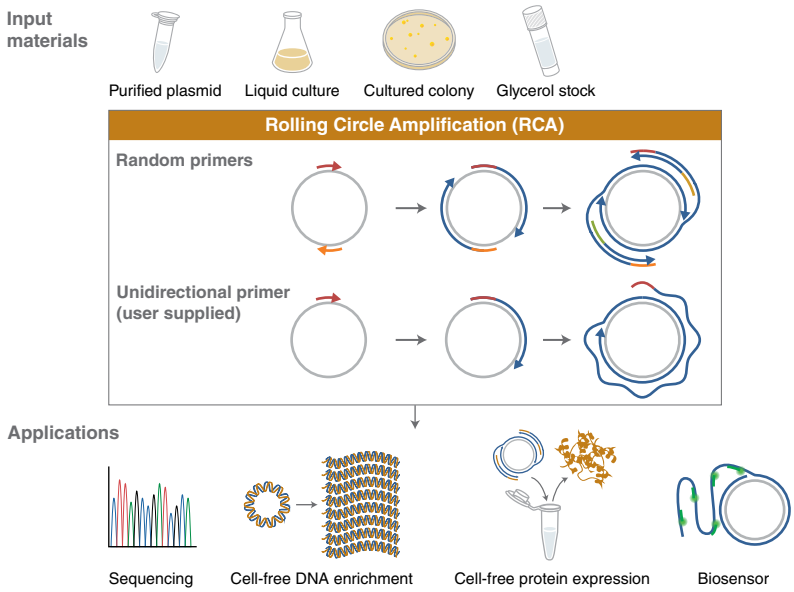
#E1603S 100 reactions
#E1603L 500 reactions

- *High sensitivity: as little as 1 fg of input plasmid DNA needed*
- *Robust and simple workflow generates high yield in a short reaction time*
- *Flexible input material format: purified circular DNA or plasmid/fosmid containing bacterial colony, glycerol stock, or liquid culture*
- *Optimal reaction temperature of 42°C*

Description: Rolling Circle Amplification (RCA) is a robust and highly sensitive isothermal amplification approach to continuously amplify circular DNA, generating long, repetitive copies of the circular sequence.This kit features phi29-XT DNA Polymerase, an engineered polymerase that generates more product in a shorter amount of time than wild-type phi29 DNA polymerase. It also possesses greater sensitivity than the wild-type enzyme while sharing the high processivity, strong strand-displacement, and high-fidelity qualities that are ideal for RCA applications. It is also more thermostable, with an optimal reaction temperature of 42°C. This kit includes exonuclease-resistant random hexamer primers to universally amplify any circular DNA sequence.

Kit Includes:

- phi29-XT DNA Polymerase
- phi29-XT Reaction Buffer
- Exonuclease-resistant Random Primers
- Deoxynucleotide (dNTP) Solution Mix



Overview of the phi29-XT RCA DNA Amplification Kit. *The phi29-XT RCA DNA Amplification Kit (NEB #E1603) is a fast, simple to use and highly versatile kit containing all the required components for rolling circle amplification (RCA) using a random primer mix. The kit delivers high yields of DNA products from a variety of starting materials including purified circular DNA or bacterial cells. This kit is ideal for various DNA applications such as DNA sequencing, cell-free DNA enrichment, cell-free protein expression and DNA biosensors.*

phi29 DNA Polymerase

#M0269S 250 units
#M0269L 1,250 units

- *Extreme processivity*
- *Extreme strand displacement*
- *Replication requiring a high degree of strand displacement and/or processive synthesis*
- *High-fidelity replication at moderate temperatures*

Description: phi29 DNA Polymerase is the replicative polymerase from the *Bacillus subtilis* phage phi29 (φ29). This polymerase has exceptional strand displacement and processive synthesis properties. The polymerase has an inherent 3´→ 5´ proofreading exonuclease activity.

Applications:

- Replication requiring a high degree of strand displacement and/or processive synthesis
- High fidelity replication at moderate temperatures

Reagents Supplied:

- phi29 DNA Polymerase Reaction Buffer
- Recombinant Albumin, Molecular Biology Grade

Source: An *E. coli* strain that carries the phi29 DNA Polymerase gene from bacteriophage phi29

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 0.5 pmol of dNTP into acid insoluble material in 10 minutes at 30°C.

Concentration: 10,000 units/ml

Heat Inactivation: 65°C for 10 minutes

NEW

LunaScript® Multiplex One-Step RT-PCR Kit

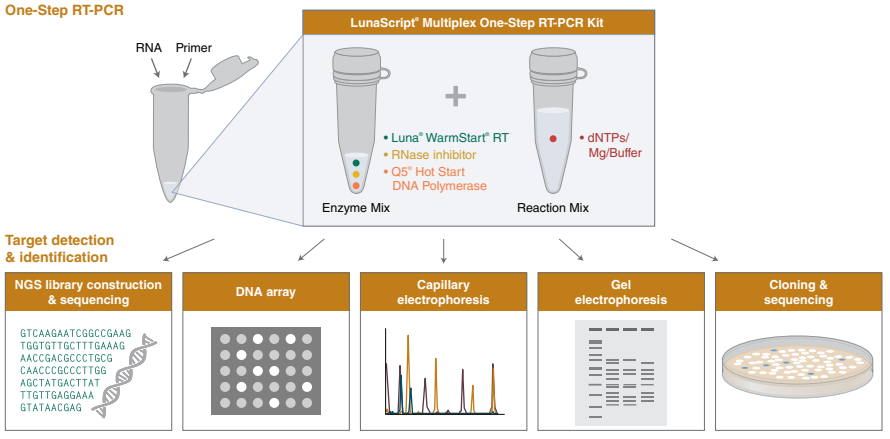
#E1555S 50 reactions
#E1555L 250 reactions

- *Detect as low as 0.01 pg of human total RNA*
- *Multiplexing capacity supports use in ARTIC workflows*
- *Set up reactions at room temperature*
- *Save time, plastics, and minimize contamination with a closed-tube, one-step RT-PCR Protocol*

The LunaScript Multiplex One-Step RT-PCR Kit offers a streamlined protocol for cDNA synthesis and PCR amplification in a single reaction. The 5X reaction mix contains dNTPs and is optimized for multiple target detection in a simple workflow. The 25X enzyme mix features Luna WarmStart Reverse Transcriptase and Q5 Hot Start High-Fidelity DNA Polymerase. The dual-temperature control of enzyme activities by aptamer-based inhibition enables room temperature reaction setup, with preassembled reactions stable at room temperature for up to 24 hours.

Kit Includes:

- LunaScript® Multiplex One-Step RT-PCR Enzyme Mix
- LunaScript® Multiplex One-Step RT-PCR Reaction Mix
- Nuclease-free Water



Multiplex RNA target detection and identification from a single RT-PCR reaction. *The LunaScript Multiplex One-Step RT-PCR Kit (NEB #E1555) requires only RNA template and gene-specific primers to enable multiplex cDNA target synthesis and amplification in a single reaction. Amplified cDNA products can be detected or identified by downstream applications including next-generation sequencing, DNA arrays, fragment analysis, electrophoresis and traditional cloning/sequencing workflows.*

One*Taq*® One-Step RT-PCR Kit

#E5315S 30 reactions

Companion Products:

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

ProtoScript II First Strand cDNA Synthesis Kit
#E6560S 30 reactions
#E6560L 150 reactions

ProtoScript II Reverse Transcriptase
#M0368S 4,000 units
#M0368L 10,000 units
#M0368X 40,000 units

- *Combine cDNA synthesis and PCR in a single reaction*
- *Detect as little as 0.1 pg of a GAPDH target*
- *Robust amplification from 100 bp to 9 kb*
- *Faster protocols with less hands-on time*
- *Quick-Load Reaction Mix allows instant gel loading*

The One*Taq* One-Step RT-PCR Kit offers sensitive and robust end-point detection of RNA templates. cDNA synthesis and PCR amplification steps are performed in a single reaction using gene-specific primers, resulting in a streamlined RT-PCR protocol.

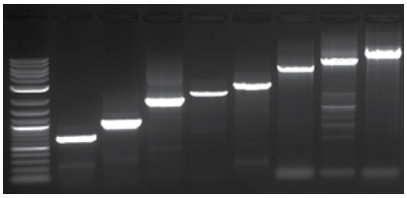
The kit combines three optimized mixes: One*Taq* OneStep Enzyme Mix, One*Taq* One-Step Reaction Mix and One*Taq* One-Step Quick-Load Reaction Mix. The Enzyme Mix combines ProtoScript II Reverse Transcriptase, Murine RNase Inhibitor and One*Taq* Hot Start DNA Polymerase. ProtoScript II Reverse Transcriptase is a mutant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. One*Taq* Hot Start DNA Polymerase is an optimized blend of *Taq* and Deep Vent DNA polymerases combined with an aptamer-based inhibitor.The One*Taq* One-Step RT-PCR Kit is capable of amplifying long transcripts up to 9 kb in length.

Two optimized reaction mixes are included, One*Taq* OneStep Reaction Mix and Quick-Load One*Taq* One-Step Reaction Mix. The reaction mixes offer robust conditions for both cDNA synthesis and PCR amplification. The unique Quick-Load One*Taq* One-Step Reaction Mix contains additional dyes, offering color indication for reaction setup as well as direct gel loading.

Both total RNA and mRNA can be used as template. The kit can detect a GAPDH target as low as 0.1 pg per reaction. It can routinely detect RNA targets up to 9 kb. The OneTaq One-Step RT-PCR Kit is capable of multiplex detection of two or three targets.

Kit Includes:

- One*Taq* One-Step Enzyme Mix
- One*Taq* One-Step Reaction Mix
- Quick-Load One*Taq* One-Step Reaction Mix
- Nuclease-free Water



Detection of RNA templates of different length. *About 100 ng of Jurkat total RNA was used in 50 µl reactions following the standard protocol. The target sizes were Lane 1: 0.7 kb, Lane 2: 1.1 kb, Lane 3: 1.9 kb, Lane 4: 2.3 kb, Lane 5: 2.5 kb, Lane 6: 5.5 kb, Lane 7: 7.6 kb and Lane 8: 9.3 kb. The marker lane (M) contains Quick-Load 1 kb Plus DNA Ladder (NEB #N0469).*

One*Taq*® RT-PCR Kit

#E5310S 30 reactions

Companion Products:

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

M-MuLV Reverse Transcriptase
#M0253S 10,000 units
#M0253L 50,000 units

One*Taq*® Hot Start 2X Master Mix with Standard Buffer
#M0484S 100 reactions
#M0484L 500 reactions

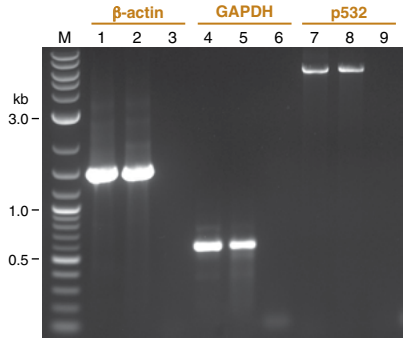
- *Robust RT-PCR reactions*
- *Convenient master mix formats*
- *OneTaq DNA Polymerase offers robust amplification for a wide range of templates*

One*Taq* RT-PCR Kit combines two powerful mixes, M-MuLV Enzyme Mix and One*Taq* Hot Start 2X Master Mix with Standard Buffer for 2-step RT-PCR applications. The two mixes require minimal handling during reaction setup and yet offer consistent and robust RT-PCR reactions.

The first strand cDNA synthesis is achieved by using two optimized mixes, M-MuLV Enzyme Mix and M-MuLV Reaction Mix. M-MuLV Enzyme Mix combines M-MuLV Reverse Transcriptase and RNase Inhibitor, Murine while M-MuLV Reaction Mix contains dNTPs and an optimized buffer. The kit also contains two optimized primers for reverse transcription and nuclease-free water. An anchored Oligo-dT primer [$d(T)_{23}$VN] forces the primer to anneal to the beginning of the polyA tail. The optimized Random Primer Mix provides random and consistent priming sites covering the entire RNA templates including both mRNAs and non-polyadenylated RNAs.

Kit Includes:

- M-MuLV Enzyme Mix
- M-MuLV Reaction Mix
- One*Taq*® Hot Start 2X Master Mix with Standard Buffer
- Random Primer Mix
- Oligo $d(T)_{23}$ VN
- Nuclease-free Water



First strand cDNA synthesis. *Synthesis was carried out in the presence of 1X M-MuLV Enzyme Mix at 42°C using 0.5 µg of human spleen total RNA in the presence of dT23VN (lanes 1, 4 and 7) or Random Hexamer Mix (lanes 2, 5 and 8). No-RT controls were lanes 3, 6 and 9. OneTaq Hot Start Master Mix was used to amplify a 1.5 kb fragment of beta-actin gene, a 0.6 kb fragment of GAPDH gene, and a 5.5 kb fragment from p532 gene in 35 cycles. The marker lane (M) contains 1 kb Plus DNA Ladder (NEB #N3200).*

PreCR® Repair Mix

#M0309S 30 reactions
#M0309L 150 reactions

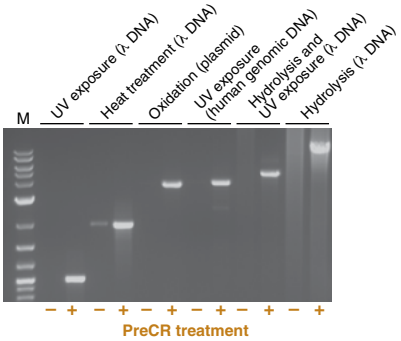
Companion Product:

β-Nicotinamide adenine dinucleotide (NAD+)
#B9007S 0.2 ml

- *Repair DNA prior to its use in DNA-related technologies*
- *Easy-to-use protocols*
- *Does not harm template*

Need to repair FFPE-treated DNA prior to next gen sequencing?
Try our NEBNext FFPE DNA Repair v2 Module (NEB #E7360).

Description: The PreCR Repair Mix is an enzyme cocktail formulated to repair damaged template DNA prior to its use in the polymerase chain reaction (PCR), microarrays, or other DNA technologies. The PreCR Repair Mix is active on a broad range of DNA damage, including those that block PCR (e.g., apurinic/apyrimidinic sites, thymidine dimers, nicks and gaps) and those that are mutagenic (e.g., deaminated cytosine and 8-oxo-guanine). In addition, it will remove a variety of moieties from the 3´ end of DNA leaving a hydroxyl group. The PreCR Repair Mix will not repair all damage that inhibits/interferes with PCR. It can be used in conjunction with any thermophilic polymerase.



Repair of different types of DNA damage with the PreCR Repair Mix. *The?gel shows amplification of damaged DNA that was either not treated (–) or treated (+) with the PreCR Repair Mix. Type of DNA damage is shown. Note: heat treated DNA is incubated at 99°C for 3 minutes. Marker (M) is the 1 kb Plus DNA Ladder (NEB #N3200).*

Applications:

- Repair of DNA prior to its use as a template in PCR or other DNA technologies.

Types of DNA Damage

DNA Damage	Cause	Repaired By PreCR Repair Mix?
Abasic sites	• Hydrolysis	Yes
Nicks	• Hydrolysis • Nucleases • Shearing	Yes
Thymidine dimers	• UV radiation	Yes
Blocked 3´ends	• Multiple	Yes
Oxidized guanine	• Oxidation	Yes
Oxidized pyrimidines	• Oxidation	Yes
Deaminated cytosine	• Hydrolysis	Yes
Fragmentation	• Hydrolysis • Nucleases • Shearing	No
Protein-DNA crosslinks	• Formaldehyde	No

Sulfolobus DNA Polymerase IV

#M0327S 100 units

- *Synthesis of DNA through DNA lesions (lesion bypass)*
- *DNA Repair*

Description: *Sulfolobus* DNA Polymerase IV is a thermostable Y-family lesion-bypass DNA Polymerase that efficiently synthesizes DNA across a variety of DNA template lesions.

Source: An *E. coli* strain that carries the gene encoding DNA polymerase IV from *Sulfolobus islandicus.*

Concentration: 2,000 units/ml

Therminator™ DNA Polymerase

#M0261S 200 units
#M0261L 1,000 units

- *Incorporation of modified nucleotides*
- *DNA sequencing by partial ribosubstitution*
- *DNA sequencing or SNP analysis using dideoxy or acyclo chain terminators*

Description: Therminator DNA Polymerase is a 9°N™ DNA Polymerase variant with an enhanced ability to incorporate modified substrates such as dideoxynucleotides, ribonucleotides and acyclonucleotides.

Source: An *E. coli* strain that carries the 9°N (D141A / E143A / A485L) DNA Polymerase gene, a genetically engineered form of the native DNA polymerase from *Thermococcus* species 9°N-7.

Concentration: 2,000 units/ml

Note: Amplification of extended regions may require optimization of reaction conditions.

DNA Polymerase I (*E. coli*)

#M0209S 500 units
#M0209L 2,500 units

- *Nick translation of DNA*
- *Second strand cDNA synthesis*

Description: DNA Polymerase I (*E. coli*) is a DNA-dependent DNA polymerase with inherent 3´→ 5´ and 5´→ 3´ exonuclease activities. The 5´→ 3´ exonuclease activity removes nucleotides ahead of the growing DNA chain, allowing nick translation.

Source: An *E. coli* strain that carries an overexpressed copy of the *polA* gene.

Concentration: 10,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Note: DNase I is not included with this enzyme and must be added for nick translation reactions.

DNA Polymerase I, Large (Klenow) Fragment

NEB2 RR 25° 75°

#M0210S 200 units
#M0210L 1,000 units

for high (10X) concentration
#M0210M 1,000 units

- *Generates probes using random primers*
- *Removal of 3´ overhangs or fill-in of 5´ overhangs to form blunt ends*
- *Second strand cDNA synthesis*

Description: DNA Polymerase I, Large (Klenow) Fragment was originally derived as a proteolytic product of *E. coli* DNA Polymerase I that retains polymerase and 3´→ 5´ exonuclease activity, but lacks 5´→ 3´ exonuclease activity. Klenow retains the polymerization fidelity of the holoenzyme without degrading 5´ termini.

Source: An *E. coli* strain that contains the *E. coli polA* gene that has had its 5'→3' exonuclease domain removed.

Concentration: 5,000 and 50,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Note: Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times may result in recessed ends due to the 3´→ 5´ exonuclease activity of the enzyme.

Klenow Fragment (3'→5' exo-)

NEB2 RR 37° 75°

#M0212S 200 units
#M0212L 1,000 units

for high (10X) concentration
#M0212M 1,000 units

- *Generates probes using random primers*
- *Random priming labeling*
- *Second strand cDNA synthesis*

Description: Klenow Fragment (3´→ 5´ exo$^-$) is an N-terminal truncation of DNA Polymerase I that retains polymerase activity, but has lost the 5´→ 3´ exonuclease activity, and has mutations (D355A, E357A) that abolish the 3´→ 5´ exonuclease activity.

Source: An *E. coli* strain containing a plasmid with a fragment of the *E.coli polA* (D355A, E357A) gene starting at codon 324.

Concentration: 5,000 and 50,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Note: Klenow Fragment (3´→ 5´ exo-) is not suitable for generating blunt ends because it lacks the 3´→ 5´ exonuclease necessary to remove non-templated 3´ additions.

T4 DNA Polymerase

NEBr21 RR 75°

#M0203S 150 units
#M0203L 750 units

Companion Product:

Quick Blunting Kit
#E1201S 20 reactions
#E1201L 100 reactions

- *Gap filling (no strand displacement activity)*
- *Removal of 3´ overhangs or fill-in of 5´ overhangs to form blunt ends*
- *Probe labeling using replacement synthesis*
- *Single-strand deletion subcloning*

Description: T4 DNA Polymerase catalyzes the synthesis of DNA in the 5´→ 3´ direction and requires the presence of template and primer. This enzyme has a 3´→ 5´ exonuclease activity which is much more active than that found in *E. coli* DNA Polymerase I. Unlike DNA Polymerase I, T4 DNA Polymerase does not have a 5´→ 3´ exonuclease function.

Source: Purified from a strain of *E. coli* that carries the T4 DNA Polymerase gene.

Concentration: 3,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Note: Elevated temperatures, excessive amounts of enzyme, failure to supplement with dNTPs or long reaction times may result in recessed ends due to the 3´→ 5´ exonuclease activity of the enzyme.

T7 DNA Polymerase (unmodified)

NEBU RR 37° 75° rAlbumin

#M0274S 300 units
#M0274L 1,500 units

- *Gap-filling reaction (no strand displacement)*

Description: T7 DNA Polymerase catalyzes the replication of T7 phage DNA during infection. The protein dimer has two catalytic activities: DNA polymerase activity and strong 3´→ 5´ exonuclease. The high fidelity and rapid extension rate of the enzyme make it particularly useful in copying long stretches of DNA template.

Source: T7 DNA Polymerase consists of two subunits: T7 gene 5 protein (84 kilodaltons) and *E.coli* thioredoxin (12 kilodaltons) (1,4-7). Each protein is cloned and overexpressed in a T7 expression system in *E. coli* (4).

Concentration: 10,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Note: The high polymerization rate of the enzyme makes long incubations unnecessary. T7 DNA Polymerase is not suitable for DNA sequencing.

NEB also offers a selection of Nucleotides and Deoxynucleotides, as well as Reaction Buffers sold separately.

Bsu DNA Polymerase, Large Fragment

NEB2 · Cloned at NEB · RR · 37° · 75°

#M0330S 200 units
#M0330L 1,000 units

- *Random primer labeling*
- *Second strand cDNA synthesis*
- *Single dA tailing*
- *Strand displacement DNA synthesis*

Description: *Bsu* DNA Polymerase I, Large Fragment retains the 5´→ 3´ polymerase activity of the *Bacillus subtilis* DNA polymerase I, but lacks the 5´→ 3´ exonuclease domain. This large fragment naturally lacks 3´→ 5´ exonuclease activity.

Source: An *E. coli* strain that contains a genetic fusion of the *Bacillus subtilis* DNA polymerase I gene (starting from codon 297 thus lacking the 5´→ 3´ exonuclease domain), and the gene coding for maltose binding protein (MBP). The fusion protein is purified to near homogeneity and the MBP portion is cleaved off *in vitro*. The remaining DNA polymerase is purified free of MBP.

Concentration: 5,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Note: *Bsu* DNA Polymerase, Large Fragment is not suitable for generating blunt ends because it lacks the 3´→ 5´ exonuclease necessary to remove non-templated 3´ additions. *Bsu* DNA Polymerase, Large Fragment retains 50% activity at 25°C and is twice as active as Klenow Fragment (3´→ 5´ exo–) at this temperature.

Terminal Transferase

NEBU · RR · 37° · 75°

#M0315S 500 units
#M0315L 2,500 units

- *Addition of homopolymer tails to the 3´ ends of DNA*
- *Labeling the 3´ ends of DNA with modified nucleotides (e.g., ddNTP, DIG-dUTP)*
- *TUNEL assay (in situ localization of apoptosis)*
- *TdT dependent PCR*

Description: Terminal Transferase (TdT) is a template independent polymerase that catalyzes the addition of deoxynucleotides to the 3´ hydroxyl terminus of DNA molecules. Protruding, recessed or blunt-ended double or single-stranded DNA molecules serve as a substrate for TdT. The 58.3 kDa enzyme does not have 5´ or 3´ exonuclease activity. The addition of Co^{2+} in the reaction makes tailing more efficient.

Source: An *E. coli* strain that carries the cloned Terminal Transferase gene from calf thymus.

Concentration: 20,000 units/ml

Heat Inactivation: 75°C for 20 minutes

Polymerase Reaction Buffers

Q5 Reaction Buffer Pack
#B9027S 6 ml

Phusion HF Buffer Pack
#B0518S 6 ml

Phusion GC Buffer Pack
#B0519S 6 ml

Standard *Taq* Reaction Buffer Pack
#B9014S 6 ml

Standard *Taq* (Mg-free) Reaction Buffer Pack
#B9015S 6 ml

ThermoPol Reaction Buffer Pack
#B9004S 6 ml

Isothermal Amplification Buffer Pack
#B0537S 6 ml

Isothermal Amplification Buffer II Pack
#B0374S 6 ml

Description: Q5 Reaction Buffer and High GC Enhancer are provided with both Q5 and Q5 Hot Start High-Fidelity DNA Polymerases.

Phusion High-Fidelity DNA Polymerase is supplied with 5X Phusion HF Buffer, 5X Phusion GC Buffer, DMSO, and 50 mM $MgCl_2$.

Standard *Taq* Reaction Buffer is provided with Taq DNA Polymerase as an alternative to the ThermoPol Reaction Buffer.

ThermoPol Reaction Buffer is provided with *Taq*, Vent, Deep Vent, *Bst* Full Length and *Bst* Large Fragment, Sulfolobus IV and Terminator DNA Polymerases; this buffer contains 2 mM $MgSO^4$ when the buffer is diluted to its final 1X concentration.

Isothermal Amplification Buffer is supplied with *Bst* 2.0 and *Bst* 2.0 WarmStart DNA Polymerases.

Isothermal Amplification Buffer II is supplied with *Bst* 3.0 DNA Polymerase.

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

Nucleotides

Acyclonucleotide Set
#N0460S 0.5 µmol

Deoxynucleotide (dNTP) Solution Set
#N0446S 25 µmol

Deoxynucleotide (dNTP) Solution Mix
#N0447S 8 µmol
#N0447L 40 µmol

Ribonucleotide Solution Set
#N0450S 10 µmol
#N0450L 50 µmol

Ribonucleotide Solution Mix
#N0466S 10 µmol
#N0466L 50 µmol

7-deaza-dGTP
#N0445S 0.3 µmol
#N0445L 1.5 µmol

Adenosine 5'-Triphosphate (ATP)
#P0756S 1 ml
#P0756L 5 ml

5-methyl-dCTP
#N0356S 1 µmol

dATP Solution
#N0440S 25 µmol

dUTP Solution
#N0459S 25 µmol

dGTP Solution
#N0442S 25 µmol

Description:
Deoxynucleotide Solution Set:
Four separate solutions of ultrapure deoxynucleotide (dATP, dCTP, dGTP and dTTP). Each deoxynucleotide is supplied at a concentration of 100 mM.

Deoxynucleotide Solution Mix:
An equimolar solution of ultrapure dATP, dCTP, dGTP and dTTP. Each deoxynucleotide is supplied at a concentration of 10 mM.

Ribonucleotide Solution Set:
Four separate solutions of ATP, CTP, GTP and UTP, pH 7.5, as sodium salts.

Ribonucleotide Solution Mix:
A buffered equimolar solution of ribonucleotide triphosphates rATP, rCTP, rGTP and rUTP. Each nucleotide is supplied at a concentration of 25 mM (total rNTP concentration equals 100 mM).

7-deaza-dGTP:
7-deaza contains a 5 mM solution of 7-deaza-dGTP as a dilithium salt.

5-methyl-dCTP:
5mdCTP is supplied as a triethylammonium salt in Milli-Q® water.

dATP Solution:
dATP Solution contains a 100 mM solution of dATP as a sodium salt at pH 7.4.

dUTP Solution:
dUTP Solution contains a 100 mM solution of dUTP as a sodium salt at pH 7.5.

dGTP Solution:
dGTP Solution contains a 100 mM solution of dGTP as a sodium salt at pH 7.5.

Acyclonucleotide Set: Four separate tubes of acyNTPs (acyATP, acyCTP, acyGTP and acyTTP). Acyclonucleotides are supplied dry, as the triethylammonium salt. Addition of 50 µl of distilled or de-ionized (Milli-Q) water will result in a final concentration of 10 mM acyNTP.

Acyclonucleotides (acyNTPs) act as chain terminators and are thus useful in applications that normally employ dideoxynucleotides such as DNA sequencing and SNP detection. AcyNTPs are especially useful in applications with archaeal DNA Polymerases, more specifically with Therminator DNA Polymerase. Therminator DNA Polymerase is an engineered enzyme with an increased capacity to incorporate analogs with altered sugars, such as ribonucleotides, dideoxynucleotides, 2´ deoxynucleotides and especially acyclo-base analogs.

Bryan K. and Brian P. are members of our U.S. Sales Team and support customers on the West Coast. Bryan K. just joined NEB in 2023 as a Senior Field Representative. Brian P. joined NEB in 2010 and is the Regional Manager of our California, Nevada, and Utah team.

cDNA Synthesis Selection Chart

cDNA Synthesis	NEB #	Features	Size
Kits			
LunaScript® RT SuperMix Kit	E3010S E3010L	• Ideal for cDNA synthesis of targets up to 3 kb (two-step RT-qPCR, amplicon sequencing) • Single tube supermix contains random hexamer and oligo-dT primers, dNTPs, Murine RNase Inhibitor, and Luna Reverse Transcriptase • Visible blue tracking dye for easy reaction setup • Fast 13-minute protocol	25 reactions 100 reactions
LunaScript® RT Master Mix Kit (Primer-free)	E3025S E3025L	• Ideal for first strand cDNA synthesis • Compatible with random primers, oligo dT primers and gene-specific primers • 5X master mix contains dNTPs, Murine RNase Inhibitor and Luna Reverse Transcriptase • Visible blue tracking dye for easy reaction setup • Fast 13-minute protocol	25 reactions 100 reactions
ProtoScript® II First Strand cDNA Synthesis Kit	E6560S E6560L	• Generates cDNA up to 10 kb in length • Contains ProtoScript II Reverse Transcriptase, an enzyme with increased thermostability and reduced RNase H activity • Convenient 2-tube kit • Includes dNTPs, Oligo-dT primer and Random Primer Mix	30 reactions 150 reactions
ProtoScript® First Strand cDNA Synthesis Kit	E6300S E6300L	• Generates cDNA at least 5 kb in length • Contains M-MuLV Reverse Transcriptase • Convenient 2-tube kit • Includes dNTPs, Oligo-dT primer and Random Primer Mix	30 reactions 150 reactions
Template Switching RT Enzyme Mix	M0466S M0466L	• Incorporates a universal adaptor sequence at the 3´ end of cDNA during the RT reaction • Enzyme mix and buffer are optimized for efficient template switching • RT enzyme mix includes RNase Inhibitor • High sensitivity for cDNA amplification – enables transcriptome analysis by RNA-seq from single cells or as low as 2 pg of human total RNA • Robust and simple workflow for 5´ Rapid Amplification of cDNA Ends (RACE) • Retains the complete 5´ end of transcripts for 2nd Strand cDNA Synthesis	20 reactions 100 reactions
Standalone Reagents			
Induro™ Reverse Transcriptase	M0681S M0681L	• Fast and processive intron-encoded RT for generating long transcripts (> 12 kb in under 10 mn.) • Increased reaction temperatures (50–60°C) • Increased inhibitor tolerance	4,000 units 10,000 units
ProtoScript® II Reverse Transcriptase An alternative to SuperScript® II	M0368S M0368L M0368X	• RNase H– mutant of M-MuLV Reverse Transcriptase with increased thermostability and reduced RNase H activity • Increased reaction temperatures (37–50°C)	4,000 units 10,000 units 40,000 units
M-MuLV Reverse Transcriptase	M0253S M0253L	• Robust reverse transcriptase for a variety of templates • Standard reaction temperatures (37–45°C)	10,000 units 50,000 units
AMV Reverse Transcriptase	M0277S M0277L	• Robust reverse transcriptase for a broad temperature range (37–52°C) • Can be used for templates requiring higher reaction temperatures	200 units 1,000 units
WarmStart® RTx Reverse Transcriptase	M0380S M0380L	• Permits room temperature reaction setup • Increased reaction temperatures (50–65°C) • Optimized for RT-LAMP isothermal detection	50 reactions 250 reactions

More information about our reverse transcriptases and cDNA synthesis kits can be found in the RNA analysis chapter.
SUPERSCRIPT® is a registered trademark of Thermo Fisher Scientific.

Monarch® PCR & DNA Cleanup Kit (5 µg)

#T1030S	50 preps
#T1030L	250 preps

Companion Products:

Monarch DNA Cleanup Columns (5 µg)
#T1034L 100 columns

Monarch DNA Wash Buffer
#T1032L 25 ml

Monarch Plasmid Miniprep Kit
#T1010S 50 preps
#T1010L 250 preps

Monarch DNA Cleanup Binding Buffer
#T1031L 175 ml

Monarch DNA Gel Extraction Kit
#T1020S 50 preps
#T1020L 250 preps

Monarch DNA Elution Buffer
#T1016L 25 ml

- *Elute in as little as 6 µl*
- *Prevent buffer retention and salt carry-over with optimized column design*
- *Purify small DNA and oligos with a slight protocol modification*
- *Save time with fast, user-friendly protocol*
- *Purchase optimized kit formats or buffers & columns separately for your convenience*

Description: The Monarch PCR & DNA Cleanup Kit (5 µg) is a rapid and reliable method for the purification and concentration of up to 5 µg of high-quality, double-stranded DNA from enzymatic reactions such as PCR, restriction digestion, ligation and reverse transcription. This method employs a bind/wash/elute workflow with minimal incubation and spin times, resulting in purification in less than 5 minutes. DNA Cleanup Binding Buffer is used to dilute the samples and ensure they are compatible for loading onto the proprietary silica matrix under high salt conditions. The DNA Wash Buffer ensures enzymes, short primers (≤ 40 nt), detergents and other low-molecular weight reaction components (e.g., nucleotides, DMSO, betaine) are removed, thereby allowing low-volume elution of concentrated, high-purity DNA. Eluted DNA is ready for use in restriction digests, DNA sequencing, ligation and other enzymatic manipulations. The unique column design ensures no buffer retention and no carryover of contaminants, allowing elution of sample in volumes as low as 6 µl. A slight protocol modification enables purification of small DNA and oligonucleotides.

Applications:

- PCR cleanup
- Enzymatic reaction cleanup
- cDNA cleanup
- Labeling cleanup
- Plasmid cleanup
- Oligonucleotide cleanup

Kit Includes:

- DNA Elution Buffer
- DNA Cleanup Columns (5 µg)
- Collection Tubes (2ml)
- DNA Wash Buffer
- DNA Cleanup Binding Buffer

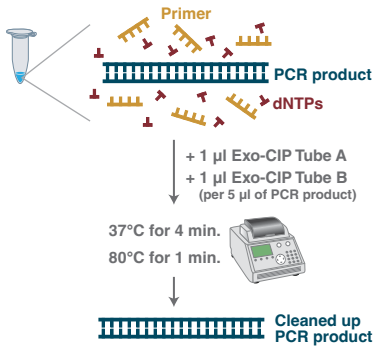
Exo-CIP™ Rapid PCR Cleanup Kit

#E1050S	100 reactions
#E1050L	400 reactions

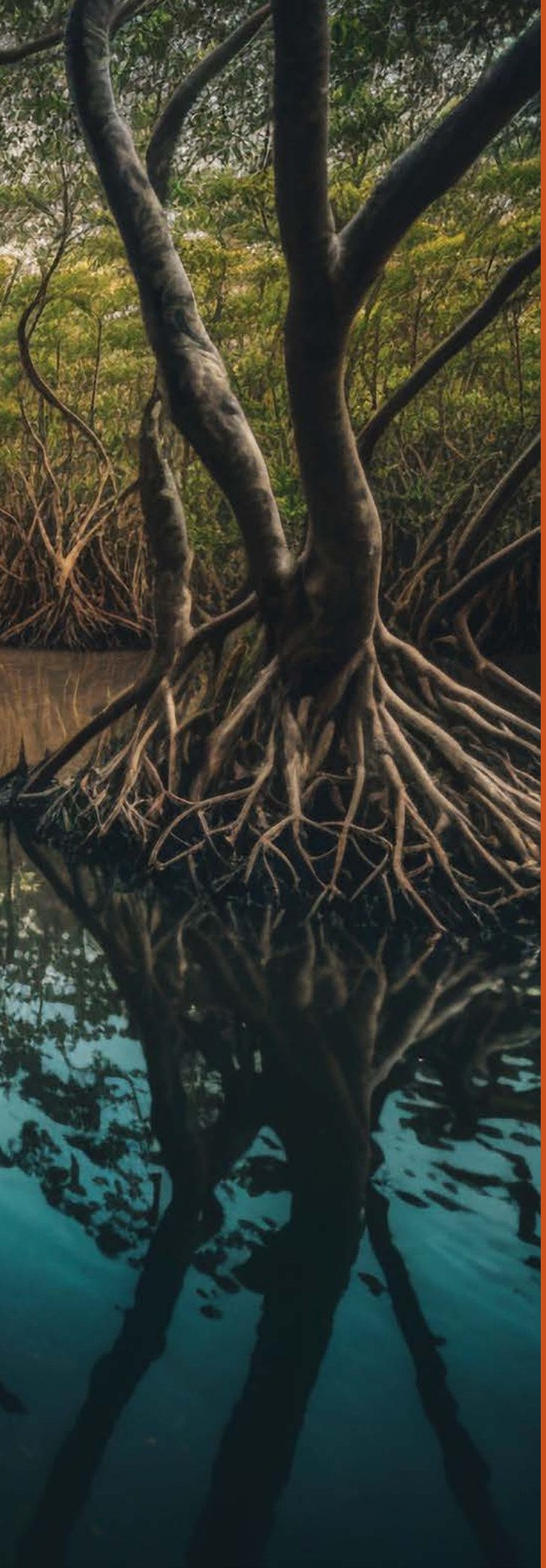
Description: The Exo-CIP Rapid PCR Cleanup Kit contains optimized formulations of thermolabile Exonuclease I and thermolabile Calf Intestinal Phosphatase and is used to rapidly degrade residual PCR primers and dephosphorylate excess dNTPs after amplification. Degradation occurs in only 4 minutes at 37°C, and is immediately followed by rapid inactivation of the enzymes by heating for 1 minute at at 80°C. In just 5 minutes, the PCR product is ready for downstream analysis such as Sanger sequencing, SNP detection, or library preparation for NGS. The Exo-CIP Rapid PCR Cleanup Kit is compatible with all commonly-used reaction buffers.

Kit Includes:

- Exo-CIP Tube A
- Exo-CIP Tube B



Exo-CIP Rapid PCR Cleanup Kit workflow. *1 µl of Exo-CIP Tube A (thermolabile Exo I) and 1 µl of Exo-CIP Tube B (thermolabile CIP) are added to the PCR product to degrade excess primers and dNTPs. The mixture is incubated at 37°C for 4 minutes, followed by a 1 minute incubation at 80°C to irreversibly inactivate both enzymes. The cleaned PCR product is ready for downstream applications or analysis.*



Restoring mangroves to maximize their carbon-capturing power

Mangrove forests were once viewed as unworthy of conservation, but studies show that these biodiversity hotspots capture nearly five times as much carbon per hectare than terrestrial forests, all the while underpinning the livelihoods of coastal communities.

Coastal mangroves store blue carbon – the carbon sequestered by tidal marshes, sea grasses and marine ecosystems. Mangrove forests are made up of many species of ferns, shrubs and trees, and species composition affects the level of carbon sequestration. They are anchored in rich, organic soil that can store large amounts of carbon; a low level of oxygen saturation in the soil results in a slower decay of organic material and, ultimately, a greater amount of stored carbon.

The remarkable carbon-capturing benefit is only one of the vital reasons to protect and restore mangrove forests, which are among the most productive ecosystems on Earth. Mangroves have extensive, intertwined root systems that stabilize and capture the sediment below, acting as a pollutant biofiltration system and protecting the coast from erosion. They have even weakened tsunami wave energy and prevented debris from causing destruction on shore. Mangrove forests are also an essential resource for fishing communities because they are a breeding environment and habitat for various marine species.

Nevertheless, these biodiverse wetlands are destroyed to make space for alternative land use. Shrimp farming is the biggest driver of their destruction, releasing large amounts of stored carbon into the atmosphere. It is estimated that up to one billion tons of CO_2 are released annually when they are cut down. These intertidal forests are cleared at a rate that far exceeds rainforest loss, and when they are cleared, the soil dries, releasing even more carbon.

Restoring mangrove forests involves intimate knowledge of the complexity of the species diversity found there, and the local hydrology that results in the root systems being submerged in water during high tide, while exposed and dry during low tide. The humid, high-rainfall climate in Southeast Asia is ideal for mangrove growth, and as a result, one-third of the world's mangrove forests are found here, but they are also found in South and Central America, West and Central Africa, northeast India, and northern Australia. In Gasi Bay, Kenya, a community-based project, Mikoko Pamoja (meaning 'Mangroves Together' in Swahili), was founded in 2012 in a community that relies on fishing as their primary income. Previously, mangrove logging provided extra income, but it was observed that this affected fish availability. A growing awareness of the dire consequences of logging inspired the community to take action and begin planting and conserving mangroves. The project is subsidized by environmentally-conscious, primarily international companies that use this investment to offset their carbon emissions when conducting carbon audits. The investment supports the planting project, community education, health and employment. The incredible success of the Mikoko Pamoja project is an example of the importance of creating benefits for the local community to instill a vested interest in the project. The Mikoko Pamoja project will next incorporate seagrasses into their conservation efforts.

Mangroves are not the mosquito-ridden wetlands they were once thought to be, In fact, they are a key player in our effort to reduce atmospheric carbon. Restoration projects around the world are working to prevent the loss of these incredible carbon sinks and the associated biodiversity, as well as protect the livelihood of the local communities.

Learn more about the Mikoko Pamoja Project.

Mangrove tree in the water.
Credit: Raihan, Adobe Stock

DNA Modifying Enzymes & Cloning Technologies

The trusted source for DNA-modifying enzymes & cloning technologies.

Molecular biology, which also includes synthetic biology, is a fundamental area of research and development. Central to these advances has been the use of DNA modifying enzymes and novel cloning technologies. Some common examples of DNA-modifying enzymes include kinases, ligases, methylases, exonucleases and endonucleases, while newer cloning technologies include NEBuilder® HiFi DNA Assembly and NEBridge® Golden Gate Assembly.

Almost 50 years as a leader in enzyme technologies gives you confidence in the products and support you'll receive. NEB continues to serve the scientific community by providing the tools to carry out the most innovative research, from start to finish. All NEB products pass stringent quality control assays to ensure the highest level of functionality and purity.

NEB offers several online tools to aid in your cloning experiments, including:

- **NEBcloner®** – find the right products and protocols for each step of your traditional cloning experiment, including double digests and mutagenesis
- **NEBioCalculator®** – use this tool for your scientific calculations and conversions
- **NEBuilder Assembly Tool** – use this tool for help with your DNA assembly primer design
- **Exo Selector** – find the right exonuclease for your workflows
- **NEBridge™ Golden Gate Assembly Tool** – use this tool for help with construct design for Golden Gate Assembly
- **NEBridge Ligase Fidelity Tools** – utilize ligation preferences for the design of high-fidelity Golden Gate Assembly

To view the full list of online tools available, visit **www.neb.com/nebtools**.

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Visit **www.neb.com/FastCloning** to accelerate your cloning experiments with reagents from NEB.

Visit **ClonewithNEB.com** to view our online tutorials explaining each of the steps in the cloning workflow.

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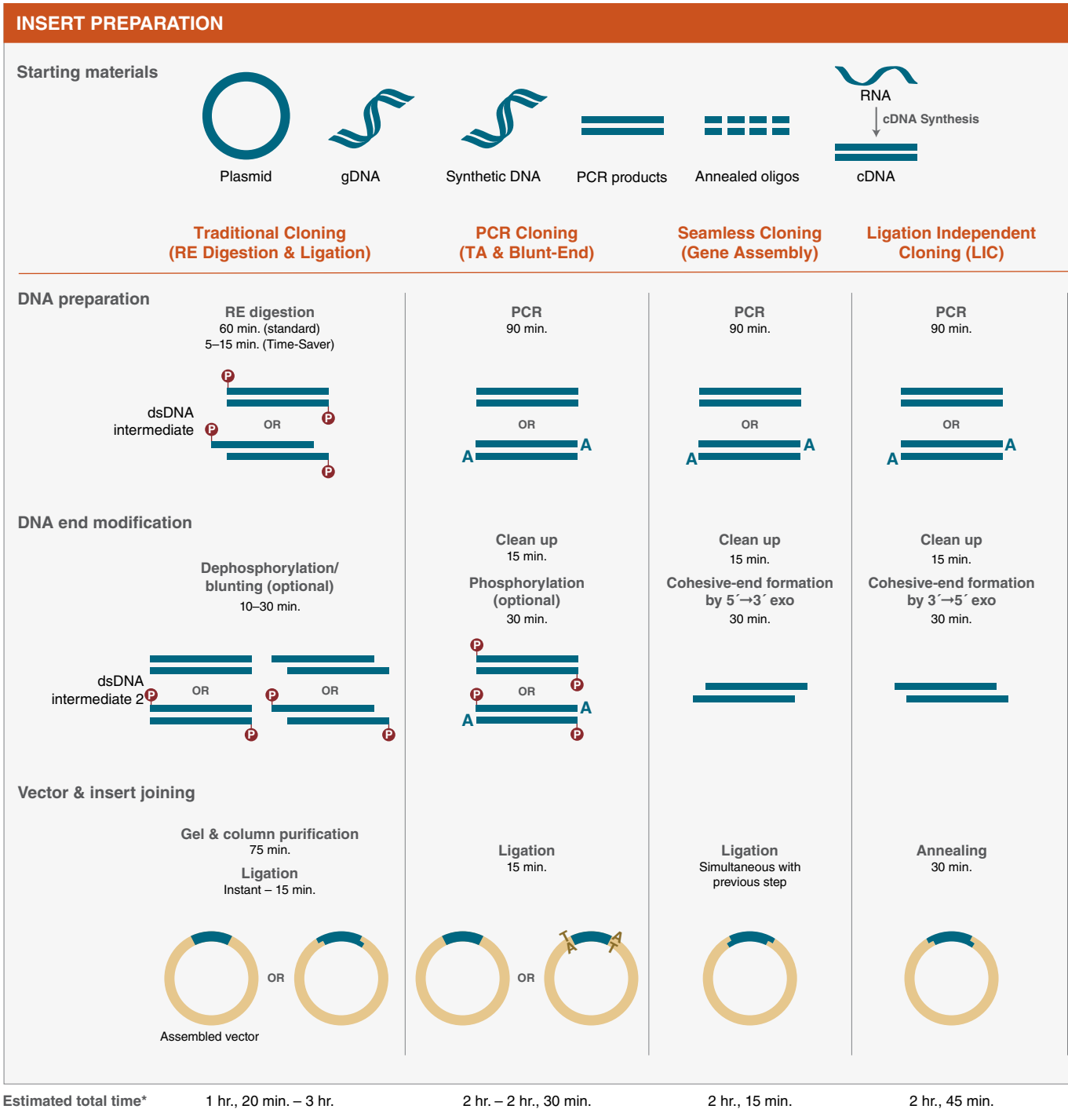
RI Recombinant Enzyme

Cloning Workflow Comparison

The figure below compares the steps for the various cloning methodologies, along with the time needed for each step in the workflows.



* Note that times are based on estimates for moving a gene from one plasmid to another. If the source for gene transfer is gDNA, add 2 hours to calculation for the traditional cloning method. Total time does not include transformation, isolation or analysis.

** 70 minutes for recombination occurs on second day

rCutSmart or r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · rAlbumin Recombinant Albumin · SAM Requires SAM · 37° Incubation Temperature · 65° Heat Inactivation

Recombinational (Gateway/Creator/Univector)

PCR
90 min.

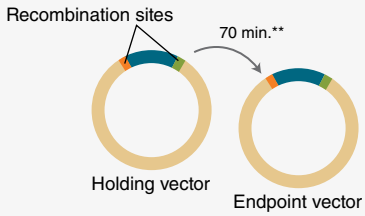
Clean up
15 min.

RE digestion
60 min. (standard)
5–15 min. (Time-Saver)

Clean up
15 min.

Site-specific recombination
60 min.

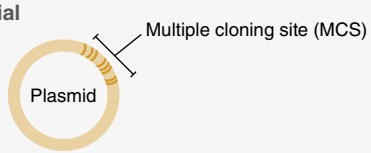
Proteinase K treatment
10 min.



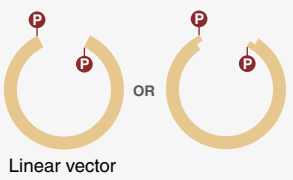
3 hr., 15 min. – 5 hr., 20 min.

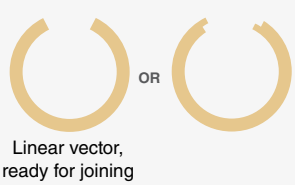
VECTOR PREPARATION

Starting material



	Restriction Enzyme (RE) Digestion	PCR
DNA preparation	RE digestion 60 min. (standard) 5–15 min. (Time-Saver)	PCR 2 hr. Clean up 15 min.
DNA end modification	Dephosphorylation (optional) 10–30 min. Clean up 15 min. OR Gel & column purification 75 min.	T-addition (optional) 1.5 hr.
Estimated total time	20 min. – 2 hr., 25 min.	2 hr., 15 min – 3 hr., 45 min.





NEBcloner®
For help with choosing the right product for each step in the cloning workflow, visit **NEBcloner.neb.com**

NEBuilder® HiFi DNA Assembly Master Mix & Cloning Kit

NEBuilder HiFi DNA Assembly Master Mix
#E2621S 10 reactions
#E2621L 50 reactions
#E2621X 250 reactions

NEBuilder HiFi DNA Assembly Cloning Kit
#E5520S 10 reactions

NEBuilder HiFi DNA Assembly Bundle for Large Fragments
#E2623S 20 reactions

- *Simple and fast seamless cloning in as little as 15 minutes*
- *Use one system for both "standard-size" cloning and larger gene assembly products (up to 11 fragments and 20+ kb)*
- *Clone into any vector with no additional sequence added (scarless)*
- *DNA can be used immediately for transformation or as template for PCR or RCA*
- *Adapts easily for multiple DNA manipulations, including site-directed mutagenesis*
- *Enjoy less screening/re-sequencing of constructs, with virtually error-free, high-fidelity assembly*
- *Use NEBuilder HiFi in successive rounds of assembly, as it removes 5´- and 3´-end mismatches*
- *Bridge two ds-fragments with a synthetic ssDNA oligo for simple and fast construction (e.g., linker insertion or gRNA library)*
- *No PCR cleanup step required*
- *No licensing fee requirements from NEB for NEBuilder products*

To learn how simple NEBuilder HiFi is, visit **NEBuilderHiFi.com**

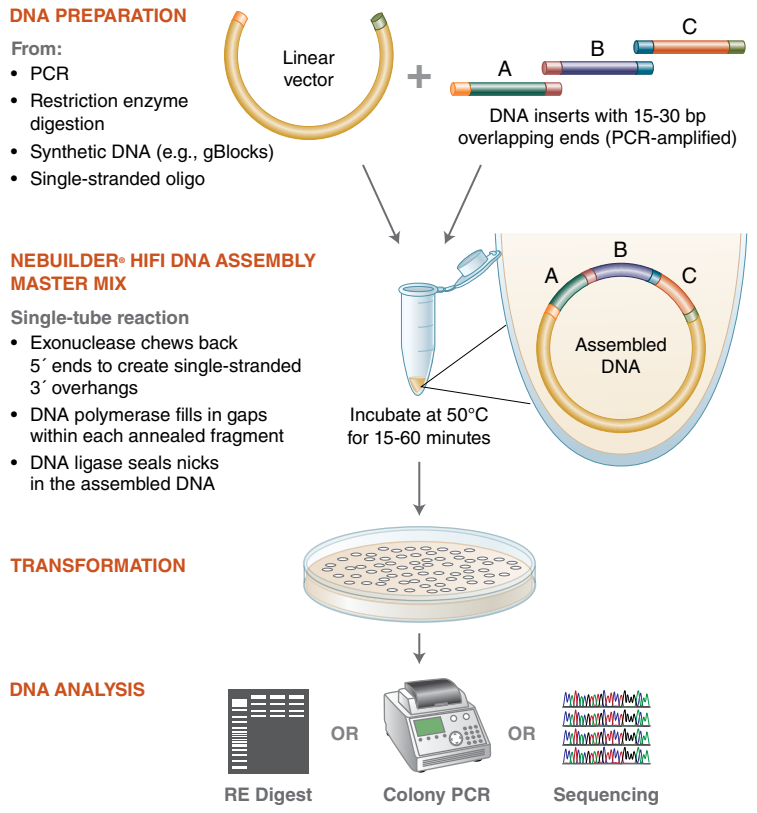
Description: NEBuilder HiFi DNA Assembly Master Mix was developed to improve the efficiency and accuracy of DNA assembly. This method allows for seamless assembly of multiple DNA fragments, regardless of fragment length or end compatibility. This method has been used to assemble either single-stranded oligonucleotides or different sizes of DNA fragments with varied overlaps (15–80 bp). It has utility for the synthetic biology community, as well as those interested in one-step cloning of multiple fragments due to its ease of use, flexibility and simple master-mix format. The reaction features different enzymes that perform in the same buffer. The end result is a double-stranded, fully sealed DNA molecule that can serve as template for PCR, RCA or a variety of other molecular biology applications, including direct transformation of *E. coli*.

NEBuilder HiFi Kits can be purchased with NEB 5-alpha Competent *E. coli* (Cloning Kit, NEB #E5520) or as a bundle with NEB 10-beta Competent E. coli (Bundle for Large Fragments, NEB #E2623). NEB 5-alpha competent cells are excellent for routine assemblies of 15 kb or less. NEB recommends NEB 10-beta competent cells for assemblies larger than 15 kb.

The NEBuilder HiFi DNA Assembly Master Mix Includes:
- NEBuilder HiFi DNA Assembly Master Mix
- NEBuilder Positive Control

The NEBuilder HiFi DNA Assembly Cloning Kit Includes:
- NEBuilder HiFi DNA Assembly Master Mix
- NEBuilder Positive Control
- NEB 5-alpha Competent *E. coli* (High Efficiency)
- SOC Outgrowth Medium
- pUC19 Control DNA

The NEBuilder HiFi DNA Assembly Bundle for Large Fragments Includes:
- NEBuilder HiFi DNA Assembly Master Mix
- NEBuilder Positive Control
- NEB 10-beta Competent *E. coli* (High Efficiency)
- NEB 10-beta/Stable Outgrowth Medium
- pUC19 Control DNA



Overview of the NEBuilder HiFi DNA Assembly Cloning Method.

NEBuilder® Assembly Tool

Speed up your experimental design with our primer design tool at **NEBuilder.neb.com**

How does NEBuilder HiFi DNA Assembly work?

Gibson Assembly® Master Mix & Cloning Kit

Gibson Assembly Master Mix
#E2611S 10 reactions
#E2611L 50 reactions

Gibson Assembly Cloning Kit
#E5510S 10 reactions

- *High efficiency assembly, particularly for longer or greater numbers of fragments*
- *Flexible sequence design with no need to engineer cloning sites*
- *Assemble multiple DNA fragments and transform in just under 2 hours*
- *Clone into any vector with no additional sequence added (scarless)*
- *No PCR clean-up step required*

Description: Gibson Assembly Master Mix was developed by Dr. Daniel Gibson and his colleagues at the J. Craig Venter Institute and licensed to NEB by Synthetic Genomics, Inc. It allows for successful assembly of multiple DNA fragments, regardless of fragment length or end compatibility.

Gibson Assembly efficiently joins multiple overlapping DNA fragments in a single-tube isothermal reaction. The Gibson Assembly Master Mix includes three different enzymatic activities that perform in a single buffer:

- The exonuclease creates a single-stranded 3´ overhang that facilitates the annealing of fragments that share complementarity at one end
- The polymerase fills in gaps within each annealed fragment
- The DNA ligase seals nicks in the assembled DNA

The Gibson Assembly Cloning Kit has been optimized for the assembly and cloning of up to 6 fragments.

The Gibson Assembly Master Mix Includes:

- Gibson Assembly Master Mix
- NEBuilder Positive Control

The Gibson Assembly Cloning Kit Includes:

- Gibson Assembly Master Mix
- NEBuilder Positive Control
- NEB 5-alpha Competent *E. coli* (High Efficiency)
- SOC Outgrowth Medium
- pUC19 Control DNA

View our online tutorials at **NEBGibson.com**

Synthetic Biology/DNA Assembly Selection Chart

	NEBuilder HiFi DNA Assembly NEB #E2621 NEB #E5520 NEB #E2623	**NEB Gibson Assembly** NEB #E2611 NEB #E5510	**NEBridge Golden Gate Assembly Kits (BsaI-HFv2/BsmBI-v2)** NEB #E1601 NEB #E1602 **NEBridge Ligase Master Mix** NEB #M1100	**USER Enzyme** NEB #M5505 **Thermolabile USER II Enzyme** NEB #M5508
Properties				
Removes 5´ or 3´ End Mismatches	★★★	★	N/A	N/A
Assembles with High Fidelity at Junctions	★★★	★★	★★★	★★★
Tolerates Repetitive Sequences at Ends	★	★	★★★	★★★
Generates Fully Ligated Product	★★★	★★★	★★★	NR
Joins dsDNA with Single-stranded Oligo	★★★	★★	NR	NR
Assembles Low Amounts of DNA with High Efficiency	★★★	★★	★★	★★
Accommodates Flexible Overlap Lengths	★★★	★★★	★	★★
Applications				
2 Fragment Assembly (Simple cloning)	★★★	★★★	★★★	★★★
3-6 Fragment Assembly (one pot)	★★★	★★★	★★★	★★★
7-11 Fragment Assembly (one pot)	★★★	★★	★★★	★★★
12-50+ Fragment Assembly (one pot) [1]	★	★	★★★	NR
Template Construction for *In vitro* Transcription	★★★	★★★	★★★	★★★
Synthetic Whole Genome Assembly	★★★	★	★★★	★
Multiple Site-directed Mutagenesis	★★★	★★	★★	★★
Library Generation	★★★	★★★	★★★	★★
Metabolic Pathway Engineering	★★★	★★	★★★	★★★
TALENs	★★	★★	★★★	★★
Short Hairpin RNA (shRNA) Cloning	★★★	★★	★	★
gRNA Library Generation	★★★	★★	★	★
Large Fragment (> 10 kb) Assembly	★★★	★★★	★★★	★★
Small Fragment (< 100 bp) Assembly	★★★	★	★★★	★★★
Use in Successive Rounds of Restriction Enzyme Assembly	★★★	★	NR	★

[1] Please visit **neb.com/GoldenGate** for more information

KEY	
★★★	Optimal, recommended product for selected application
★★	Works well for selected application
★	Will perform selected application, but is not recommended
N/A	Not applicable to this application
NR	Not recommended

NEBridge® Golden Gate Assembly Kits

NEBridge Golden Gate Assembly Kit (BsaI-HF v2)
#E1601S 20 reactions
#E1601L 100 reactions

NEBridge Golden Gate Assembly Kit (BsmBI-v2)
#E1602S 20 reactions
#E1602L 100 reactions

NEW
NEBridge Ligase Master Mix
#M1100S 50 reactions

Companion Products:

NEB 10-beta Competent *E. coli* (High Efficiency)
#C3019H 20 x 0.05 ml
#C3019I 6 x 0.2 ml
#C3019P 1 96-well plate

NEB 5-alpha Competent *E. coli* (High Efficiency)
#C2987H 20 x 0.05 ml
#C2987I 6 x 0.2 ml
#C2987P 1 96-well plate
#C2987R 384-well plate
#C2987U 96 x 0.05 ml

NEB Cloning Competent *E. coli* Sampler
#C1010S 8 x 0.05 ml

- *Seamless cloning – no scar remains following assembly*
- *Ordered assembly of multiple fragments (2-50+) in a single reaction**
- *Can also be used for cloning of single inserts and library preparations*
- *Efficient with regions of high GC content and areas of repeats*
- *Compatible with a broad range of fragment sizes (< 100 bps to > 15 kb)*

* NEB has tested 50+ fragments with NEB #E1601/1602 and 25+ fragments with NEB #M1100.

NEBridge Golden Gate Assembly Tool
Speed up your experimental design with our assembly tool at **GoldenGate.neb.com**

For help designing high-fidelity Golden Gate Assemblies, try **NEBridge Ligase Fidelity Tools** at **LigaseFidelity.neb.com.**

- **Ligase Fidelity Viewer™ (v2)** – visualize overhang ligation junctions
- **GetSet™** – predict high-fidelity junction sets
- **SplitSet™** – split DNA sequence for scarless high-fidelity assembly.

How does Golden Gate Assembly work?

Description: The NEBridge Golden Gate Assembly Kits (BsaI-HFv2 and BsmBI-v2) contain an optimized mix of Type IIS restriction enzyme and T4 DNA Ligase. Together these enzymes can direct the assembly of multiple inserts using the Golden Gate method. The kits include pGGAselect destination plasmid, which provides a backbone for the assembly, features convenient restriction enzyme sites for subcloning, and has T7/SP6 promoter sequences to enable *in vitro* transcription.

NEBridge Ligase Master Mix performs high efficiency and high-fidelity Golden Gate Assemblies with a broad assortment of Type IIS restriction enzymes which can be ordered separately.

Golden Gate Assembly is a method for efficient and seamless assembly of DNA fragments using Type IIS restriction enzymes and T4 DNA Ligase. Type IIS restriction enzymes bind to their recognition sites but cut the DNA downstream from that site at a positional, not sequence-specific, cut site. Thus, a single Type IIS restriction enzyme can generate DNA fragments with unique overhangs (see Figure below). Ordered assembly of digested fragments proceeds through annealing of complementary overhangs on adjacent fragments. The final assembly product no longer contain Type IIS restriction enzyme recognition sites, so no further digestion is possible, allowing the assembly product to accumulate over time.

While particularly useful for multi-fragment assemblies, the Golden Gate method can also be used for cloning single inserts and inserts from diverse populations to create libraries. Golden Gate is also useful for assembling repetitive elements (e.g., gene circuits and CRISPR guide arrays).

Advances in Ligase Fidelity: Research at NEB has led to increased understanding of ligase fidelity, including the development of a comprehensive method for profiling end-joining ligation fidelity in order to predict which overhangs have improved fidelity. This research allows careful choice of overhang sets, which is especially important for achieving complex Golden Gate Assemblies. To learn more, visit www.neb.com/goldengate.

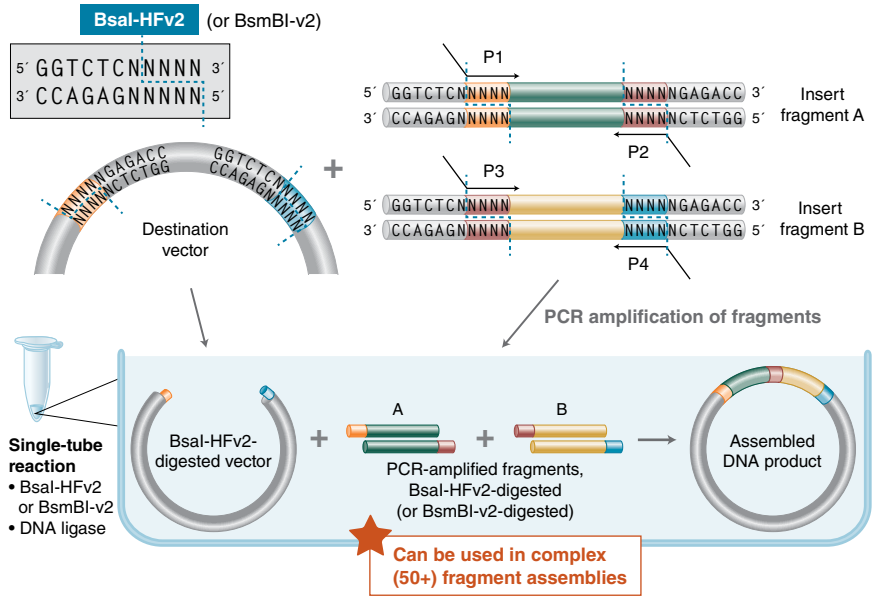
NEBridge Golden Gate Assembly Kit (BsaI-HFv2) Includes:
- NEBridge Golden Gate Assembly Mix
- T4 DNA Ligase Reaction Buffer (10X)
- pGGAselect Destination Plasmid

NEBridge Golden Gate Assembly Kit (BsmBI-v2) Includes:
- NEBridge Golden Gate Assembly Mix
- T4 DNA Ligase Reaction Buffer (10X)
- pGGAselect Destination Plasmid

NEBridge Ligase Master Mix Includes:
- NEBridge Ligase Master Mix (3X)
- Use with your choice of NEB Type IIS restriction enzyme

* Note: Assemblies up to 24 fragments have been routinely achieved with both precloned and amplicon insert test systems. Assemblies of 35+ fragments have only used amplicon inserts to date.

Type IIS Enzymes used in Golden Gate:
- BbsI (NEB #R0539)
- BbsI-HF (NEB #R3539)
- BsaI-HFv2 (NEB #R3733)
- BsmBI-v2 (NEB #R0739)
- BspQI (NEB #R0712)
- BtgZI (NEB #R0703)
- Esp3I (NEB #R0734)
- PaqCI (NEB #R0745)
- SapI (NEB #R0569)



In its simplest form, Golden Gate Assembly requires a Type IIS recognition site, in this case, BsaI-HFv2 (GGTCTC), or BsmBI-v2 (CGTCTC) added to both ends of a dsDNA fragment. *After digestion, these sites are left behind, with each fragment bearing the designed 4-base overhangs that direct the assembly.*

NEB® PCR Cloning Kit (with or without competent cells)

NEB PCR Cloning Kit
#E1202S 20 reactions

NEB PCR Cloning Kit (Without Competent Cells)
#E1203S 20 reactions

- *In vitro transcription with both SP6 & T7 promoters*
- *Easy cloning of all PCR products, including blunt and TA ends*
- *Fast cloning with 5-minute ligation step*
- *Simplified screening with low/no colony background and no blue/white selection*
- *Save time by eliminating purification steps*
- *More flanking restriction sites available for easy subcloning, including choice of two single digest options*
- *BsaI site removed to allow cloning of Golden Gate modules*

Description: The NEB PCR Cloning Kit contains optimized Cloning Master Mixes with a proprietary ligation enhancer and a linearized vector that uses a novel mechanism for background colony suppression to give a low background. It allows simple and quick cloning of any PCR amplicon, whether the amplification reactions are performed with proofreading DNA polymerases, such as Q5® or Phusion® which produce blunt ends; or nonproofreading DNA polymerases, such as *Taq* or *Taq* mixes (One*Taq*, LongAmp *Taq*) which produce single-base overhangs. This is possible due to "invisible" end polishing components in the master mix that are active during the ligation step. The kit also allows direct cloning from amplification reactions without purification, and works well whether or not the primers used in the PCR possess 5´-phosphate groups.

- Provided analysis primers allow for downstream colony PCR screening or sequencing
- Ready-to-use kit components include 1 kb control amplicon, linearized cloning vector and single-use competent *E. coli* (NEB #E1202 only)
- Longer shelf life (12 months), as compared to some commercially available products

Kit Includes:

- Linearized pMiniT™ 2.0 Vector
- Cloning Mix 1
- Cloning Mix 2
- Amplicon Cloning Control (1 kb)
- Cloning Analysis Forward Primer
- Cloning Analysis Reverse Primer
- NEB 10-beta Competent *E. coli* (Cloning Efficiency) (NEB #E1202 only)
- NEB 10-beta/Stable Outgrowth Medium (NEB #E1202 only)
- pUC19 Control DNA

Phusion® is a registered trademark of Thermo Fisher Scientific

Quick Blunting™ Kit

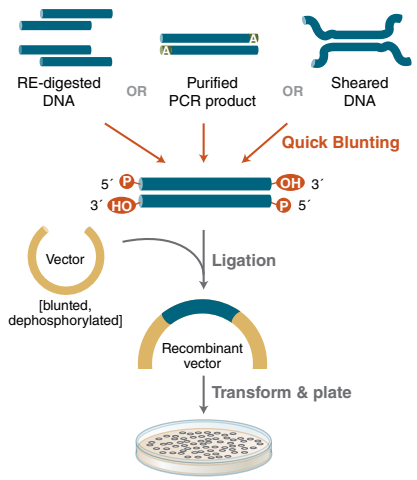
#E1201S 20 reactions
#E1201L 100 reactions

Companion Product:

Quick Blunting and Quick Ligation Kits
#E0542S 20 reactions

- *Restriction enzyme-digested DNA is blunted in less than 30 minutes*
- *Reactions are performed at room temperature in a ready-to-use mix*
- *Suitable for restriction enzyme-digested DNA, sheared or nebulized DNA or PCR product*

Description: The Quick Blunting Kit is used to convert DNA with incompatible 5´or 3´overhangs to 5´phosphorylated, blunt-ended DNA for efficient blunt-end ligation into DNA cloning vectors. DNA is blunted using T4 DNA Polymerase (NEB #M0203) which has both 3´→ 5´ exonuclease activity and 5´→ 3´ polymerase activity. T4 Polynucleotide Kinase (NEB #M0201) is included in the enzyme mix for phosphorylation of the 5´ends of blunt-ended DNA for subsequent ligation into a cloning vector. This kit is optimized for blunting up to 5 µg of DNA in a single reaction.



Applications:

- Prepare sheared, nebulized or restriction enzyme digested DNA for blunt-ended ligation into a plasmid, cosmid, fosmid or BAC vector
- Prepare PCR products for efficient blunt-end cloning

Kit Includes:

- Blunting Enzyme Mix
- 10X Blunting Buffer
- Deoxynucleotide Solution Mix (1 mM)

Note: PCR generated DNA must be purified before blunting by using a commercial purification kit, such as Monarch® PCR & DNA Cleanup Kit (NEB #T1030), phenol extraction/ethanol precipitation, or gel electrophoresis. Restriction enzyme digested DNA can be blunted directly without purification.

How does the NEB PCR Cloning Kit work?

Q5® Site-Directed Mutagenesis Kit (with or without competent cells) RR

Q5 Site-Directed Mutagenesis Kit
#E0554S 10 reactions

Q5 Site-Directed Mutagenesis Kit
(Without Competent Cells)
#E0552S 10 reactions

KLD Enzyme Mix
#M0554S 25 reactions

- *Robust exponential amplification generates high yields of desired mutations from a wide range of templates.*
- *Low error rate of Q5 High-Fidelity DNA Polymerase reduces screening time.*
- *Room temperature reaction setup*
- *Use of standard primers eliminates need for phosphorylated or purified oligos*
- *Easy-to-use master mix format*

NEBaseChanger®

For help with primer design, visit
NEBaseChanger.neb.com

Description: The Q5 Site-Directed Mutagenesis Kit allows rapid site-specific mutagenesis of double-stranded plasmid DNA in less than 2 hours. The kit utilizes the robust Q5 Hot Start High-Fidelity DNA Polymerase along with custom mutagenic primers to create substitutions, deletions and insertions in a wide variety of plasmids. Transformation into high-efficiency NEB 5-alpha Competent *E. coli*, provided with (NEB #E0554), ensures robust results with plasmids up to 14 kb in length.

Applications:

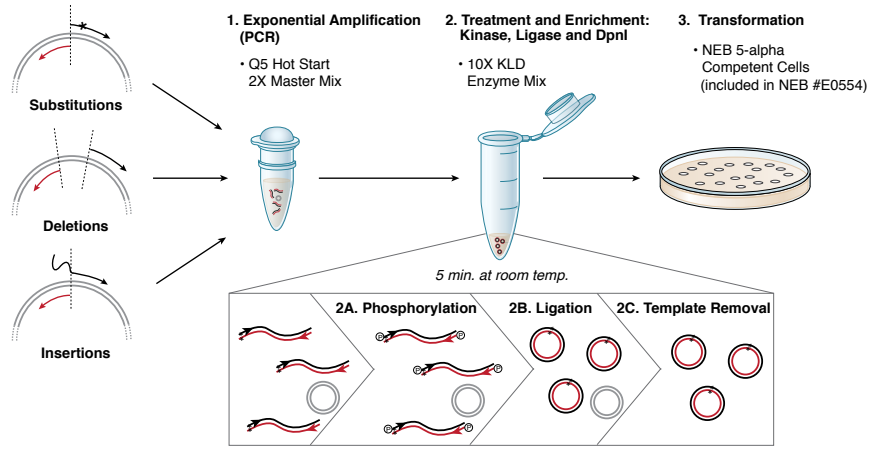
- Generation of mutations, insertions or deletions in plasmid DNA

Q5 Site-Directed Mutagenesis Kit Includes:

- Q5 Hot Start High-Fidelity Master Mix (2X)
- KLD Enzyme Mix (10X)
- Control SDM Plasmid
- Control SDM Primer Mix
- pUC19 Vector (NEB #E0554 only)
- SOC Outgrowth Medium (NEB #E0554 only)
- NEB 5-alpha Competent *E. coli* (High Efficiency) (NEB #E0554 only)

KLD Enzyme Mix Includes:

- KLD Enzyme Mix (10X)
- KLD Reaction Buffer (2X)



Q5 Site-Directed Mutagenesis Kit Overview. *The first step is an exponential amplification using Q5 Hot Start High-Fidelity DNA Polymerase. The second step is a unique enzyme mix containing a kinase, ligase and DpnI. Together, these enzymes allow for rapid circularization of the PCR product and removal of the template DNA. The last step is a high-efficiency transformation into chemically competent cells.*

Shelley started her career at NEB in 1993 as a summer student. She currently works as a Quality Control Analyst II in the Quality Control Laboratory. Shelley is known for her infectious smile and great attitude, and is a member of the Engage in Giving group at NEB.

Learn more about the benefits of the Q5 SDM Kit.

rCutSmart or r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · rAlbumin Recombinant Albumin · SAM Requires SAM · 37° Incubation Temperature · 65° Heat Inactivation

DNA Ligase Selection Chart

NEB offers a variety of ligases for DNA research. Many of these enzymes are recombinant, and all offer the quality and value you have come to expect from our products. While more than one ligase may work for your application, the following selection chart presents our recommendations for optimal performance.

Visit **NEBStickTogether.com** for more information on DNA Ligases.

	Blunt/ TA Ligase Master Mix	Instant Sticky-end Master Mix	Electro-Ligase	T4 DNA Ligase	Hi-T4 DNA Ligase	Immo-bilized T4 DNA Ligase	Salt-T4® DNA Ligase	Quick Ligation Kit	NEBridge Ligase Master Mix	T3 DNA Ligase	T7 DNA Ligase	HiFi *Taq* DNA Ligase	*Taq* DNA Ligase	9°N™ DNA Ligase	NEBNext Quick Ligation Module	SplintR Ligase	*E. coli* DNA Ligase
DNA Applications																	
Ligation of sticky ends	★★	★★★	★★	★★	★★	★★★	★★	★★★	★	★★	★★	★	★	★			★
Ligation of blunt ends	★★★	★	★★	★★	★★	★	★★	★★★	★	★★							
T/A cloning	★★★	★	★★	★★	★★		★★	★★		★	★						
Electroporation			★★★	★★	★★												
Golden Gate Assembly				★★★	a				★★★		★						
Ligation of sticky ends only											★★★						
Repair of nicks in dsDNA	★★	★★	★★	★★★	★★★		★★★	★★		★★	★★	★★	★★	★★		★★	★★
High-complexity library cloning	★★	★★	★★	★★★				★★									
Adapter Ligation	★★★	★★	★★	★		▲		★★		★					▲		
Ligation-Dependent DNA Sequence & SNP Detection (LCR, LDR & related methods)												★★★	★★	★★			
Ligation-Dependent RNA Sequence & SNP Detection				★												★★★	
Ligation of adjacent ssDNAs on an RNA splint																★★★	
NGS Applications																	
NGS Library Prep dsDNA-dsDNA (ligation)	▲			▲						▲					▲		
Features																	
Salt tolerance (>2X that for T4 DNA Ligase)							●			●							
Ligation in 15 min. or less	●	●		●	●		●	●		●	●	●	●	●	●	●	
Master Mix Formulation	●	●							●						●		
Thermostable												●	●	●			
Thermotolerant					●							●	●	●			
Recombinant	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Reusable/ Removable						●											

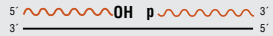
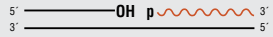
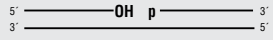
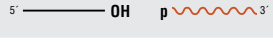
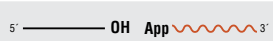
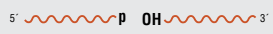
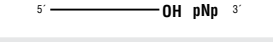
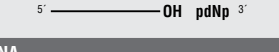
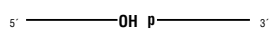
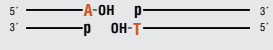
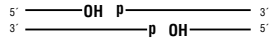
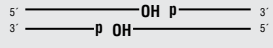
KEY	
★★★	Optimal, recommended ligase for selected application
★★	Works well for selected application
★	Will perform selected application, but is not recommended
▲	Please consult the specific NGS protocol to determine the optimal enzyme for your needs
a	Not yet tested

Find an overview of ligation.

Substrate-based Ligase Selection Chart

This chart provides our recommendation for a choice of ligase to use in a reaction, based upon the type of substrate present. DNA, RNA and hybrid substrates are represented and require specific enzymes to achieve the highest-efficiency ligation.

DNA
RNA

	Recommended Ligase	Comments
Nicked DNA/RNA		
	T4 RNA Ligase 2	
	T4 RNA Ligase 2	
	T4 RNA Ligase 2	
	T4 DNA Ligase Immobilized T4 DNA Ligase	
	N/A	No ligase optimized for this activity
	T3 DNA Ligase	
	SplintR Ligase	100-1,000-fold higher efficiency than T4 DNA Ligase
	T4 DNA Ligase Immobilized T4 DNA Ligase	For high temperatures, we recommend *Taq* DNA Ligase. For highest fidelity, we recommend HiFi *Taq* DNA Ligase.
ssDNA/RNA		
	N/A	See CircLigase™
	N/A	No ligase optimized for this activity
	T4 RNA Ligase 1	Supplement with ATP
	T4 RNA Ligase 1	
	T4 RNA Ligase 2 Truncated KQ	
	T4 RNA Ligase 2 Truncated KQ	
	Thermostable 5´ App DNA/RNA Ligase	We recommend a Proteinase K cleanup
	Thermostable 5´ App DNA/RNA Ligase	We recommend a Proteinase K cleanup
	RtcB Ligase	Supplement with GTP and Mn^{2+}
	RtcB Ligase	Supplement with GTP and Mn^{2+}
	T4 RNA Ligase 1	
	T4 RNA Ligase 1	Reported to work, but ligates inefficiently. Consider pdCp.
	T4 RNA Ligase 1	
	T4 RNA Ligase 1	
dsDNA		
	Blunt T/A Ligase Master Mix	
	Blunt T/A Ligase Master Mix	
	Quick Ligation Kit or Instant Sticky-end Ligase Master Mix	For ligating ends under high salt conditions, we recommend Salt-T4 DNA Ligase. For ligation at temperatures up to 50°C, we recommend Hi-T4 DNA Ligase. For ligation of cohesive ends ONLY, we recommend T7 DNA Ligase.
	Quick Ligation Kit or Instant Sticky-end Ligase Master Mix	For ligating ends under high salt conditions, we recommend Salt-T4 DNA Ligase. For ligation at temperatures up to 50°C, we recommend Hi-T4 DNA Ligase. For ligation of cohesive ends ONLY, we recommend T7 DNA Ligase.

CIRCLIGASE™ is a trademark of EpiCentre Technologies Corp.

rCutSmart or r1.1 Recommended Buffer · Cloned at NEB · RR Recombinant Enzyme · rAlbumin Recombinant Albumin · SAM Requires SAM · 37° Incubation Temperature · 65° Heat Inactivation

T4 DNA Ligase Products

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5´ phosphate and 3´ hydroxyl termini in duplex DNA or RNA. This enzyme will join blunt-end and cohesive-end termini, as well as repair single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids. T4 DNA Ligase is available in a variety of formulations and variants. The table below lists products available from NEB.

Product	NEB #	Features	Reaction Conditions	Size
T4 DNA Ligase	Regular concentration: M0202S M0202L High (5X) concentration: M0202T M0202M	• Standalone enzyme ideal for a variety of ligation reactions • Ligation can be performed in supplied buffer, or in any of the four restriction endonuclease NEBuffers, or in T4 Polynucleotide Kinase Buffer if supplemented with 1 mM ATP	1X T4 DNA Ligase Reaction Buffer. Incubate at 16°C. Heat inactivate at 65°C for 10 minutes.	Regular concentration: S: 20,000 units L: 100,000 units High (5X) concentration: T: 20,000 units M: 100,000 units
Master Mixes				
Instant Sticky-end Ligase Master Mix	M0370S M0370L	• Ready-to-use 2X solution of T4 DNA Ligase and a proprietary ligation enhancer in an optimized reaction buffer • Specifically formulated to rapidly ligate cohesive-end (2-4 bp) substrates and improve transformation	1X Instant Sticky-end Ligase Master Mix with DNA substrates in a 10 µl reaction volume	50 reactions 250 reactions
Blunt/TA Ligase Master Mix	M0367S M0367L	• Ready-to-use 2X solution of T4 DNA Ligase and a proprietary ligation enhancer in an optimized reaction buffer • Specifically formulated to improve ligation and transformation of both-blunt-end and single-base overhang substrates	1X Blunt/TA Ligase Master Mix with DNA substrates in a 10 µl reaction volume	50 reactions 250 reactions
NEW **NEBridge Ligase Master Mix**	M1100S	• Ideal for high-efficiency and high-fidelity Golden Gate Assembly with a broad assortment of Type IIS restriction enzymes • Available in 3X master mix format	1X NEBridge Ligase Master Mix, Type IIS restriction enzyme and DNA fragments in a 15 or 30 µl reaction volume	50 reactions
Formulations				
Quick Ligation Kit	M2200S M2200L	• Ligation of cohesive- or blunt-end DNA fragments in 5 minutes at room temperature (25°C)	1X Quick Ligation Reaction Buffer. Incubate at room temperature (25°C).	30 reactions 150 reactions
ElectroLigase	M0369S	• Combines T4 DNA Ligase and an optimized, ready-to-use 2X reaction buffer containing a proprietary ligation enhancer and no PEG • Promotes robust ligation of all types of DNA ends (blunt, sticky, TA) and is suitable for electroporation, without desalting or purification	1X ElectroLigase Reaction Buffer with DNA substrates and 1 µl ElectroLigase in an 11 µl reaction volume incubated at 25°C	50 reactions
Immobilized T4 DNA Ligase	M0569S	• Enzyme is covalently linked to a magnetic bead, and can be removed from a reaction and reused • Enables ligated product to be used directly with no heat inactivation step	1X T4 DNA Ligase Reaction Buffer. Incubate at 25°C. Place on magnet for 3 minutes to remove.	1 mg
Variants				
Hi-T4™ DNA Ligase	M2622S M2622L	• Enables ligation with improved thermostability • Active in temperatures up to 50°C	1X T4 DNA Ligase Reaction Buffer. Incubate at 25°C. Heat inactivate at 65°C for 10 minutes.	20,000 units 100,000 units
Salt-T4® DNA Ligase	M0467S M0467L	• Enables ligation with improved salt tolerance • Active in reactions as high as 300 mM salt with no loss in activity	1X T4 DNA Ligase Reaction Buffer. Incubate at 25°C. Heat inactivate at 65°C for 10 minutes.	20,000 units 100,000 units

T3 DNA Ligase

NEBU RR 25° No

#M0317S 100,000 units
#M0317L 750,000 units

- *Ligation of sticky or blunt ends*
- *Increased salt tolerance*
- *Repair of nicks in dsDNA*

Description: T3 DNA Ligase is an ATP-dependent ds-DNA ligase from bacteriophage T3. Cohesive ends, blunt ends, and nick sealing can all be efficiently catalyzed by T3 DNA Ligase. Blunt end ligation is enhanced by the addition of PEG 6000 to the reaction. T3 DNA Ligase exhibits a higher tolerance (2-fold) for NaCl in the reaction compared to T4 DNA Ligase, making the enzyme a versatile choice for *in vitro* molecular biology protocols requiring DNA ligase activity.

Reaction Conditions: StickTogether DNA Ligase Buffer, 25°C.

Unit Definition: One unit is defined as the amount of enzyme required to give 50% ligation of 100 ng HindIII fragments of λ DNA in a total reaction volume of 20 µl in 1 minute at 25°C in 1X StickTogether DNA Ligase Buffer.

Concentration: 3,000,000 units/ml

Note: ATP is an essential cofactor for the reaction. This contrasts with *E. coli* DNA Ligase which requires NAD. T3 DNA Ligase is also active in buffers without PEG 6000, such as our T4 DNA Ligase Buffer and NEBuffers, for applications in which PEG 6000 is detrimental. Please remember to supplement the reaction with 1 mM ATP (final concentration). In these buffers T3 DNA Ligase exhibits an approximately 10-fold reduction in activity. In applications where a high concentration of NaCl needs to be maintained, we suggest using a reaction buffer without PEG 6000.

T7 DNA Ligase

NEBU RR 25° No

#M0318S 100,000 units
#M0318L 750,000 units

- *Ligation of sticky ends only*
- *Repair of nicks in dsDNA*

Description: T7 DNA Ligase is an ATP-dependent ds-DNA ligase from bacteriophage T7. It will catalyze the formation of a phosphodiester bond between adjacent 5´ phosphate and 3´ hydroxyl groups of duplex DNA. Cohesive end ligation and nick sealing can be efficiently catalyzed by T7 DNA Ligase. However, unlike T4 and T3 DNA Ligases, blunt end ligation is not efficiently catalyzed by T7 DNA Ligase, making it a good choice for applications in which blunt and sticky ends of DNA are present but only the sticky ends are to be joined.

Reaction Conditions: StickTogether DNA Ligase Buffer, 25°C.

Unit Definition: One unit is defined as the amount of enzyme required to give 50% ligation of 100 ng HindIII fragments of λ DNA in a total reaction volume of 20 µl in 30 minutes at 25°C in 1X StickTogether DNA Ligase Buffer.

Concentration: 3,000,000 units/ml

Note: ATP is an essential cofactor for the reaction. T7 DNA Ligase is also active in buffers without PEG 6000, such as our T4 DNA Ligase Buffer and NEBuffer r1.1–r4.1, for applications in which PEG 6000 is detrimental. Please remember to supplement the reaction with 1 mM ATP (final concentration). Using these buffers, the activity of T7 DNA Ligase is reduced approximately 10-fold.

E. coli DNA Ligase

NEBU RR 16° 65°

#M0205S 200 units
#M0205L 1,000 units

- *Selective ligation of nicks in dsDNA without significant joining of dsDNA fragments regardless of end type*
- *cDNA synthesis*

Description: *E. coli* DNA Ligase catalyzes the formation of a phosphodiester bond between the 5´-phosphate and 3´-hydroxyl of two adjacent DNA strands in duplex DNA with cohesive ends. It is not appreciably active on blunt-ended substrates. *E. coli* DNA Ligase uses NAD as a cofactor and can be heat-inactivated. *E. coli* DNA Ligase is active at a range of temperatures (4–37°C).

Reaction Conditions: *E. coli* DNA Ligase Reaction Buffer, 16°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to give 50% ligation of HindIII fragments of λ DNA (5' DNA termini concentration of 0.12 µM, 300 µg/ml) in a total reaction volume of 20 µl in 30 minutes at 16°C in 1X *E. coli* DNA Ligase Reaction Buffer.

Concentration: 10,000 units/ml

Note: Requires NAD^+ (nicotinamide adenine dinucleotide) as a cofactor, in contrast to other ligases which use rATP. Ligation of blunt-ended fragments is extremely inefficient. For ligation of blunt-ended fragments we recommend Blunt/TA Ligase Master Mix (NEB #M0367).

HiFi *Taq* DNA Ligase

NEBU RR No

#M0647S 50 reactions

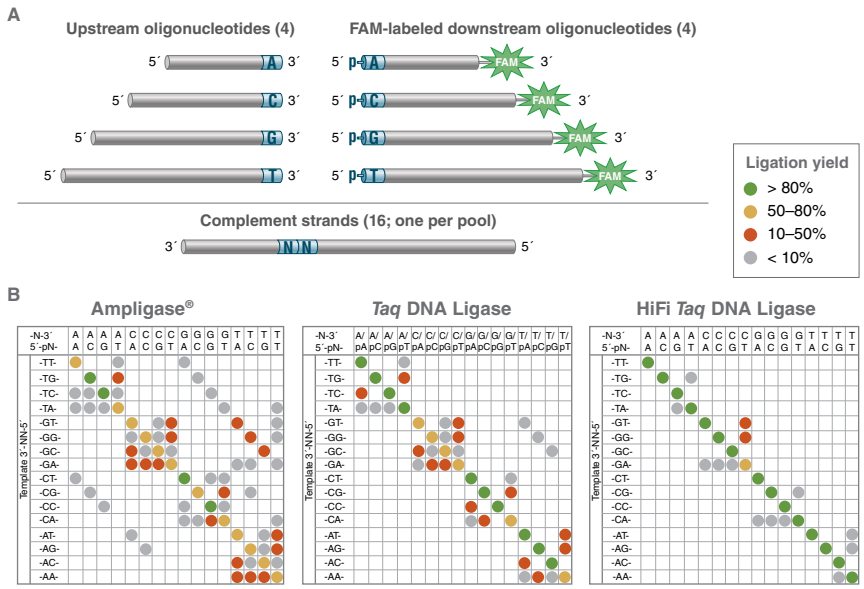
- *High fidelity, thermostable*
- *Repair of nicks in dsDNA*
- *Allele-specific gene detection using ligase-dependent methods, including the Ligase Chain Reaction (LCR) and Ligase Detection Reaction (LDR)*
- *Ligation of padlock probes*

Thermostable Ligase
Reaction Temperature Calculator

For help with calculating ligation temperature, visit **LigaseCalc.neb.com**

Description: An optimized blend of a thermostable DNA Ligase and a proprietary additive, HiFi *Taq* DNA Ligase efficiently seals nicks in DNA with unmatched high fidelity. The formation of a phosphodiester bond between juxtaposed 5´ phosphate and 3´ hydroxyl termini of two adjacent oligonucleotides that are hybridized to a complementary target DNA is enhanced in the improved reaction buffer and mismatch ligation is dramatically reduced. The improved formulation allows higher resolution discrimination between ligation donors and acceptors, enabling precise detection of SNPs and other allele variants. HiFi *Taq* DNA Ligase is active at elevated temperatures (37–75°C).

Reaction Conditions: HiFi *Taq* DNA Ligase Reaction Buffer.



HiFi Taq DNA Ligase displays increased fidelity. *(A) Schematic of multiplexed substrate pools. Each substrate pool contained a single splint with a defined NN at the ligation junction (e.g., AA, AC, AG…) along with all four upstream probes and all four FAM-labeled downstream probes. Each probe that encodes the base at the ligation junction is of unique length allowing for separation and analysis by capillary electrophoresis. A total of 16 substrate pools were prepared, one for each unique splint. (B) Comparison of the ligation fidelity of Ampligase (Epicentre), Taq DNA Ligase and HiFi Taq DNA Ligase. Fidelity measurements were performed using 1 ?l of ligase in a 50 ?l reaction mixture in the supplied buffers at 1X concentration. Reactions were incubated 30 min at 55°C, using multiplexed substrate pools as outlined in (A). Rows represent a single template sequence, while columns indicate a particular ligation product resulting from a specific pair of probes ligating with the indicated bases at the ligation junction. A dot indicates detection of a product (see legend above). The diagonal from the top left to the bottom right represents Watson-Crick ligation products; all other spaces indicate mismatch ligation products. While Taq DNA Ligase and Ampligase perform similarly under these conditions, with a range of mismatch products detectable, HiFi Taq DNA Ligase shows dramatically fewer mismatch products while maintaining high yields.*

Taq DNA Ligase

NEBU RR 45° No

#M0208S 2,000 units
#M0208L 10,000 units

- *Thermostable*
- *Repair of nicks in dsDNA*
- *Used in Gibson Assembly method*
- *Allele-specific gene detection using Ligase Detection Reaction and Ligase Chain Reaction*

Description: *Taq* DNA Ligase is a thermostable ligase that catalyzes the formation of a phosphodiester bond between the 5´-phosphate and 3´ -hydroxyl termini of two adjacent DNA strands. The strands to be ligated need to be hybridized and accurately paired, with no gap, to a complementary DNA strand; allowing resolution of single nucleotide variants. *Taq* DNA Ligase uses NAD as a cofactor and is active at elevated temperatures (37–75°C).

Reaction Conditions: *Taq* DNA Ligase Reaction Buffer, 45°C

Unit Definition: (Cohesive End Unit)
One unit is defined as the amount of enzyme required to give 50% ligation of the 12-base pair cohesive ends of 1 µg of BstEII-digested λ DNA in a total reaction volume of 50 µl in 15 minutes at 45°C.

Concentration: 40,000 units/ml

Note: 1X *Taq* DNA Ligase Reaction Buffer requires NAD^+ as a cofactor. NAD^+ is supplied in the 10X *Taq* DNA Ligase Reaction Buffer; the buffer should be stored at -80°C to extend the half life of the NAD^+ cofactor. *Taq* DNA ligase will not ligate short 4-base overlaps (typical of restriction enzyme digests), while it efficiently ligates 12-base pair overhangs.

9°N™ DNA Ligase

#M0238S 2,500 units

- *Repair of nicks in DNA while incubating at high temperatures*
- *Thermostable*
- *Allele-specific gene detection using Ligase Detection Reaction and Ligase Chain Reaction*

Description: 9°N DNA Ligase is a thermostable ligase that catalyzes the formation of a phosphodiester bond between the 5´-phosphate and 3´-hydroxyl of two adjacent DNA strands that are hybridized and accurately paired, with no gap, to a complementary DNA strand. 9°N DNA Ligase uses ATP as a cofactor and it is active at elevated temperatures (45–70°C).

Reaction Conditions: 9°N DNA Ligase Reaction Buffer, 45°C

Unit Definition: (Cohesive End Unit) One unit is defined as the amount of enzyme required to give 50% ligation of the 12-base pair cohesive ends of 1 µg of BstEII-digested λ DNA in a total reaction volume of 50 µl in 15 minutes at 45°C. A cohesive end unit is equivalent to the nick-closing unit (1).

Concentration: 40,000 units/ml

Note: 9°N will not ligate short 4-base overlaps (typical of restriction enzyme digests), while it efficiently ligates 12-base pair overlaps.

SplintR® Ligase

#M0375S 1,250 units
#M0375L 6,250 units

- *Ligation of adjacent, single-stranded DNA splinted by a complimentary RNA*
- *Characterization of miRNAs and mRNAs, including SNPs*

Description: SplintR Ligase, also known as PBCV-1 DNA Ligase or *Chlorella* virus DNA Ligase, efficiently catalyzes the ligation of adjacent, single-stranded DNA oligonucleotides splinted by a complementary RNA strand. This previously unreported activity may enable novel approaches for characterization of miRNAs and mRNAs, including SNPs. SplintR is ideally suited for many target enrichment workflows with applications in next-generation sequencing and molecular diagnostics. The robust activity of the enzyme and its affinity for RNA-splinted DNA substrates (apparent Km = 1 nM) enable sub-nanomolar detection of unique RNA species within a complex mixture, making SplintR ligase a superior choice for demanding RNA detection technologies.

Reaction Conditions: SplintR Ligase Reaction Buffer, 25°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme needed to ligate (to 50% completion) 2 picomoles of a tripartite FAM-labeled DNA:RNA hybrid substrate in a 20 µl reaction at 25°C in 15 minutes in 1X SplintR Ligase Reaction Buffer.

Concentration: 25,000 units/ml

Note: If dilution of enzyme for storage is needed, we recommend using Diluent A (NEB #B8001).

T4 Polynucleotide Kinase & T4 Polynucleotide Kinase (3´ phosphatase minus)

T4 Polynucleotide Kinase
#M0201S 500 units
#M0201L 2,500 units

T4 Polynucleotide Kinase
(3' phosphatase minus)
#M0236S 200 units
#M0236L 1,000 units

- *5´ phosphorylation of DNA/RNA for subsequent ligation*
- *End labeling DNA or RNA for probes and DNA sequencing*
- *Removal of 3´ phosphoryl groups with T4 Polynucleotide Kinase (NEB #M0201)*
- *T4 PNK (3´ phosphatase minus) (NEB #M0236) can be used for the 5´ phosphorylation of 3´ phosphorylated mononucleotide to generate a substrate (pNp) that can be added to the 3´ end of DNA or RNA*
- *5´ end labeling of 3´ phosphorylated oligos with T4 PNK (3´ phosphatase minus) (NEB #M0236)*

Description: T4 Polynucleotide Kinase catalyzes the transfer and exchange of P_i from the γ position of ATP to the 5´ hydroxyl terminus of polynucleotides (double- and single-stranded DNA and RNA), as well as nucleoside 3´ monophosphates. The T4 Polynucleotide Kinase (NEB #M0201) also catalyzes the removal of 3´ phosphoryl groups from 3´ phosphoryl polynucleotides, deoxynucleoside 3´ monophosphates and deoxynucleoside 3´ diphosphates. The modified version (NEB #M0236) exhibits full kinase activity with no 3´ phosphatase activity.

Reaction Conditions: 1X T4 Polynucleotide Kinase Reaction Buffer. Incubate at 37°C. Heat inactivation: 65°C for 20 minutes.

Notes: Fresh buffer is required for optimal activity (in older buffers, loss of DTT due to oxidation lowers activity).

CTP, GTP, TTP, UTP, dATP or dTTP can be substituted for ATP as a phosphate donor.

Protocols for phosphorylation (radioactive and non-radioactive) of DNA & RNA can be found at www.neb.com.

The efficiencies of blunt and recessed 5´ end phosphorylation can be improved by heating to 70°C for 5 minutes, then chilling on ice prior to kinase addition and by adding PEG-8,000 to 5% (w/v).

T4 Polynucleotide Kinase requires ATP for activity, but the supplied reaction buffer does not contain ATP to allow for high specific activity radiolabeling reactions.

Often, a kinase reaction is followed by a ligation reaction. In such cases, the T4 PNK reaction is performed in ligase buffer at 37°C for 30 minutes. The product of this reaction can be used directly in the ligation reaction without a buffer change or heat inactivation UNLESS there is a need to keep other DNA fragments dephosphorylated during ligation. When this is desirable, PNK should be heat inactivated prior to ligation.

Unit Definition: One Richardson unit is defined as the amount of enzyme catalyzing the incorporation of 1 nmol of acid-insoluble [^{32}P] in 30 minutes at 37°C.

Concentration: 10,000 units/ml

5-hydroxymethyluridine DNA Kinase

#M0659S 1,000 units

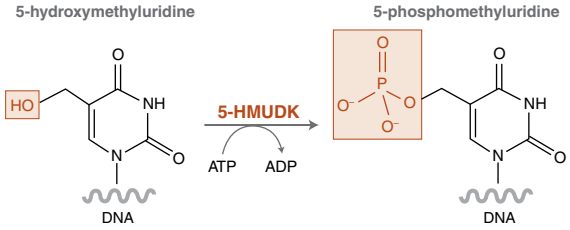
This is an **Enzyme for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. To view the full list, visit **www.neb.com/EnzymesforInnovation.**

Description: 5-hydroxymethyluridine DNA Kinase (5-HMUDK) transfers the gamma phosphate from ATP to the hydroxymethyl moiety of 5-hydroxymethyluridine in polymeric DNA.

Reaction Conditions: T4 DNA Ligase Reaction Buffer, 37°C. Heat inactivation: 80°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg of *Bacillus subtilis* bacteriophage SP8 genomic DNA in 30 minutes at 37°C in a total reaction volume of 20 µl against subsequent cleavage by NcoI-HF restriction endonuclease.

Concentration: 20,000 units/ml



Phosphatase Selection Chart

	Quick CIP* NEB #M0525	Shrimp Alkaline Phosphatase (rSAP) NEB #M0371	Antarctic Phosphatase NEB #M0289
FEATURES			
100% heat inactivation	2 minutes at 80°C	5 minutes at 65°C	2 minutes at 80°C
High specific activity	•	•	
Improved stability	•	•	
Works directly in NEBuffers	•	•	•
Requires additive			• (Zn^{2+})
Quick Protocol (10 minutes)	•		

* Note: NEB recommends Quick CIP for most applications.

Quick CIP

#M0525S 1,000 units
#M0525L 5,000 units

- *Dephosphorylation of 5´ and 3´ ends of DNA and RNA*
- *Dephosphorylation of cloning vector DNA to prevent recircularization during ligation*
- *Removal of dNTPs in PCR reactions prior to sequencing or SNP analysis*
- *Dephosphorylation of DNA prior to end-labeling using T4 Polynucleotide Kinase*

Description: Quick CIP is a heat-labile recombinant version of calf intestinal alkaline phosphatase (CIP). Quick CIP nonspecifically catalyzes the dephosphorylation of 5´ and 3´ ends of DNA and RNA phosphomonoesters. It also hydrolyses ribo- and deoxyribonucleoside triphosphates (NTPs and dNTPs). Quick CIP is useful in many applications that require the dephosphorylation of DNA or RNA ends. In cloning, dephosphorylation prevents re-ligation of linearized plasmid DNA. The enzyme can quickly dephosphorylate 5´ protruding, 5´ recessed, and blunt ends in just 10 minutes. Quick CIP may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing or SNP analysis.

Quick CIP is completely and irreversibly inactivated by heating it at 80°C for 2 minutes, unlike wild type CIP, which is not heat-inactivatable. This makes removal of Quick CIP prior to ligation or end-labeling unnecessary.

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 80°C for 2 minutes.

Unit Definition: One unit is defined as the amount of enzyme that hydrolyzes 1 µmol of p-Nitrophenyl Phosphate, PNPP (NEB #P0757) in a total reaction volume of 1 ml in 1 minute at 37°C.

Concentration: 5,000 units/ml

Find an overview of dephosphorylation.

Shrimp Alkaline Phosphatase (rSAP)

rCutSmart RR 37° 65°

#M0371S 500 units
#M0371L 2,500 units

- *Dephosphorylation of DNA and RNA*
- *Dephosphorylation of cloning vector DNA to prevent recircularization during ligation*
- *Removal of dNTPs and pyrophosphate from PCR reactions prior to sequencing or SNP analysis*
- *Dephosphorylation of DNA prior to 5´ end-labeling using T4 Polynucleotide Kinase*

Description: Shrimp Alkaline Phosphatase (rSAP) is a heat labile alkaline phosphatase purified from a recombinant source. rSAP is identical to the native enzyme, and contains no affinity tags or other modifications. rSAP nonspecifically catalyzes the dephosphorylation of 5´ and 3´ ends of DNA and RNA phosphomonoesters. Also, rSAP hydrolyzes ribo-, as well as deoxyribonucleoside triphosphates (NTPs and dNTPs). rSAP is useful in many applications that require the dephosphorylation of DNA or RNA ends. In cloning, dephosphorylation prevents re-ligation of linearized plasmid DNA. rSAP may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing or SNP analysis. rSAP is completely and irreversibly inactivated by heating at 65°C for 5 minutes, thereby making removal of rSAP prior to ligation or end-labeling unnecessary.

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 5 minutes.

Unit Definition: One unit is defined as the amount of enzyme that hydrolyzes 1 µmol of *p*-Nitrophenyl Phosphate, PNPP (NEB #P0757) in a total reaction volume of 1 ml in 1 minute at 37°C.

Concentration: 1,000 units/ml

Antarctic Phosphatase

NEB RR 37° 80°

#M0289S 1,000 units
#M0289L 5,000 units

- *Dephosphorylation of DNA and RNA*
- *Dephosphorylation of cloning vector DNA to prevent recircularization during ligation*
- *Removal of dNTPs and pyrophosphate from PCR reactions prior to sequencing or SNP analysis*
- *Dephosphorylation of DNA prior to 5´ end-labeling using T4 Polynucleotide Kinase*

Description: Antarctic Phosphatase catalyzes the dephosphorylation of 5´ and 3´ ends of DNA and RNA phosphomonoesters. Antarctic Phosphatase also hydrolyzes ribo-, as well as deoxyribonucleoside triphosphates (NTPs and dNTPs). Antarctic Phosphatase is useful in many applications that require the dephosphorylation of DNA or RNA ends. In cloning, dephosphorylation prevents religation of linearized plasmid DNA. The enzyme acts on 5´ protruding, 5´ recessed and blunt ends. Antarctic Phosphatase may also be used to degrade unincorporated dNTPs in PCR reactions to prepare templates for DNA sequencing. The enzyme is completely and irreversibly inactivated by heating at 80°C for 2 minutes, thereby making removal of Antarctic Phosphatase prior to ligation or end-labeling unnecessary.

Reaction Conditions: Antarctic Phosphatase Reaction Buffer, 37°C. Heat inactivation: 80°C for 2 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will dephosphorylate 1 µg of pUC19 vector DNA cut with a restriction enzyme generating 5´ recessed ends in 30 minutes at 37°C. Dephosphorylation is defined as > 95% inhibition of recircularization in a self-ligation reaction and is measured by transformation into *E. coli*.

Concentration: 5,000 units/ml

Pyrophosphatases

RR No

Pyrophosphatase, Inorganic (*E. coli*)
#M0361S 10 units
#M0361L 50 units

Pyrophosphatase, Inorganic (yeast)
#M2403S 10 units
#M2403L 50 units

Thermostable Inorganic Pyrophosphatase
#M0296S 250 units
#M0296L 1,250 units

NudC Pyrophosphatase
#M0607S 250 pmol

- *Increasing RNA yield in transcription reactions*
- *Enhancing DNA replication*

Description: Inorganic pyrophosphatase (PPase) catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate.

$P_2O_7^{-4} + H_2O \rightarrow 2HPO_4^{-2}$

Source: Pyrophosphatase, Inorganic (*E. coli*) is prepared from a clone of the *E. coli* inorganic pyrophosphatase gene.

Pyrophosphatase, Inorganic (yeast) is an *E. coli* strain containing a genetic fusion of the *Saccharomyces cerevisiae ppa* gene and the gene coding for *Mycobacterium xenopi* GyrA intein. Developed by BioHelix Corporation, now a wholly owned subsidiary of Quidel Corporation, and produced at New England Biolabs.

Thermostable Inorganic Pyrophosphatase is an *E. coli* strain carrying a plasmid encoding a pyrophosphatase from the extreme thermophile *Thermococcus litoralis*.

NudC is a NUDIX pyrophosphatase that efficiently hydrolyzes NAD^{+-} and NADH-capped RNA, generating a ligatible 5´ monophosphate on the RNA (NAD^+ decapping or deNADding). Deletion of the nudC gene has been shown to increase the fraction of NAD^{+-} capped RNA in *E. coli*.

Unit Definition: One unit is defined as the amount of enzyme that will generate 1 µmol of phosphate per minute from inorganic pyrophosphate under standard reaction conditions.

1 µM of NudC hydrolyzes 200 µM or more NAD^+ into NMN^+ and AMP in 1X NEBuffer r3.1 and 5 mM DTT at 37°C for 30 min.

Concentration: Pyrophosphatase, Inorganic (*E. coli*) and Pyrophosphatase, Inorganic (yeast): 100 units/ml. Thermostable Inorganic Pyrophosphatase: 2,000 units/ml. NudC Pyrophosphatase: 10 µM

NudC Pyrophosphatase is an Enzyme for Innovation (EFI). EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. To view the full list, visit **www.neb.com/EnzymesforInnovation.**

Apyrase

#M0398S 10 units
#M0398L 50 units

- *Highly efficient degradation of ATP to ADP and ADP to AMP*
- *Removal of deoxynucleotides in DNA pyrosequencing between cycles*
- *Conversion of 5´ triphosphorylated RNA to ligatable monophosphorylated*
- *Conversion of 5´ triphosphorylated RNA to 5´ exonuclease XRN-1 (NEB #M0338) sensitive monophosphorylated RNA*

Description: Apyrase (recombinant, *E. coli*) is a highly active ATP-diphosphohydrolase that catalyses the sequential hydrolysis of ATP to ADP and ADP to AMP releasing inorganic phosphate. It is a recombinant version of one of several isoforms of apyrase. It can also hydrolyse 5´ tri- and diphosphate ribonucleosides and deoxyribonuclesides to their respective 5´ monophosphates. Apyrase can catalyze the conversion of 5´ triphosphorylated RNA to 5´ monophosphorylated RNA by sequential removal of γ and β phosphates.

Reaction Conditions: Apyrase Reaction Buffer, 30°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that catalyses the release of 1 µmol of inorganic phosphate from ATP (1 mM, NEB #P0756) in 1X Apyrase Reaction Buffer in 1 minute at 30°C in a total reaction of 50 µl.

Concentration: 500 units/ml

Note: Apyrase has a higher ratio of activity for ATP:ADP (14:1). Apyrase is a calcium-activated enzyme. It is approximately 50% active when Mg^{2+} substitutes Ca^{2+} in Apyrase Reaction Buffer. As a metal-dependent enzyme, Apyrase can be inhibited by EGTA and EDTA. The activity of Apyrase is approximately 30% in NEBuffers r1.1, r2.1, r3.1 and rCutSmart™ Buffer. Apyrase does not remove 5´ caps from eukaryotic mRNA.

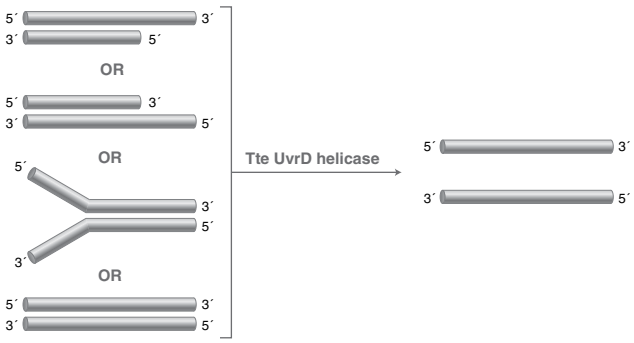
Tte UvrD Helicase

#M1202S 1 µg

- *Unwinds double-stranded DNA*
- *Thermostable to 65°C*
- *Reduces non-specific product formation in isothermal amplification (e.g., LAMP)*

This is an **Enzyme for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. To view the full list, visit **www.neb.com/EnzymesforInnovation.**

Description: *Tte* UvrD Helicase is a repair helicase from the thermophilic organism *Thermoanaerobacter tengcongensis*. It is capable of unwinding double-stranded DNA without a requirement for a specific flap or overhang structure. *Tte* UvrD Helicase is active on a wide range of DNA substrates and, along with its thermostability (active to 70°C), *Tte* UvrD Helicase has been demonstrated to be a useful additive for improving specificity of isothermal amplification reactions.



Reaction Conditions: Isothermal Amplification Buffer Pack, 65°C. Heat inactivation: 80°C for 5 minutes.

Concentration: 20 µg/ml

Marcel is the IT Manager for NEB Germany and has been with the company for 13 years. Learn more about Marcel's role at NEB in his video reel.

#NEBiographies

Exonucleases and Non-specific Endonucleases: Properties

Enzyme	Polarity	Activity on ssDNA		Activity on dsDNA[1]					Partial Digestion to Generate ss Extension[2]	Products Produced[3]	Inhibition by Phosphoro-thioate[4]	Notes
		Linear	Circular	Linear 5´ Ext	Linear 3´ Ext	Linear Blunt	Nicked (Circular/ Linear)	Circular (Supercoiled)				
Exonuclease I (*E. coli*)	3´→5´	+	—	—	—[15]	—[5]	—	—	No	dNMP, dinucleotide[6]	Yes	15, 5, 6
Thermolabile Exonuclease I	3´→5´	+	—	—	—[15]	—[5]	—	—	No	dNMP, dinucleotide[6]	Yes	15, 5, 6
***Msz* Exonuclease I**	3´→5´	+	—	—	—[15]	—[5]	—	—	No	dNMP, dinucleotide[6]	Yes	15, 5, 6
Exonuclease T	3´→5´	+	—	—	—[7]	—[5]	—	—	No	dNMP,dinucleotide, short oligo	Yes	5, 7
Exonuclease VII	both	+	+[8]	—[17]	—[17]	—	—	—	No	short oligos	No	8
RecJ$_f$	5´→3´	+	—	—[15]	—	—[5]	—	—	No	dNMP, ssDNA	Yes	5, 15
Mung Bean Nuclease	Endonuclease	+	+	—	—	—	—	—	No	dNMP, ssDNA	No	
Nuclease P1	Endonuclease	+	+	—	—	—	—	—	No	5' mononucleotides	No	
Exonuclease III (*E. coli*)	3´→5´	+/-[16]	—	+	+/-[14]	+	+	—	5´	dNMP, ssDNA	Yes	14
T7 Exonuclease	5´→3´	—	—	+/-	+	+	+	—	3´	dNMP, dinucleotide, ssDNA[9]	Yes	9
Exonuclease V (RecBCD)	both	+	+	+	+	+	—	—	Yes	Short oligos	No	
Exonuclease VIII, truncated	5´→3´	+/-[10]	—	+	+	+	—	—	3´	dNMP, ssDNA	No	10
Lambda Exonuclease	5´→3´	+/-[10]	—	+/-[11]	+	+	+/-[11]	—	3´	dNMP, dinucleotide, ssDNA,	Yes	10, 11
T5 Exonuclease	5´→3´	+	+	+	+	+	+	—	3´	dNMP to 6 mer	No	
DNase I (RNase-free)	Endonuclease	+	+	+	+	+	+	+	NA	dinucleotides, trinucleotides, oligonucleotides, ssDNA, dsDNA,	No	
DNase I-XT	Endonuclease	+	+	+	+	+	+	+	NA	dinucleotides, trinucleotides, oligonucleotides, ssDNA, dsDNA,	NO	
Micrococcal Nuclease	Endonuclease	+	+	+	+	+	+	+	NA	diphosphonucleotides, ssDNA, dsDNA 3´-monophospho-nucleotides[13]	No	13

Footnotes

(1) The ability to act on short extensions, blunt ends and nicks distinguishes these enzymes; some of these ends are conveniently generated by restriction digestion. The 5´ and 3´ extensions tested were 4 nt in length

(2) Partial digestion of dsDNA by Lambda Exonuclease, T7 Exonuclease and Exonuclease III will produce dsDNA products with ss extensions. Complete digestion produces ssDNA as products.

(3) Complete hydrolysis of the preferred substrate will generate the listed products

(4) To inhibit exonucleases, use of at least 5 phosphorothioate (pt) bonds in a row is recommended. These bonds must be placed at the end of the DNA corresponding to the Polarity of the enzyme; 5´ end for 5´→ 3´ nucleases, the 3´ end for 3´→ 5´ nucleases, and at both ends if the nucleases cannot initiate at both ends. Endonucleases cannot be inhibited by pt bonds unless the entire sequence has pt bonds between all nucleotides.

(5) Depending upon the DNA sequence and amount of exonuclease, RecJ$_f$, Thermolabile Exonuclease I, Exonuclease I, *Msz* Exonuclease I, and Exonuclease T may remove a few nucleotides from blunt termini.

(6) Thermolabile Exonuclease I, Exonuclease I, and *Msz* Exonuclease I release dNMP from ssDNA, except from the last hydrolytic step where a dinucleotide is produced.

(7) Exonuclease T can be used to make 3´ extensions blunt, however, the yield is low.

(8) Exonuclease VII will not be able to digest circular ssDNA when EDTA is present in the reaction. In the absence of Mg^{++} the enzyme will act as a pure exonuclease.

(9) It has been reported that the initial first product hydrolyzed from dsDNA by T7 Exonuclease is a dinucleotide. Subsequent hydrolytic cleavage releases dNMP.

(10) Lambda Exonuclease and Exonuclease VIII, truncated only cut ssDNA if the 5´ contains a phosphate

(11) Lambda Exonuclease has a strong preference for initiating on dsDNA containing a 5´ phosphate. Thus if linear dsDNA has a 5´ phosphate at one end and lacks a 5´ phosphate on the other end, then Lambda Exonuclease will preferentially degrade the DNA that contains the phosphorylated end.

(12) BAL-31 Nuclease has been reported as having both ss endonuclease activity as well as 3´ to 5´ exonuclease activity. Thus any linear DNA is substrate for this enzyme.

(13) Products of Micrococcal Nuclease degradation have 3´ phosphates. Also cuts RNA whereas DNase I does not.

(14) Exonuclease III will be inhibited by overhangs >4 nucleotides

(15) RecJ$_f$ is not suitable for making 5´ extensions blunt. Thermolabile Exonuclease I, Exonuclease I, and *Msz* Exonuclease I are not suitable for making 3´ extensions blunt. These enzymes require longer length ssDNA extensions to initiate than those generated by restriction enzymes.

(16) Exonuclease III exhibits 5-10X less activity on linear ssDNA versus linear dsDNA

(17) For information on removing ssDNA extensions from dsDNA see the Blunting Selection chart

Table Legend

+	Activity, preferred substrate
—	No significant activity
+/-	Activity greatly reduced relative to preferred substrate
NA	Not applicable
ss	Single-stranded
ds	Double-stranded
ext	Extension
dNMP	Deoxyribonucleoside monophosphate

Exonucleases and Endonucleases: Common Applications

Application	Recommended Enzyme(s)	Notes
Removal of 3´ overhangs	• Quick Blunting™ Kit	
5´ overhang Fill in Treatment	• Quick Blunting™ Kit	
Removal of single-stranded primers for nested PCR reactions	• Thermolabile Exonuclease I	
Removal of primers post PCR prior to DNA sequencing or SNP detection	• Exonuclease I • Thermolabile Exonuclease I • Exonuclease VII • *Msz* Exonuclease I	• Quick Heat inactivation versus Exonuclease I For 3´ chemically modified primers • Quick Heat inactivation versus Exonuclease I for 3´chemically modified primers
Mapping positions of introns in genomic DNA	• Exonuclease VII	
Removal of primers with or without 3´ or 5´ terminal phosphorothioate bonds	• Exonuclease VII	
Generating ssDNA from linear dsDNA: **If 5´ → 3´ polarity required** **If 3´ → 5´ polarity required**	• Lambda Exonuclease • Exonuclease III	• Strand targeted for removal requires one 5´ end with phosphate • Strand targeted for removal requires a 5´ overhang, a blunt end, or a 3´ overhang (with less than 4 bases)
Preparation of nested deletions in double-stranded DNA	• Exonuclease III (*E. coli*) plus Exonuclease VII	
Site-directed mutagenesis	• Exonuclease III (*E. coli*) • T7 Exonuclease	• Removes nicked-strand DNA from 3´ to 5´ • Removes nicked-strand DNA from 5´ to 3´
Nick-site extension	• T7 Exonuclease	
Degradation of denatured DNA from alkaline-based plasmid purification methods for improving DNA cloning	• T5 Exonuclease	
Removal of chromosomal/linear DNA in plasmid preparations	• T5 Exonuclease • Exonuclease V (RecBCD)	• Degrades linear ss + dsDNA, nicked DNA • Degrades linear ss + dsDNA: PREFERRED as Exo V will save nicked plasmids resulting in higher yields especially for low-copy number plasmid prep
Removal of unligated products (linear dsDNA) from ligated circular double-stranded DNA	• T5 Exonuclease • Exonuclease V (RecBCD)	• Only the un-nicked form of ligated circular double-stranded remains • Both nicked and unnicked-form of ligated circular double-stranded DNA remains
Removal of residual gDNA after purification of low copy plasmid	• Exonuclease V (RecBCD)	
Removal of contaminated DNA from RNA samples	• DNase I • DNase I-XT	
Removal of template DNA from IVT reactions	• DNase I-XT	
Conversion of single-stranded DNA or RNA to 5´-mononucleotides	• Nuclease P1	
Analysis of base composition, potential damage and modification of nucleic acids	• Nuclease P1	
Preparation of double-stranded DNA fragments with 5´-OH and 3´-phosphate	• Micrococcal Nuclease	
Degradation of nucleic acids (both DNA and RNA) in crude cell-free extracts	• Micrococcal Nuclease	
Preparation of rabbit reticulocyte	• Micrococcal Nuclease	
Chromatin Immunoprecipitation (ChIP) analysis	• Micrococcal Nuclease	

What are exonucleases and their applications?

DNase I (RNase-Free)

NEW

DNase I-XT

DNase I (RNase-free)
#M0303S 1,000 units
#M0303L 5,000 units

NEW

DNase I-XT
#M0570S 1,000 units
#M0570L 5,000 units

- *Removal of contaminating genomic DNA from RNA samples*
- *Degradation of DNA templates in transcription reactions*

Description: DNase I is a DNA-specific endonuclease that degrades ds- and ss-DNA to release short oligos with 5´ phosphorylated and 3´-hydroxylated ends.

DNase I-XT is a salt tolerant enzyme that exhibits optimal activity between 50-100 mM salt and retains 65% and ~40% activity in 200 and 300 mM salt, respectively. Increased salt tolerance makes it ideal for DNA template removal from an *in vitro* transcription (IVT) reaction.

Both enzymes are RNase-free, allowing for complete removal of DNA from RNA preps, while maintaining RNA integrity.

Reaction Conditions: 1X DNase I Reaction Buffer, 37°C. DNase I can be heat inactivated at 75°C for 10 minutes, while DNase I-XT cannot.

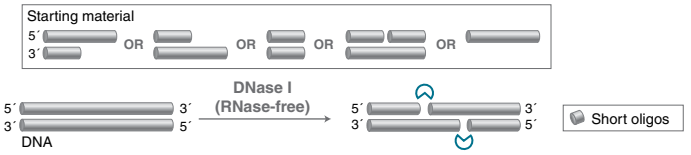
Reagents Supplied:

- DNase I Reaction Buffer (NEB #M0303)
- DNase I-XT Reaction Buffer (NEB #M0570)

Unit Definition: DNase I – One unit is defined as the amount of enzyme which will completely degrade 1 µg of pBR322 DNA in a total reaction volume of 50 µl in 10 minutes at 37°C. DNase I-XT – One unit is defined as the amount of enzyme required to release 210 pmol of FAM from a 35-mer FAM-BHQ1 labeled hairpin oligonucleotide in 1 minute at 30°C in a 50 µl reaction volume.

Concentration: 2,000 units/ml

Notes: DNase I-XT is supplied with an optimized reaction buffer. Use with supplied buffer, and not DNase I Reaction Buffer. Likewise, due to the sub-optimal salt concentration, do not use DNase I-XT Buffer with DNase I. When using DNase I, EDTA should be added to a final concentration of 5 mM to protect RNA from being degraded during enzyme inactivation.



Lambda Exonuclease

#M0262S 1,000 units
#M0262L 5,000 units

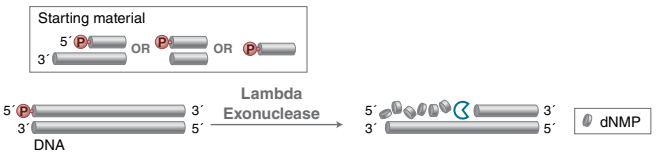
Description: Lambda exonuclease is a highly processive DNA-specific exonuclease that catalyzes the removal of nucleotides from linear or nicked dsDNA in a 5´→ 3´ direction. The preferred substrate is 5´ phosphorylated dsDNA, although it will also degrade ssDNA and non-phosphorylated substrates at a reduced rate.

Reaction Conditions: Lambda Exonuclease Reaction Buffer, 37°C. Heat inactivation: 75°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 10 nmol of acid-soluble deoxyribonucleotide from double-stranded substrate in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X Lambda Exonuclease Reaction Buffer with 1 µg sonicated duplex [^{3}H]-DNA.

Concentration: 5,000 units/ml

Note: 5´-OH ends are digested 20X slower than 5´-PO_4 ends. ssDNA is digested 100X slower than dsDNA.



Exonuclease I (*E. coli*)

#M0293S 3,000 units
#M0293L 15,000 units

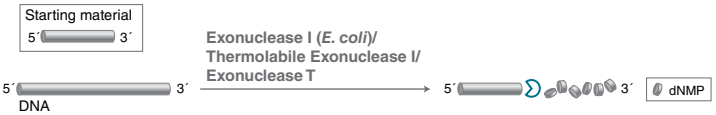
- *Removal of single-stranded primers in PCR reactions prior to Sanger DNA sequencing or SNP analysis*
- *Removal of single-stranded primers for nested PCR reactions*
- *Removal of linear ssDNA, leaving behind dsDNA in the sample*

Description: Exonuclease I is a DNA-specific exonuclease that catalyzes the removal of nucleotides from linear single-stranded DNA in the 3´ to 5´ direction.

Reaction Conditions: Exonuclease I Reaction Buffer, 37°C. Heat inactivation: 80°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will catalyze the release of 10 nmol of acid-soluble nucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X Exonuclease I Reaction Buffer with 0.17 mg/ml single-stranded [^{3}H]-DNA.

Concentration: 20,000 units/ml



Thermolabile Exonuclease I

NEB r3.1 RR 37° 80°

#M0568S 3,000 units
#M0568L 15,000 units

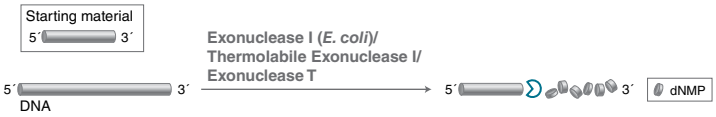
- *Removal of single-stranded primers in PCR reactions prior to DNA sequencing or SNP analysis*
- *Removal of single-stranded primers for nested PCR reactions*
- *Removal of linear ssDNA, leaving behind dsDNA in the sample*

Description: Thermolabile Exonuclease I is a DNA-specific exonuclease that catalyzes the removal of nucleotides from single-stranded DNA in the 3´ to 5´ direction, and can be heat inactivated at 80°C in 1 minute.

Reaction Conditions: NEBuffer r3.1, 37°C. Heat inactivation: 80°C for 1 minutes.

Unit Definition: One unit of Thermolabile Exonuclease I is defined as the amount of enzyme that will catalyze the release of 2 nmol of acid-soluble nucleotide in a total reaction volume of 100 µl in 6 minutes at 37°C in 50 mM Tris-HCl (pH 7.9), 100 mM NaCl, 10 mM $MgCl_2$ and 100 µg/ml BSA with 0.17 mg/ml single-stranded [3H]-*E. coli* DNA.

Concentration: 20,000 units/ml



Exonuclease III (*E. coli*)

NEB1 RR 37° 70°

#M0206S 5,000 units
#M0206L 25,000 units

- *Site-directed mutagenesis*
- *Preparation of ssDNA for dideoxy sequencing*
- *Preparation of nested deletions in dsDNA*

Description: Exonuclease III is a dsDNA-specific exonuclease that catalyzes the removal of nucleotides from linear or nicked dsDNA in the 3´→ 5´ direction. A limited number of nucleotides are removed during each binding event, resulting in coordinated progressive deletions within the population of DNA molecules.

Initiation occurs at the 3´ termini of linear double-stranded DNA with 5´ overhangs or blunt ends and 3´ overhangs containing less than four bases.

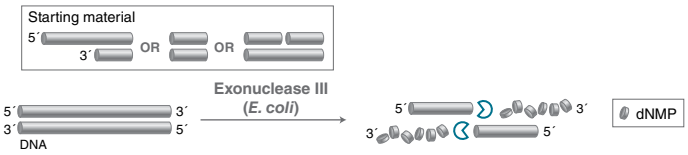
Exonuclease III has also been reported to have RNase H, 3´ phosphatase and AP-endonuclease activities.

Reaction Conditions: NEBuffer 1, 37°C. Heat inactivation: 70°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble total nucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X NEBuffer 1 with 0.15 mM sonicated duplex [3H]-DNA.

Concentration: 100,000 units/ml

Note: Phosphorothioate linkages are not cleaved by Exonuclease III. Unidirectional deletions can also be created by protecting one terminus by incorporation of α-phosphorothioate-containing nucleotide.



Exonuclease V (RecBCD)

NEB4 RR 37° 70°

#M0345S 1,000 units
#M0345L 5,000 units

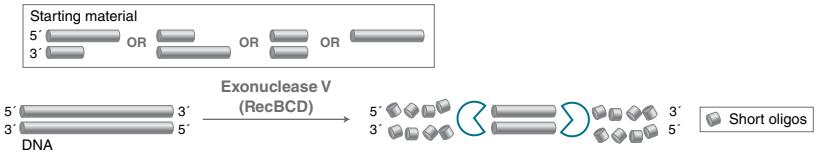
- *Degradation of contaminating linear DNA in plasmid samples*
- *Removal of residual gDNA after purification of low copy plasmid*

Description: Exonuclease V, (RecBCD) is a DNA-Specific exonuclease that also acts as an endonuclease on ssDNA. Activity initiates at both the 5´ and 3´ ends and is processive, generating oligonucleotides. Activity requires ATP and divalent cations. Mg^{2+} is required for the exonuclease activity, while Ca^{2+} inhibits the exonuclease activity and allows dsDNA unwinding (helicase activity).

Reaction Conditions: NEBuffer 4, 37°C. Supplement with 1 mM ATP. Heat inactivation: 70°C for 30 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from double-stranded DNA in 30 minutes at 37°C in a total reaction volume of 50 µl.

Concentration: 10,000 units/ml



Exonuclease VII

#M0379S 200 units
#M0379L 1,000 units

- *Removal of primers with or without 3´ or 5´ terminal phosphorothioate bonds*
- *Mapping positions of introns in genomic DNA*
- *Removal of ssDNA, leaving behind the dsDNA in a sample*

Description: Exonuclease VII, (Exo VII) is a DNA-specific exonuclease that cleaves linear ssDNA in both 5´→3´ and 3´→5´ direction. The preferred substrate is linear ssDNA.

Reaction Conditions: Exonuclease VII Reaction Buffer, 37°C. Heat inactivation: 95°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will catalyze the release of 1 nmol of acid-soluble nucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C.

Concentration: 10,000 units/ml

Exonuclease VIII, truncated

#M0545S 1,000 units

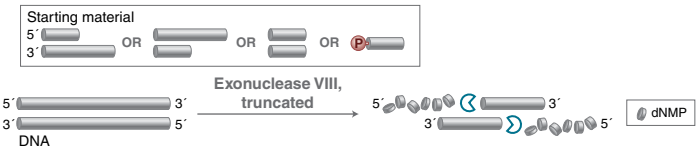
- *Removal of linear dsDNA, leaving behind circular DNA in the sample*

Description: Exonuclease VIII, truncated, is a dsDNA-specific exonuclease. Exonuclease VIII, truncated initiates nucleotide removal from the 5´ termini of linear double-stranded DNA in the 5´ to 3´ direction. The enzyme does not degrade supercoiled dsDNA and circular ssDNA.

Reaction Conditions: NEBuffer 4, 37°C. Heat inactivation: 70°C for 15 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide from double-stranded DNA in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X NEBuffer 4 with 0.15 mM sonicated duplex [^{3}H] DNA.

Concentration: 10,000 units/ml



Exonuclease T

#M0265S 250 units
#M0265L 1,250 units

- *Removal of 3´ overhangs of dsDNA to generate blunt-ends (sequence dependent)*
- *Removal of single-stranded primers in PCR reactions prior to DNA sequencing of SNP analysis*
- *Removal of single-stranded primers for nested PCR reactions*
- *Removal of ssDNA, leaving behind dsDNA in the sample*

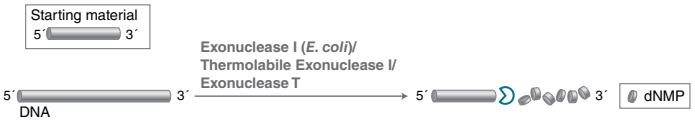
Description: Exonuclease T (Exo T), also known as RNase T, is a single-stranded RNA or DNA specific nuclease that requires a free 3´ terminus and removes nucleotides in the 3´→ 5´ direction. Exo T can be used to generate blunt ends from RNA or DNA having 3´ extensions.

Reaction Conditions: NEBuffer 4, 25°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to release 1 nmol of single dT nucleotides from 50 pmol of Fam-labeled polythymidine substrate in 30 minutes at 25°C.

Concentration: 5,000 units/ml

Note: Exonuclease T has a different activity on RNA vs. DNA. For RNA, 1 unit of Exonuclease T is required to completely digest 1.0 pmol of rA20 under standard reaction conditions as measured by gel electrophoresis.



Thermostable FEN1

NEBU RR 65° Mg

#M0645S 1,600 units

This is an **Enzyme for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. To view the full list, visit **www.neb.com/EnzymesforInnovation.**

Thermostable Flap Endonuclease I, FEN1, is a thermostable DNA and RNA endonuclease that catalyzes the cleavage of 5´ DNA flaps from branched dsDNA substrates, creating a 5´ phosphate terminus. FEN1 products can be ligated by DNA ligase to create dsDNA.

Reaction Conditions: ThermoPol Reaction Buffer, 65°C.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 10 pmol of 5´ flap containing oligonucleotide substrate in a total reaction volume of 10 µl for 10 min at 65°C.

Concentration: 32,000 units/ml

Micrococcal Nuclease

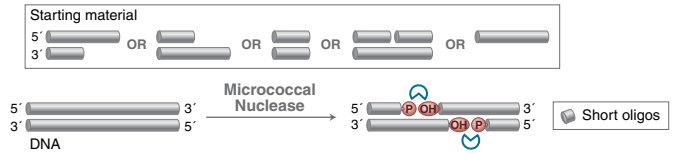
NEBU RR 37° Mg BSA

#M0247S 320,000 gel units

- *Preparation of double-stranded DNA fragments with 5´-OH and 3´-phosphate*
- *Studies of chromatin structure*
- *Degradation of nucleic acids in crude cell-free extracts*
- *Preparation of rabbit reticulocyte*

Description: Micrococcal Nuclease is a DNA and RNA endonuclease that degrades ds-and ss-DNA and RNA. Both DNA and RNA are degraded to 3´ phosphomononucleotides and dinucleotides.

Reaction Conditions: Micrococcal Nuclease Reaction Buffer, 37°C. Supplement with 100 µg/ml Purified BSA.



Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of Lambda DNA in 15 minutes at 37°C, to the extent that the accumulation of low molecular DNA fragments is <400 base pairs as determined by agarose gel electrophoresis.

Concentration: 2,000,000 gel units/ml

Note: 1-5 mM Ca^{2+} is required for activity. The enzyme is active in the pH range 7-10, with optimal activity at 9.2, as long as salt concentration is less than 100 mM. Enzyme can be inactivated by addition of excess EGTA.

NEW

Msz Exonuclease I

rCutSmart 55° 80°

#M0527S 1,000 units

This is an **Enzyme for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. To view the full list, visit **www.neb.com/EnzymesforInnovation.**

Msz Exonuclease I is a DNA specific exonuclease that catalyzes the removal of nucleotides from linear single-stranded DNA in the 3´ to 5´ direction, with optimal activity between 45°C and 60°C.

Reaction Conditions: rCutSmart Buffer, 55°C. Heat inactivation: 80°C for 1 minutes.

Unit Definition: One unit of *Msz* Exonuclease I is defined as the amount of enzyme that will catalyze the release of 2 nmol of acid-soluble nucleotide in a total reaction volume of 100 µl of 0.17 mg/ml single-stranded [^{3}H]-*E. coli* DNA in 15 minutes at 55°C in 50 mM Potassium Acetate, 20 mM Tris Acetate, 10 mM Magnesium Acetate, 100 µg/ml Recombinant Albumin, pH 7.9@25°C.

Concentration: 10,000 units/ml

Mung Bean Nuclease

NEBuffer 3.0

#M0250S 1,500 units
#M0250L 7,500 units

- *Removal of both 3´ and 5´ single-stranded overhangs from dsDNA to create blunt ends*
- *Cleavage of ssDNA and RNA*
- *Cleavage of the single-stranded region in a DNA hairpin*
- *Mapping of RNA transcripts*

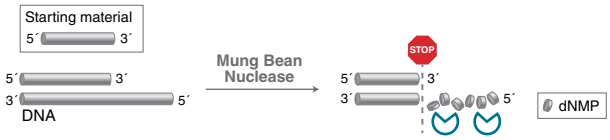
Description: Mung Bean Nuclease is a single-strand specific DNA and RNA endonuclease which will degrade single-stranded extensions from the ends of DNA and RNA molecules, leaving blunt, ligatable ends.

Reaction Conditions: Mung Bean Nuclease Reaction Buffer, 30°C.

Unit Definition: One unit is defined as the amount of enzyme required to digest 1 µg of M13mp18 single-stranded DNA to fragments less than 1 kb in length in a total reaction volume of 80 µl in 1X Mung Bean Nuclease Reaction Buffer when incubated for 15 minutes at 37°C.

Concentration: 10,000 units/ml

Note: Do not attempt to heat inactivate, DNA will "breathe" before enzyme inactivates, causing undesirable degradation.



Nuclease P1

NEBuffer r1.1 37°C 75°C

#M0660S 10,000 units

- *Conversion of ssDNA or RNA to 5´ mononucleotides*
- *Analysis of the base composition of nucleic acids*
- *Studies of the potential damage and modification of DNA*

Description: Nuclease P1 (from *P. citrinum*) is a zinc-dependent ssDNA or RNA specific endonuclease which hydrolyzes 3´→ 5´ phosphodiester bonds in RNA and ssDNA with no base specificity. Nuclease P1 also exhibits 3´-phosphomonoesterase activity.

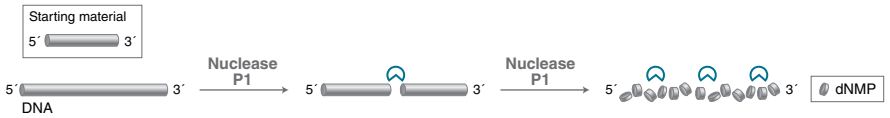
Although a single-strand specific nuclease, Nuclease P1 does display some activity toward double-stranded DNA (dsDNA) in Nuclease P1 Reaction Buffer. If preferentially degrading single-stranded nucleic acids (ssDNA or RNA) in the presence of dsDNA, we recommend using Nuclease P1 in 1X NEBuffer r1.1, to limit activity on dsDNA while maintaining single-strand nuclease activity.

Reaction Conditions: Nuclease P1 Reaction Buffer, 37°C. Heat inactivation: 75°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to liberate 1.0 µg of acid soluble nucleotides from Torula Yeast total RNA per min at 37°C in 1X Nuclease P1 Reaction Buffer.

Concentration: 100,000 units/ml

Note: Substrate specificity for Nuclease P1 is as follows: 3´ AMP > RNA > ssDNA >> dsDNA. The rate of hydrolysis of 2´-AMP is 3,000-fold less than that of 3´-AMP.



$RecJ_f$

NEBuffer 2 rCutSmart 37°C 65°C

#M0264S 1,000 units
#M0264L 5,000 units

- *Degradation of single-stranded DNA from the 5´-end*
- *Removal of 5´ protruding single-stranded termini at the ends of linear dsDNA (blunt ends are not exclusively created)*

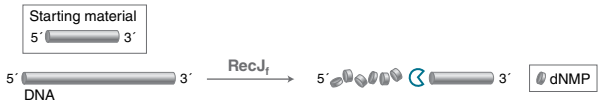
Description: $RecJ_f$ is a ssDNA-specific exonuclease that catalyzes the removal of nucleotides from linear ssDNA in the 5´→ 3´ direction. The preferred substrate is dsDNA with 5´ single-stranded overhangs > 6 nucleotides long.

DNA substrate containing a 22 base 5´ extension results in products that are a mixture of DNA fragments that have blunt-ends, 5´ extensions (1–5 nucleotides) and recessed 5´ ends (1–8 nucleotides). $RecJ_f$ does not require a 5´ phosphate.

Reaction Conditions: NEBuffer 2, 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to produce 0.05 nmol TCA soluble deoxyribonucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X NEBuffer 2 with 1.5 µg sonicated single-stranded [^{3}H]-labeled *E. coli* DNA.

Concentration: 30,000 units/ml

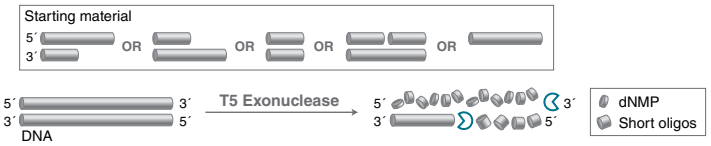


T5 Exonuclease

#M0663S 1,000 units
#M0663L 5,000 units

- *Removal of incomplete ligation products from ligated circular dsDNA*
- *Degradation of denatured DNA from alkaline-based plasmid purification methods for improved DNA cloning*
- *Degradation of contaminating linear and nicked DNA in plasmid samples*

Description: T5 Exonuclease is a dsDNA-specific exonuclease and ssDNA endonuclease. It initiates at the 5´ termini of linear or nicked dsDNA, and cleaves in the 5´→ 3´ direction. T5 Exonuclease is able to initiate nucleotide removal from the 5´ termini or at gaps and nicks of linear or circular dsDNA. However, the enzyme does not degrade supercoiled dsDNA.



Reaction Conditions: NEBuffer 4, 37°C.

Unit Definition: 1 unit of T5 Exonuclease is defined as the amount of enzyme required to cause the change of 0.00032 A260 nm/min at 37° C in rCutSmart Buffer.

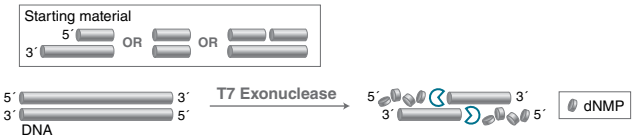
Concentration: 10,000 units/ml

T7 Exonuclease

#M0263S 1,000 units
#M0263L 5,000 units

- *Site-directed mutagenesis*
- *Nick-site extension*

Description: T7 Exonuclease is a dsDNA specific exonuclease that catalyzes removal of nucleotides from linear or nicked dsDNA in the 5´ to 3´ direction. It initiates at the 5´ termini or at gaps and nicks of double-stranded DNA. It will degrade both 5´ phosphorylated or 5´ dephosphorylated DNA. It has also been reported to degrade RNA and DNA from RNA/DNA hybrids in the 5´ to 3´ direction, but it is unable to degrade either ds- or ssRNA.



Reaction Conditions: NEBuffer 4, 25°C.

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of acid-soluble deoxyribonucleotide in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X NEBuffer 4 with 0.15 mM sonicated duplex [^{3}H]-DNA.

Concentration: 10,000 units/ml

Nucleoside Digestion Mix

#M0649S 50 reactions

- *Convenient one-step protocol*
- *Digests both DNA and RNA to single nucleosides*
- *Low-glycerol formulation significantly reduces glycerol-induced ion suppression during MS analysis*

Description: The Nucleoside Digestion Mix is a mixture of enzymes that provides a convenient one-step method to generate single nucleosides from DNA or RNA. Optimized for quantitative analysis by liquid chromatography-mass spectrometry (LC-MS), this reagent eliminates the need for sequential multi-step, time-consuming digestion protocols. The Nucleoside Digestion Mix digests ssDNA, dsDNA, DNA/RNA hybrids and RNA (except mRNA cap structures) containing epigenetically modified (m5C, hm5C, f5C, ca5C, m4C, m6A, etc.), unnatural, or damaged bases. Moreover, the low-glycerol formulation (< 1%) significantly reduces glycerol-induced ion suppression during mass spectrometry analysis.

Reaction Conditions: Nucleoside Digestion Mix Reaction Buffer, 37°C.

DNA Repair Enzymes and Structure-specific Endonucleases: Properties

NEB carries an array of reliable DNA repair enzymes, for use in multiple applications.

Enzyme	Major Substrate [1,2]	Cleavage Site	Product(s) Produced	Termini Created From Cleavage		Major Activity	Thermostable
				5´-Terminus	3´-Terminus		
APE 1	AP site	1st phosphodiester bond 5´ to AP site	1 nt gap	dR5P	OH	Endonuclease	
Mismatch Endonuclease I	T:T, G:G and G:T Mismatches in dsDNA	3rd phosphodiester bond on the 5´ side of the mismatched base in both strands	5 bp overhang	P	OH	Endonuclease	
T7 Endo I	Cruciforms, mismatches, Holliday junctions, across DNA nicks	Phosphodiester bond 5´ to structure	Nick	P	OH	Endonuclease	
Endo III	AP site, damaged pyrimidines, Tg	N-glycosidic bond, 1st phosphodiester bond 3´ to AP site	1nt gap	P	PA	Glycosylase & AP lyase	
Tma Endo III	AP site, damaged pyrimidines, Tg	N-glycosidic bond, 1st phosphodiester bond 3´ to AP site	1nt gap	P	PA	Glycosylase & AP lyase	Yes
Endo IV	AP site	1st phosphodiester bond 5´ to AP site	1nt gap	dR5P	OH	Endonuclease	
Tth Endo IV	AP site	1st phosphodiester bond 5´ to AP site	1nt gap	dR5P	OH	Endonuclease	Yes
Endo V	dI4, dU, AP site	2nd phosphodiester bond 3´ to dI	Nick	P	OH	Endonuclease	
T4 PDG	CPD, AP site	N-glycosidic bond, phosphodiester bond 3´ to AP site	AP site, 1nt gap	P6		Glycosylase & AP lyase	
Endo VIII	AP site4	Phosphodiester bond 3´ and 5´ to AP site	1nt gap	P	P	AP lyase	
Thermostable FEN1	5´ DNA flap3	Phosphodiester bond at base of flap	Nick	P	OH (on flap)	Endonuclease	Yes
Fpg	8-oxoG, oxidized purines	N-glycosidic bond, phosphodiester bond 3´ and 5´ to AP site	AP site, 1nt gap	P	P	Glycosylase & AP lyase	
hAAG	3mA, 7mG, dI, dX	N-glycosidic bond	AP site	N/A	N/A	Glycosylase	
hSMUG1	dU4, 5-hmU, 5-hoU, 5fU	N-glycosidic bond	AP site	N/A	N/A	Glycosylase	
RNaseHII	rN in dsDNA	phosphodiester bond 5´ to ribo	Nick	P	OH	Endonuclease	
UDG	dU4	N-glycosidic bond	AP site	N/A	N/A	Glycosylase	
Afu UDG	dU4	N-glycosidic bond	AP site	N/A	N/A	Glycosylase	Yes
Antarctic Thermolabile UDG	dU4	N-glycosidic bond	AP site	N/A	N/A	Glycosylase	
USER Enzyme	dU	N-glycosidic bond; phosphodiester bond 3´ & 5´ to AP site	1 nt gap	P	P	Glycosylase & AP lyase	No
Thermolabile USER II	dU	N-glycosidic bond; phosphodiester bond 3´ & 5´ to AP site	1 nt gap	P	PA	Glycosylase & AP lyase	No
Thermostable USER III	dU	N-glycosidic bond; phosphodiester bond 3´ & 5´ to AP site	1 nt gap	dR5P	OH	Glycosylase & AP lyase	Yes

Table Legend:

3mA	3-methyladenine
5fU	5-formyluridine
5-hmU	5-hydroxymethyluridine
5-hoU	5-hydroxyuridine
7mG	7-methylguanine
8-oxoG	8-oxo-7, 8-dihydroguanine
AP	apurinic/apyrimidinic sites
CPDs	Cyclobutane pyrimidine dimers
dI	deoxyinosine
dR5P	deoxyribose-5´-phosphate
dU	deoxyuridine
dX	deoxyxanthosine
NA	not applicable
OH	Hydroxyl
P	Phosphate
PA	3´ phospho-a, b-unsaturated aldehyde
rN	ribonucleotides
Tg	Thymine Glycol

Footnotes:

(1) Activity is on dsDNA unless noted otherwise.
(2) Minor activies, substrates, and references can be found at www.neb.com.
(3) 5´ flaps of 1-40 nt in length have been confirmed substrates.
(4) Enzyme has robust activity on ssDNA in addition to dsDNA.
(5) Antarctic Thermolabile UDG can be heat inactivated.
(6) CPD still covalently attached.

What are endonucleases and their applications?

DNA Repair Enzymes on Damaged and Non-standard Bases

NEB carries an array of endonucleases and glycosylases for Base-excision repair (BER) for use in multiple applications. The following table indicates the level of repair on either double-stranded or single-stranded DNA oligos for various damaged and non-standard bases.

Double-stranded DNA Oligos (34-mers)

Repair Enzyme	AP:A	DHT:A	5-hmU:A	5-hmU:G	I:T	6-MeA:T	8-OG:C	8-OG:G	U:A	U:G	Thymine Glycol:A
APE 1	++++	+	-	-	-	-	-	-	-	-	-
Endo III	++++	+	-	-	-	-	-	-	-	-	-
Tma Endo III	++++	++	-	-	-	-	+	+	-	-	++
Endo IV	++++	+	-	-	-	-	-	-	-	-	-
Tth Endo IV	++++	+	-	-	-	-	-	+	-	-	-
Endo V*	+++	+	+	+	++++	+	++	+	+	++++	++
Endo VIII	++++	++	-	-	-	-	-	-	-	-	++++
Fpg	+	+	-	-	-	-	++++	++++	-	-	+
hAAG	-	-	-	-	++++	-	-	-	-	-	-
T4 PDG	++++	-	-	-	-	-	-	-	-	-	-
UDG	N/A	-	-	-	-	-	-	-	++++	+	-
Afu UDG	N/A	-	-	-	-	-	-	-	++++	+	-
hSMUG1	N/A	-	+++	+++	-	-	-	-	++++	++++	-

Standard reaction conditions were used to titer the enzymes with the alternate base
*Nicks only, does not remove damage

Single-stranded DNA Oligos (34-mers)

Repair Enzyme	AP	DHT	5-hmU	I	6-MeA	8-OG	U
APE 1	++	-	-	-	-	-	-
Endo III	++	-	-	-	-	-	-
Tma Endo III	++	+	-	-	-	-	-
Endo IV	-	-	-	-	-	-	-
Tth Endo IV	-	-	-	-	-	-	-
Endo V	+	-	-	++++	-	+	-
Endo VIII	+++	-	-	-	-	-	-
Fpg	+	+	-	-	-	++++	-
hAAG	-	-	-	+	-	-	-
T4 PDG	-	-	-	-	-	-	-
UDG	N/A	-	-	-	-	-	++++
Afu UDG	N/A	-	-	-	-	-	++++
hSMUG1	N/A	-	++	-	-	-	+++

Table Legend:

AP apurinic/apyrimidinic site. The AP site is created by treating a uracil containing oligo with UDG.
DHT 5,6-dihydrothymine
5-hmU 5-hydroxymethyluracil
I Inosine
6-MeA 6-methyladenine
8-OG 8-oxoguanine
U uridine
AP:A apurinic/apyrimidinic site base paired with adenine
DHT:A 5,6 dihydrothymine base paired with an adenine
5-hmU:A 5-hydroxymethyluracil base paired with an adenine
5-hmU:G 5-hydroxymethyluracil base paired with a guanine
I:T Inosine base paired with a thymine
6-MeA:T 6-methyladenine base paired with a thymine
8-OG:C 8-oxoguanine base paired with a cytosine
8-OG:G 8-oxoguanine base paired with a guanine
U:A uridine base paired with an adenine
U:G uridine base paired with a guanine

Level of Repair:

++++ 100%
+++ 50%
++ 10% –25%
+ <10%
– no detectable enzyme activity (<0.7%) Some data were based on oligo data and visualization on a gel using ethidium bromide staining. Depending on the reaction conditions and sensitivity of detection, results may vary. Please be aware that star-activity (non-specific cleavage) may occur if enzyme is in excess.

N/A not applicable

APE 1

#M0282S 1,000 units
#M0282L 5,000 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*
- *Modified nick translation*

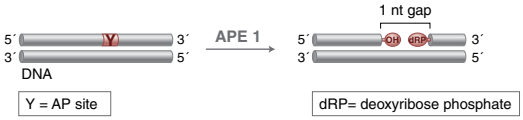
Description: Human apurinic/apyrimidinic (AP) endonuclease, APE 1, also known as HAP 1 or Ref-1, shares homology with *E. coli* Exonuclease III. APE 1 catalyzes the cleavage of DNA phosphodiester backbone at AP sites via hydrolysis leaving a 1 nucleotide gap with 3´-hydroxyl and 5´-deoxyribose phosphate termini. APE 1 has also been reported to have weak DNA 3´-diesterase, 3´ to 5´ exonuclease and RNase H activities.

Reaction Conditions: NEBuffer 4, 37°C.
Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 20 pmol of a 34 mer oligonucleotide duplex containing a single AP site* in a total reaction volume of 10 µl in 1 hour at 37°C.

*An AP site is created by treating 20 pmol of a 34 mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C.

Concentration: 10,000 units/ml



NEW

Mismatch Endonuclease I

#M0678S 4,000 units

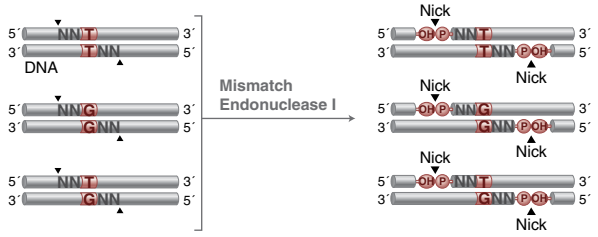
- *Catalyzes the cleavage of some DNA mismatches (T:T, G:G and G:T)*

Description: Mismatch Endonuclease I is a Mg^{2+} dependent DNA endonuclease that specifically cleaves mismatched base pairs (T:T, G:G and T:G mismatches). Mismatch Endonuclease I cleaves the 3rd phosphodiester bond on the 5´ side of the mismatched base in both strands, leaving a 5-base pair overhang. While Mismatch Endonuclease I prefers the cleave T:T, G:G and T:G mismatches, it will also readily cleave T:I, G:I and G:U DNA mismatches.

Reaction Conditions: NEBuffer r2.1, 37°C.
Heat inactivation: 70°C for 5 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 50% of 0.2 pmol of a fluorescently labeled 60mer oligonucleotide duplex containing a single T:T mismatch in 30 minutes at 37°C in a total reaction volume of 20 µl in 1X NEBuffer r2.1.

Concentration: 80,000 units/ml



T7 Endonuclease I

#M0302S 250 units
#M0302L 1,250 units

- *Recognition of mismatched DNA*
- *Resolve four-way junction or branched DNA*
- *Detection or cleavage of heteroduplex and nicked DNA*
- *Random cleavage of linear DNA for shotgun cloning*
- *Key enzyme for genome editing mutation detection*
- *Also available: EnGen® Mutation Detection Kit (NEB #E3321)*

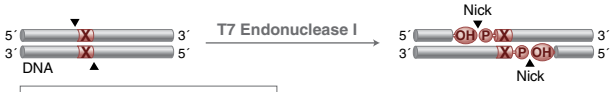
Description: T7 Endonuclease I is a DNA endonuclease that catalyzes the cleavage of DNA mismatches and non-β DNA structures, including Holliday junctions and cruciform, leaving 3´-OH and 5´ phosphate. It is best at C mismatches and does not recognize all DNA mismatches, and to a lesser extent cleaves across a nick in dsDNA.

Reaction Conditions: NEBuffer 2, 37°C.

Unit Definition: One unit is defined as the amount of enzyme required to convert > 90% of 1 µg of supercoiled cruciform pUC(AT) to > 90% linear form in a total reaction volume of 50 µl in 1 hour at 37°C.

Concentration: 10,000 units/ml

Note: It is important to control the amount of enzyme and the reaction time used for cleavage of a particular substrate. Temperatures above 42°C cause an increase in nonspecific nuclease activity and should be avoided.



Endonuclease III (Nth)

#M0268S 1,000 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

Description: Endonuclease III (Nth) protein from *E. coli* acts both as a *N*-glycosylase and an AP-lyase. The *N*-glycosylase activity releases damaged pyrimidines, including thymine glycol and 5, 6-dihydroxythymine, generating an AP site. The AP lyase activity cleaves an AP site via β-elimination, creating a 1-nucleotide gap with 3´-α, β-unsaturated aldehyde and 5´-phosphate termini.

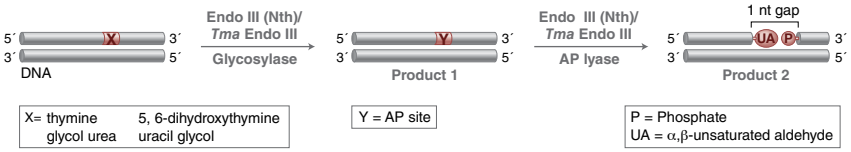
Some of the damaged bases recognized and removed by Endonuclease III (Nth) include urea, 5, 6 dihydroxythymine, thymine glycol, 5-hydroxy-5 methylhydanton, uracil glycol, 6-hydroxy-5, 6-dihdrothimine and methyltartronylurea.

Reaction Conditions: Endonuclease III (Nth) Reaction Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34 mer oligonucleotide duplex containing a single AP site* in a total reaction volume of 10 µl in 1 hour at 37°C in 1X Endonuclease III Reaction Buffer containing 10 pmol of fluorescently labeled oligonucleotide duplex.

* An AP site is created by treating 10 pmol of a 34 mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C.

Concentration: 10,000 units/ml



Tma Endonuclease III

#M0291S 500 units

- *Alkaline elution*
- *Alkaline unwinding*

Description: A thermostable homolog of the *E. coli* Endonuclease III (Nth) that acts as an *N*-glycosylase and an AP-lyase. The *N*-glycosylase activity releases damaged pyrimidines, including thymine glycol and 5,6-dihydroxythymine, generating an AP site. The AP lyase activity cleaves an AP site via β-elimination, creating a 1-nucleotide gap with 3´-α, β-unsaturated aldehyde and 5´-phosphate termini.

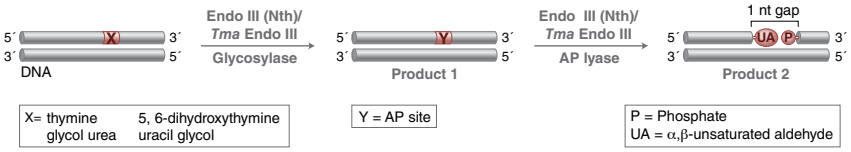
Tma Endonuclease III recognizes abasic sites, 5,6 dihydroxythymine and thymine glycol in DNA.

Reaction Conditions: ThermoPol Reaction Buffer, 65°C.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 60-mer oligonucleotide duplex containing a single AP site* in a total reaction volume of 10 µl in 1 hour at 65°C.

*An AP site is created by treating 10 pmol of a 60-mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C. .

Concentration: 10,000 units/ml



Anuj, Sue, and Julio are members of our international team (pictured left to right). Anuj joined NEB in 2022 as a Business Manager servicing our customers in India. Sue has been the General Manager of NEB Singapore since 2012. Julio joined NEB in 2022 as the Global Channel Manager in the Global Development Department.

Endonuclease IV

#M0304S 1,000 units
#M0304L 5,000 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

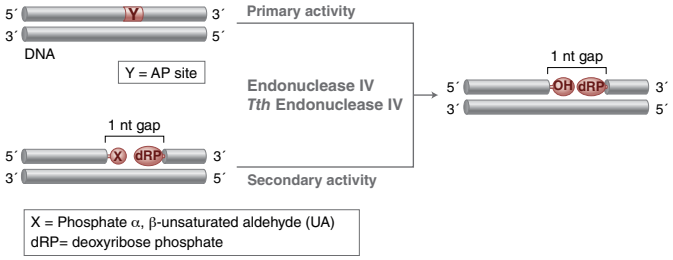
Description: Endonuclease IV can act on several types of oxidative damage in DNA. The enzyme is an apurinic/apyrimidinic (AP) endonuclease that catalyzes the cleavage of DNA phosphodiester backbone at AP sites via hydrolysis leaving a 1 nucleotide gap with 3´-hydroxyl and 5´ deoxyribose phosphate (dRP) termini. The enzyme has 3´-diesterase activity which can remove 3´ phosphate, 3´-α, β-unsaturated aldehyde, phosphoglycoaldehyde, and other 3´ blocking groups.

Reaction Conditions: NEBuffer 3, 37°C. Heat inactivation: 85°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34-mer oligonucleotide duplex containing a single AP site* in a total reaction volume of 10 µl in 1 hour at 37°C.

*An AP site is created by treating 10 pmol of a 34 mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C.

Concentration: 10,000 units/ml



Tth Endonuclease IV

#M0294S 500 units

- *Thermostable*
- *Alkaline elution*
- *Alkaline unwinding*

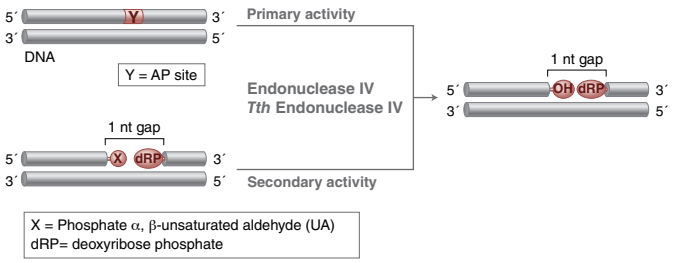
Description: *Tth* Endonuclease IV is a thermostable apurinic/apyrimidinic (AP) endonuclease that catalyzes the cleavage of DNA phosphodiester backbone at AP sites via hydrolysis, leaving a 1 nucleotide gap with 3´-hydroxyl and 5´ deoxyribose phosphate (dRP) termini. The enzyme also has a 3´-diesterase activity that can remove 3´ phosphate, 3´-α, β-unsaturated aldehyde, phosphoglycoaldehyde, and other 3´ blocking groups.

Reaction Conditions: ThermoPol Reaction Buffer, 65°C.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 60-mer oligonucleotide duplex containing a single AP site* in a total reaction volume of 10 µl in 1 hour at 65°C.

* An AP site is created by treating 10 pmol of a 60-mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C.

Concentration: 10,000 units/ml



Endonuclease V

#M0305S 250 units

- *Cleavage of oligonucleotides containing deoxyinosines*
- *Mismatch cleavage*

Description: Endonuclease V is a repair enzyme found in *E. coli* that recognizes deoxyinosine, a deamination product of deoxyadenosine in DNA. Endonuclease V, often called Deoxyinosine 3´ Endonuclease, recognizes DNA containing deoxyinosines (paired or not) on dsDNA, ssDNA with deoxyinosines and, to a lesser degree, DNA containing abasic sites (AP) or urea, base mismatches, insertion/deletion mismatches, hairpin or unpaired loops, flaps and pseudo-Y structures.

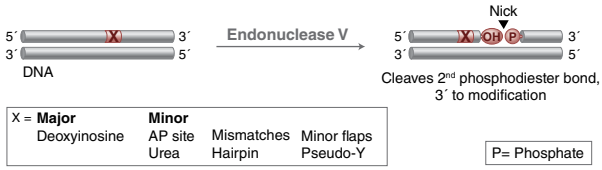
Endonuclease V catalyzes cleavage of the second phosphodiester bond 3´ to the mismatch of deoxyinosine, leaving a nick with 3´-hydroxyl and 5´-phosphate.

Reaction Conditions: NEBuffer 4, 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34 mer oligonucleotide duplex containing a single deoxyinosine site* in a total reaction volume of 10 µl in 1 hour at 37°C.

* A deoxyinosine site is synthetically prepared with a dI in the middle of one strand of a 34 mer oligonucleotide duplex.

Concentration: 10,000 units/ml



T4 PDG (T4 Endonuclease V)

#M0308S 2,000 units

- *DNA damage studies*
- *Single-cell gel electrophoresis (Comet assay)*

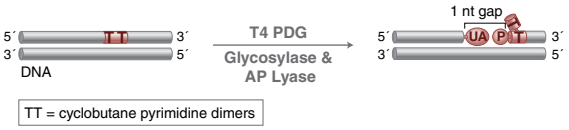
Description: T4 PDG (pyrimidine dimer glycosylase) has both DNA glycosylase and AP-lyase activity. The *N*-glycosylase activity releases cis-syn cyclobutane pyrimidine dimers, including T^T, T^C and C^C, generating an AP site. The AP lyase activity cleaves an AP site via β-elimination, creating a 1 nucleotide DNA gap with 3´-α, β-unsaturated aldehyde and 5´-phosphate termini.

Reaction Conditions: T4 PDG Reaction Buffer, 37°C. Supplement with 100X Purified BSA or rAlbumin.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the conversion of 0.5 µg of UV irradiated supercoiled pUC19 DNA to > 95% nicked plasmid in a total reaction volume of 20 µl in 30 minutes at 37°C. Nicking is assessed by agarose gel electrophoresis. Irradiated plasmid contains an average of 3-5 pyrimidine dimers.

Concentration: 10,000 units/ml

Note: For best results, incubation time should be 30 minutes or less.



Endonuclease VIII

#M0299S 1,000 units
#M0299L 5,000 units

- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

Description: Endonuclease VIII acts as both an *N*-glycosylase and an AP-lyase. The *N*-glycosylase activity releases damaged pyrimidines, including thymine glycol and uracil glycol. The AP lyase activity cleaves DNA phosphodiester backbone at AP sites via β and δ-elimination, creating a 1 nucleotide DNA gap with 5´ and 3´ phosphate termini.

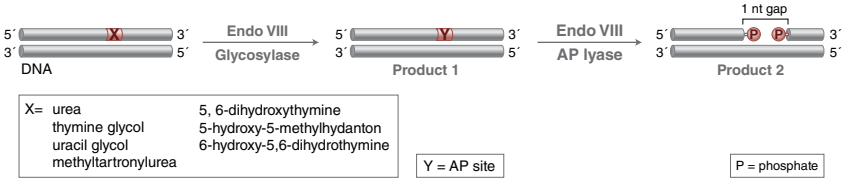
Damaged bases recognized and removed by Endonuclease VIII include urea, 5, 6- dihydroxythymine, thymine glycol, 5-hydroxy-5- methylhydanton, uracil glycol, 6-hydroxy-5, 6-dihydrothymine and methyltartronylurea. While Endonuclease VIII is similar to Endonuclease III, Endonuclease VIII has β and δ lyase activity while Endonuclease III has β lyase activity.

Reaction Conditions: Endonuclease VIII Reaction Buffer, 37°C. Heat inactivation: 75°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 1 pmol of a 34 mer oligonucleotide duplex containing a single AP site* in a total reaction volume of 10 µl in 1 hour at 37°C in 1X Endonuclease VIII Reaction Buffer containing 10 pmol of fluorescently labeled oligonucleotide duplex.

* An AP site is created by treating 10 pmol of a 34 mer oligonucleotide duplex containing a single uracil residue with 1 unit of Uracil-DNA Glycosylase (UDG) for 2 minutes at 37°C.

Concentration: 10,000 units/ml



Fpg

NEB1 RR 37° 60° BSA

#M0240S 500 units
#M0240L 2,500 units

- *Single-cell electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

Description: Fpg (formamidopyrimidine [fapy]-DNA glycosylase), also known as 8-oxoguanine DNA glycosylase, acts both as an *N*-glycosylase and an AP-lyase. *N*-glycosylase activity releases damaged purines, including 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) and 8-oxo-7,8-dihydroguanine (8-oxoG), generating an AP site. The AP lyase activity cleaves an AP site, via β and δ-elimination, creating a 1 nucleotide DNA gap with 5´ and 3´ phosphate termini.

Some of the damaged bases recognized and removed by Fpg include 7, 8-dihydro-8-oxoguanine (8-oxoguanine), 8-oxoadenine, fapy-guanine, methy-fapy-guanine, fapy-adenine, aflatoxin B1-fapy-guanine, 5-hydroxy-cytosine and 5-hydroxy-uracil.

Reaction Conditions: NEBuffer 1, 37°C. Supplement with 100 µg/ml Purified BSA or rAlbumin. Heat inactivation: 60°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 10 pmol of a 34-mer oligonucleotide duplex containing a single 8-oxoguanine base paired with a cytosine in a total reaction volume of 10 µl in 1 hour at 37°C.

Concentration: 8,000 units/ml

X = 8-oxoguanine damaged purines

Y = AP site

hAAG

NEBU RR 37° 65°

#M0313S 500 units

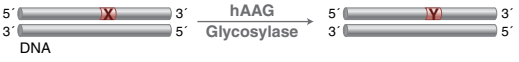
- *Single-cell gel electrophoresis (Comet assay)*
- *Alkaline elution*
- *Alkaline unwinding*

Description: Human Alkyladenine DNA Glycosylase (hAAG) excises alkylated and oxidative DNA damaged sites, including 3-methyladenine, 7-methylguanine, 1,N^6-ethenoadenine and hypoxanthine. hAAG catalyzes the hydrolysis of the *N*-glycosidic bond to release the damaged base. hAAG is also known as methylpurine DNA glycosylase (MPG) or 3-methyladenine-DNA glycosylase (ANPG).

Reaction Conditions: ThermoPol Reaction Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to create an AP site from 1 pmol of a 34-mer oligonucleotide duplex containing a single deoxyinosine site in a total reaction volume of 10 µl in 1 hour at 37°C.

Concentration: 10,000 units/ml



X = 3mA, 7mG, dI, dX

Y = AP site

hSMUG1

NEB1 RR 37° 65° rAlbumin

#M0336S 500 units

- *Oxidative DNA damage studies*
- *Single-cell gel electrophoresis (Comet assay)*

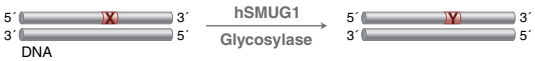
Description: Human single-strand-selective monofunctional uracil-DNA Glycosylase (hSMUG1) excises deoxyuracil and deoxyuracil-derivatives bearing an oxidized group at C5, such as 5-hydroxyuracil, 5-hydroxymethyluracil and 5-formyluracil in ssDNA and dsDNA. Major substrates include uracil, 5-hydroxyuracil, 5-hydroxymethyluracil, and 5-formyluracil.

Reaction Conditions: NEBuffer 1, 37°C. Supplement with 100 µg/ml Recombinant Albumin, Molecular Biology Grade. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to excise 1 pmol of deoxyuracil from a 34 mer oligonucleotide duplex containing a single dU site in a total reaction volume of 10 µl in 1 hour at 37°C.

Concentration: 5,000 units/ml

Note: hSMUG1 has 50% activity on 5-hydroxymethyluracil when compared to uracil. hSMUG1 has 50% activity on ssDNA compared to dsDNA.



X = dU and dU derivatives

Y = AP site

Uracil-DNA Glycosylase (UDG)

#M0280S 1,000 units
#M0280L 5,000 units

Companion Product:

Uracil Glycosylase Inhibitor (UGI)
#M0281S 200 units.
#M0281L 1,000 units

- *Eliminates PCR carry-over contamination*
- *Release of uracil from ss- or ds- DNA*

Description: *E. coli* Uracil-DNA Glycosylase (UDG) is a monofunctional DNA glycosylase that catalyzes the release of uracil from uracil-containing DNA. UDG efficiently hydrolyzes uracil from single-stranded or double-stranded DNA, but not from oligomers (6 or fewer bases).

Reaction Conditions: UDG Reaction Buffer, 37°C.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the release of 60 pmol of uracil per minute from double-stranded, uracil-containing DNA. Activity is measured by release of [^{3}H]-uracil in a 50 µl reaction containing 0.2 µg DNA (10^4-10^5 cpm/µg) in 30 minutes at 37°C.

Concentration: 5,000 units/ml

Note: UDG is active over a broad pH range with an optimum at pH 8.0, does not require divalent cation, and is inhibited by high ionic strength (> 200 mM).

Afu Uracil-DNA Glycosylase (UDG)

#M0279S 200 units

- *Eliminates PCR carryover contamination*
- *Thermostable*
- *Release of uracil from ss- or ds- DNA*

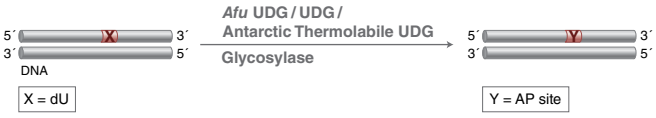
Description: A thermostable homolog of the *E. coli* Uracil-DNA Glycosylase (UDG) from *Archaeoglobus fulgidus*. *Afu* UDG catalyses the release of uracil from uracil-containing DNA. *Afu* UDG efficiently hydrolyzes uracil from ss- or ds-DNA.

Reaction Conditions: ThermoPol II (Mg-free) Reaction Buffer, 65°C.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the release of 60 pmol of uracil per minute from double-stranded, uracil-containing DNA. Activity is measured by release of [^{3}H]-uracil in a 50 µl reaction containing 0.2 µg DNA (10^4-10^5 cpm/µg) in 30 minutes at 65°C.

Concentration: 2,000 units/ml

Note: *Afu* UDG retains 50% activity in the presence of 150 mM NaCl. *Afu* UDG retains less than 1% activity after boiling for 30 minutes in standard reaction conditions. Under standard reaction conditions, uracil glycosylase inhibitor (UGI) does not inhibit *Afu* UDG.



Antarctic Thermolabile UDG

#M0372S 100 units
#M0372L 500 units

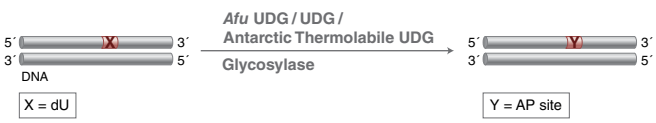
- *Eliminates PCR carry-over contamination*
- *Release of uracil from ss- or ds- DNA*
- *Component of Thermolabile User II Enzyme (NEB #M5508)*

Description: Antarctic Thermolabile UDG (Uracil-DNA Glycosylase) is a monofunctional DNA glycosylase that catalyzes the release of free uracil from uracil-containing ss-or ds-DNA. The resulting abasic sites are susceptible to the hydrolytic cleavage at the elevated temperature and high pH. This enzyme is sensitive to heat and can be rapidly and completely inactivated at temperatures above 50°C.

Reaction Conditions: Standard *Taq* Reaction Buffer Pack, 37°C. Heat inactivation: 50°C for 5 minutes.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the release of 60 pmol of uracil per minute from double-stranded, uracil-containing DNA. Activity is measured by release of [^{3}H]-uracil in a 50 µl Standard *Taq* Reaction Buffer containing 0.2 µg DNA (10^4–10^5 cpm/µg) in 30 minutes at 37°C.

Concentration: 1,000 units/ml



PreCR® Repair Mix

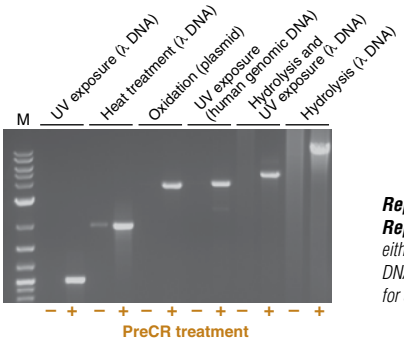
#M0309S 30 reactions
#M0309L 150 reactions

Companion Product:

β-Nicotinamide adenine dinucleotide (NAD+)
#B9007S 0.2 ml

- *Repair DNA prior to its use in DNA-related technologies*
- *Easy-to-use protocols*
- *Does not harm template*

Description: The PreCR Repair Mix is an enzyme cocktail formulated to repair damaged template DNA prior to its use in the polymerase chain reaction (PCR), microarrays, or other DNA technologies. The PreCR Repair Mix is active on a broad range of DNA damage, including those that block PCR (e.g., apurinic/apyrimidinic sites, thymidine dimers, nicks and gaps) and those that are mutagenic (e.g., deaminated cytosine and 8-oxo-guanine). In addition, it will remove a variety of moieties from the 3´ end of DNA leaving a hydroxyl group. The PreCR Repair Mix will not repair all damage that inhibits/interferes with PCR. It can be used in conjunction with any thermophilic polymerase.



Repair of different types of DNA damage with the PreCR Repair Mix. *The gel shows amplification of damaged DNA that was either not treated (–) or treated (+) with the PreCR Repair Mix. Type of DNA damage is shown. Note: heat treated DNA is incubated at 99°C for 3 minutes. Marker (M) is the 1 kb Plus DNA Ladder (NEB #N3200).*

USER® Enzyme

rCutSmart · Cloned at NEB · Recombinant Enzyme · 37° · Heat Inactivation

Thermolabile USER® II Enzyme

rCutSmart · Cloned at NEB · Recombinant Enzyme · 37° · 65° Heat Inactivation

NEW

Thermostable USER® III Enzyme

NEBU · 65° Heat Inactivation

USER Enzyme
#M5505S 50 units
#M5505L 250 units

Thermolabile USER II Enzyme
#M5508S 50 units
#M5508L 250 units

NEW
Thermostable USER III Enzyme
#M5509S 50 units

Description: USER (Uracil-Specific Excision Reagent) Enzyme generates a single nucleotide gap at the location of a uracil. USER Enzyme is a mixture of Uracil DNA glycosylase (UDG) and the DNA glycosylase-lyase Endonuclease VIII. UDG catalyses the excision of a uracil base, forming an abasic (apyrimidinic) site while leaving the phosphodiester backbone intact. The lyase activity of Endonuclease VIII breaks the phosphodiester backbone at the 3´ and 5´ sides of the abasic site so that base-free deoxyribose is released.

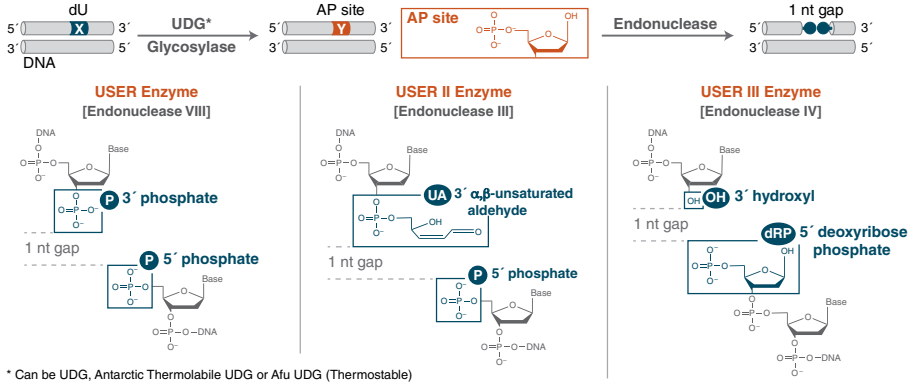
Thermolabile Uracil DNA Glycosylase (UDG) catalyzes the excision of a uracil base, forming an abasic (apyrimidinic) site while leaving the phosphodiester backbone intact. The lyase activity of Endonuclease III breaks the phosphodiester backbone at the 3´ and 5´ sides of the abasic site. In addition to generating a different 3´- terminus than USER Enzyme, Thermolabile USER II Enzyme can also be completely heat inactivated after 10 minutes at 65°C.

Thermostable USER III generates a single nucleotide gap at the location of a uracil. It is a mixture of Afu UDG and DNA glycosylase-lyase Endonuclease IV and is active between 50-75°C, with optimal activity observed at 65°C.

Reaction Conditions: USER & Thermolabile USER II: rCutSmart Reaction Buffer, 37°C. Thermolabile USER II Enzyme can be heat inactivated at 65°C for 10 minutes, while USER cannot. Thermostable USER III: ThermoPol Reaction Buffer, 65°C.

Unit Definition: One unit is defined as the amount of enzyme required to nick 10 pmol of a 34-mer oligonucleotide duplex containing a single uracil base, in 15 minutes at 37°C in a total reaction volume of 10 µl. Unit assay conditions can be found at www.neb.com.

Concentration: 1,000 units/ml



* Can be UDG, Antarctic Thermolabile UDG or Afu UDG (Thermostable)

USER Enzyme, Thermolabile USER II Enzyme and Thermostable USER III Enzyme generate different functional ends after cleavage of DNA. *The different USER Enzymes generate different 3´ and 5´ termini after cleavage. USER Enzyme (NEB #M5505) contains Endonuclease VIII and leaves a 3´ and 5´ phosphate after cleavage. Thermolabile USER II Enzyme (NEB #M5508) contains Endonuclease III and leaves a 3´-phospho-α, β-unsaturated aldehyde and 5´ phosphate after cleavage. Thermostable USER III Enzyme (NEB #M5509) contains Endonuclease IV and leaves a 3´-hydroxyl and 5´-deoxyribose phosphate.*

Cre Recombinase

NEBU RR 37° 70°

#M0298S 50 units
#M0298L 250 units

for high (15X) concentration
#M0298M 250 units

- *Excision of DNA between two loxP sites*
- *Fusion of DNA molecules containing loxP sites*
- *Inversion of DNA between loxP sites*

Description: Cre Recombinase is a Type I topoisomerase from bacteriophage P1 that catalyzes the site-specific recombination of DNA between *loxP* sites. The enzyme requires no energy cofactors, and Cre-mediated recombination quickly reaches equilibrium between substrate and reaction products. The *loxP* recognition element is a 34 base pair (bp) sequence comprised of two 13 bp inverted repeats flanking an 8 bp spacer region which confers directionality. Recombination products depend on the location and relative orientation of the *loxP* sites. Two DNA species containing single *loxP* sites will be fused. DNA between repeated *loxP* sites will be excised in circular form while DNA between opposing *loxP* sites will be inverted with respect to external sequences.

Reaction Conditions: Cre Recombinase Reaction Buffer, 37°C. Heat inactivation: 70°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme necessary to produce maximal site-specific recombination of 0.25 µg pLox2+ control DNA in 30 minutes at 37°C in a total reaction volume of 50 µl. Maximal recombination is determined by agarose gel analysis and by transformation of reactions followed by selection on ampicillin plates.

Concentration: 1,000 and 15,000 units/ml

TelN Protelomerase

NEBU 30° 75°

#M0651S 250 units

This is an **Enzyme for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. To view the full list, visit **www.neb.com/EnzymesforInnovation.**

Description: TelN Protelomerase, from phage N15, cuts dsDNA at a TelN recognition sequence (56 bp) and leaves covalently closed ends at the site of cleavage.

Reaction Conditions: ThermoPol Reaction Buffer, 30°C. Heat inactivation: 75°C for 5 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 0.5 µg of pMiniT-TelN BsaI-linearized control plasmid (313 fmol of TelN recognition sites) in a total reaction volume of 50 µl in 30 minutes at 30°C in 1X ThermoPol Reaction Buffer.

Concentration: 5,000 units/ml

Topoisomerase I (*E. coli*)

rCutSmart RR 37° 65°

#M0301S 100 units
#M0301L 500 units

- *Recognition of mismatched DNA*
- *Catalyzes relaxation of negatively-supercoiled DNA*

Description: Topoisomerase I (*E. coli*) catalyzes the relaxation of negatively-supercoiled DNA. Topoisomerase I has also been implicated in knotting and unknotting DNA, and in linking complementary rings of single-stranded DNA into double-stranded rings. The intact holoenzyme is a 97 kDa protein.

Reaction Conditions: rCutSmart Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that catalyzes the relaxation of > 95% of 0.5 µg of pUC19 RF I (negatively supercoiled) DNA in 15 minutes at 37°C in a total reaction volume of 25 µl. DNA supercoiling is assessed by agarose gel electrophoresis in the absence of ethidium bromide.

Concentration: 5,000 units/ml

β-Agarase I

NEBU RR 42° 65°

#M0392S 100 units
#M0392L 500 units

- *Extraction of DNA from agarose gels*
- *DNase and RNase free*

Description: β-Agarase cleaves the agarose subunit, unsubstituted neoagarobiose [3,6-anhydro-α-L-galactopyranosyl-1-3-D-galactose] to neoagaro-oligosaccharides. β-Agarase I digests agarose, releasing trapped DNA and producing carbohydrate molecules which can no longer gel. The remaining carbohydrate molecules and β-Agarase I will not, in general, interfere with subsequent DNA manipulations such as restriction endonuclease digestion, ligation and transformation.

Reaction Conditions: β-Agarase I Reaction Buffer, 42°C. Heat inactivation: 65°C for 15 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to digest 200 µl of molten low melting point or NuSieve agarose to nonprecipitable neoagaro-oligosaccharides in 1 hour at 42°C.

Concentration: 1,000 units/ml

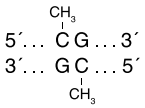
Note: Incubation at 95°C for 2 minutes or incubation at 65°C for 15 minutes inactivates 50 units of β-Agarase I. β-Agarase I retains activity for several hours at 40-45°C and is stabilized by the presence of agarose in the reaction.

CpG Methyltransferase (M.SssI)

NEB2 RR 37° 65° SAM

#M0226S 100 units
#M0226L 500 units

for high (5X) concentration
#M0226M 500 units



- *Blocking restriction enzyme cleavage*
- *Studying of CpG methylation-dependent gene expression*
- *Probing sequence-specific contacts within the major groove of DNA*
- *Altering the physical properties of DNA*
- *Uniform [^{3}H]-labeling of DNA*
- *Decreasing the number of RE cut sites, yielding an apparent increase in specificity*

Description: The CpG Methyltransferase (M.SssI) methylates all cytosine residues (C^5) within the double-stranded dinucleotide recognition sequence 5′...CG...3′.

Reaction Conditions: NEBuffer 2, 37°C. Supplement with 160 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg of λ DNA in a total reaction volume of 20 µl in 1 hour at 37°C against cleavage by BstUI restriction endonuclease.

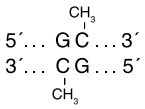
Concentration: 4,000 and 20,000 units/ml

Note: $MgCl_2$ is not required as a cofactor. In the presence of Mg^{2+}, methylation by M.Sss I becomes distributive rather than processive and also exhibits topoisomerase activity.

GpC Methyltransferase (M.CviPI)

NEBU RR 37° 65° SAM

#M0227S 200 units
#M0227L 1,000 units



- *Blocking restriction enzyme cleavage*
- *Altering the physical properties of DNA*
- *Uniform [3H]-labeling of DNA*

Description: The GpC Methyltransferase (M.CviPI) methylates all cytosine residues (C^5) within the double-stranded dinucleotide recognition sequence 5′...GC...3′.

Reaction Conditions: GC Reaction Buffer, 37°C. Supplement with 160 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg of λ DNA in a total reaction volume of 20 µl in 1 hour at 37°C against cleavage by HaeIII restriction endonuclease.

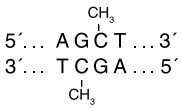
Concentration: 4,000 units/ml

Note: $MgCl_2$ is not required as a cofactor.

AluI Methyltransferase

NEBU RR 37° 65° SAM

#M0220S 100 units



Description: AluI Methyltransferase modifies the cytosine residue (C5) in the sequence to the left.

Reaction Conditions: AluI Methyltransferase Reaction Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 20 minutes.

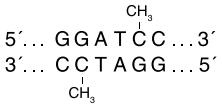
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg of λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by AluI restriction endonuclease.

Concentration: 5,000 units/ml

BamHI Methyltransferase

NEBU RR 37° No SAM

#M0223S 100 units



Description: BamHI Methyltransferase modifies the internal cytosine residue (N^4) in the sequence to the left.

Reaction Conditions: BamHI Methyltransferase Reaction Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM).

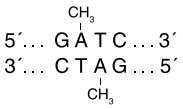
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by BamHI restriction endonuclease.

Concentration: 4,000 units/ml

dam Methyltransferase

NEBU RR 37° 65° SAM

#M0222S 500 units
#M0222L 2,500 units



Description: *dam* Methyltransferase modifies the adenine residue (N^6) in the sequence to the left.

Reaction Conditions: dam Methyltransferase Reaction Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 20 minutes.

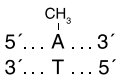
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg (*dam*-) Lambda DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by MboI restriction endonuclease.

Concentration: 8,000 units/ml

EcoGII Methyltransferase

rCutSmart RR 37° 65° SAM

#M0603S 200 units



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Description: EcoGII Methyltransferase is a non-specific methyltransferase that modifies adenine residues (N^6) in any sequence context.

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with 160 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 100 ng FAM-labeled dsDNA in 30 minutes at 37°C in a total reaction volume of 20 µl against cleavage by MboI restriction endonuclease.

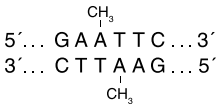
Concentration: 5,000 units/ml

Note: For use of methylation reaction the SAM should be diluted 1:200 in H_2O to a final concentration of 160 µM. EcoGII Methyltransferase is sensitive to salt. Make sure the DNA solution is low in salt concentration or that it makes up only a small percentage of the final reaction volume. If salt is a problem, reduce the salt concentration by drop dialysis.

EcoRI Methyltransferase

rCutSmart RR 37° 65° SAM

#M0211S 10,000 units



Description: EcoRI Methyltransferase modifies the internal adenine residue (N^6) in the sequence to the left.

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by EcoRI restriction endonuclease

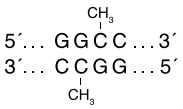
Concentration: 40,000 units/ml

Note: EcoRI Methyltransferase is inhibited by $MgCl_2$. Only 50% activity is retained at a concentration of 4 mM $MgCl_2$.

HaeIII Methyltransferase

NEBU RR 37° 65° SAM

#M0224S 500 units



Description: HaeIII Methyltransferase modifies the internal cytosine residue (C^5) in the sequence to the left.

Reaction Conditions: HaeIII Methyltransferase Reaction Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by HaeIII restriction endonuclease.

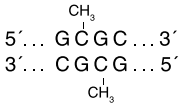
Concentration: 10,000 units/ml

Note: HaeIII Methyltransferase protects DNA against cleavage by NotI.

HhaI Methyltransferase

rCutSmart · Cloned at NEB · Recombinant Enzyme · 37° · Heat Inactivation · SAM

#M0217S 1,000 units



Description: HhaI Methyltransferase modifies the internal cytosine residue (C^5) in the sequence to the left.

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM).

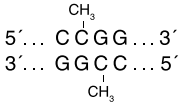
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by HhaI restriction endonuclease.

Concentration: 25,000 units/ml

HpaII Methyltransferase

rCutSmart · Cloned at NEB · Recombinant Enzyme · 37° · Heat Inactivation · SAM

#M0214S 100 units



Description: HpaII Methyltransferase recognizes the same sequence as the MspI Methyltransferase, but modifies the internal cytosine residue (C^5) in the sequence to the left.

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM). Heat inactivation: 65°C for 20 minutes.

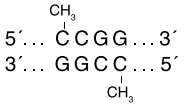
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by HpaII restriction endonuclease.

Concentration: 4,000 units/ml

MspI Methyltransferase

NEBuffer · Cloned at NEB · Recombinant Enzyme · 37° · Heat Inactivation · SAM

#M0215S 100 units



Description: MspI Methyltransferase recognizes the same sequence as the HpaII Methyltransferase, but modifies the external cytosine residue (C^5) in the sequence to the left.

Reaction Conditions: MspI Methylase Reaction Buffer, 37°C. Supplement with 80 µM S-adenosylmethionine (SAM).

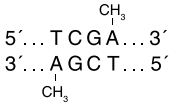
Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 37°C in a total reaction volume of 10 µl against cleavage by MspI restriction endonuclease.

Concentration: 5,000 units/ml

*Taq*I Methyltransferase

rCutSmart · Cloned at NEB · Recombinant Enzyme · 65° · Heat Inactivation · SAM

#M0219S 1,000 units



Description: *Taq*I Methyltransferase modifies the adenine residue (N^6) in the sequence to the left.

Reaction Conditions: rCutSmart Buffer, 65°C. Supplement with 80 µM S-adenosylmethionine (SAM).

Unit Definition: One unit is defined as the amount of enzyme required to protect 1 µg λ DNA in 1 hour at 65°C in a total reaction volume of 20 µl against cleavage by *Taq*I restriction endonuclease.

Concentration: 10,000 units/ml

Note: *Taq*I Methyltransferase gives 25% activity at 37°C.

RecA

#M0249S 200 µg
#M0249L 1,000 µg

- *Visualization of DNA structures with electron microscopy*
- *D-loop mutagenesis*
- *Screening libraries using RecA-coated probes*
- *Cleavage of DNA at a single predetermined site*
- *RecA mediated affinity capture for full length cDNA cloning*

Description: *E. coli* RecA is necessary for genetic recombination, reactions involving DNA repair and UV-induced mutagenesis. RecA promotes the autodigestion of the lexA repressor, umuD protein and lambda repressor. Cleavage of LexA derepresses more than 20 genes. *In vitro* studies indicate that in the presence of ATP, RecA promotes the strand exchange of single-strand DNA fragments with homologous duplex DNA. The reaction has three distinct steps: (i) RecA polymerizes on the single-strand DNA, (ii) the nucleo-protein filament binds the duplex DNA and searches for a homologous region, (iii) the strands are exchanged.

Reaction Conditions: Rec A Reaction Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Molecular Weight: 37,973 kDa.

Concentration: 2 mg/ml

T4 Gene 32 Protein

#M0300S 100 µg
#M0300L 500 µg

- *Increase yield and processivity of reverse transcriptase during RT-PCR*
- *Increase yield and specificity of PCR products from soil samples*
- *Stabilization and marking of ssDNA structures*

Description: T4 Gene 32 Protein is a single-stranded DNA (ssDNA) binding protein required for bacteriophage T4 replication and repair. It cooperatively binds to and stabilizes transiently formed regions of ssDNA and plays an important structural role during T4 phage replication. It also has been used extensively to stabilize and mark regions of ssDNA for electron microscopic examination of intracellular DNA structures. Recently, it has been shown to improve restriction enzyme digestion, improve the yield and efficiency of reverse transcription (RT) reactions during RT-PCR, enhance T4 DNA polymerase activity, as well as increase the yield of PCR products.

Reaction Conditions: NEBuffer 4, 37°C. Heat inactivation: 65°C for 20 minutes.

Molecular Weight: 33,506 daltons.

Concentration: 10 mg/ml

ET SSB

#M2401S 50 µg

- *Improve the processivity of DNA polymerase*
- *Stabilization and marking of ssDNA structure*
- *Increase the yield and specificity of PCR*
- *Increase the yield and processivity of RT during RT-PCR*
- *Improve DNA sequencing through regions with strong secondary structures*

Description: ET SSB (Extreme Thermostable Single-Stranded DNA Binding Protein) is a single-stranded DNA binding protein isolated from a hyperthermophilic microorganism, which remains fully active after incubation at 95°C for 60 minutes. Due to the extreme thermostability, ET SSB can be used in applications that require extremely high temperature conditions, such as nucleic acid amplification and sequencing.

Unit Definition: Sold by mass of pure protein as determined by OD_{280}.

Molecular Weight: 16 kDa.

Concentration: 500 µg/ml

Note: ET SSB is active in any polymerase buffer. Add 200 ng of ET SSB per 50 µl reaction.

Cloning Plasmids and DNAs

Cloning Plasmid/DNA	NEB #	Features	Concentration	MW/Size	Size
pBR322 Vector	N3033S N3033L	• Commonly used cloning vectors • Amp resistance	1,000 µg/ml	2.83 x 10^6 Da 4,361 bp	50 µg 250 µg
pUC19 Vector	N3041S N3041L	• Commonly used cloning vectors • Amp resistance	1,000 µg/ml	1.75 x 10^6 Da 2,686 bp	50 µg 250 µg
M13mp18 RF I DNA	N4018S	• Phage vectors derived from bacteriophage M13 • DNA, covalently closed circular • 13 Unique RE sites with β-gal gene • Blue/white selection	100 µg/ml	7,249 bp	10 µg
M13mp18 Single-stranded DNA	N4040S		250 µg/ml	7,249 bp	10 µg
Lambda DNA	N3011S N3011L	• Commonly used DNA substrate	500 µg/ml	31.5 x 10^6 Da 48,502 bp	250 µg 1,250 µg
Lambda DNA (dam⁻)	N3013S N3013L	• Commonly used DNA substrate	500 µg/ml	31.5 x 10^6 Da 48,502 bp	250 µg 1,250 µg
ΦX174 RF I DNA	N3021S N3021L	• Commonly used DNA substrate • Covalently closed circular form of φX174	1,000 µg/ml	3.5 x 10^6 Da 5,386 bp	30 µg 150 µg
ΦX174 RF II DNA	N3022L	• Commonly used DNA substrate • Double-stranded nicked circular form of φX174	1,000 µg/ml	3.5 x 10^6 Da 5,386 bp	150 µg
ΦX174 Virion DNA	N3023S N3023L	• Single-stranded viral DNA	1,000 µg/ml	1.7 x 10^6 Da 5,386 bp	50 µg 250 µg

NEB offers a selection of common cloning plasmids and DNAs for use as substrates. Additional information for many of these DNAs can be found in the Technical Reference section or at www.neb.com.

M13KO7 Helper Phage

#N0315S 1.8 ml

- *Production of single-stranded phagemid DNA for sequencing and mutagenesis*

Description: M13KO7 is a derivative of M13 phage with the origin of replication from P15A and the kanamycin resistance gene from Tn903 both inserted within the M13 origin of replication, which is able to replicate in the absence of phagemid DNA. In the presence of phagemid bearing a wild-type M13 or f1 origin, single-stranded phagemid is packaged preferentially and secreted into the culture medium. This allows easy production of single-stranded phagemid DNA for mutagenesis or sequencing. M13KO7 carries the kanamycin resistance marker.

Source: M13KO7 Phage supernatant is isolated from infected E. coli ER2738 by a standard procedure.

Concentration: 1 x 10^{11} pfu/ml

Note: NEB does not recommend the use of M13KO7 as a cloning vector. For cloning peptides displayed on M13 phage, we recommend the Ph.D.™ Peptide Display Cloning System.

Programmable Nucleases

Site-specific gene modification and highly-specific *in vitro* cutting is enabled by nucleases that can be easily programmed with nucleic acids. In addition to programmed with nucleic acids. In addition to RNA-guided Cas enzymes, *Tth* Argonaute can be programmed with DNA, further expanded the range of available tools.

Tth Argonaute (TtAgo)

#M0665S 50 pmol

- *Short 16-18 nucleotide 5´-phosphorylated ssDNA guides are cost effective and can be phosphorylated with T4 Polynucleotide Kinase*
- *Guide/target sequence selection is not limited by the requirement of an adjacent sequence motif*
- *Highly active on ssDNA and most dsDNA substrates (generates a nick in dsDNA substrates), with mild activity on ssRNA substrates*

Description: *Thermus thermophilus* argonaute (TtAgo) is a programmable DNA-endonuclease which requires a short 5´-phosphorylated single-stranded DNA guide to target its activity to a specific corresponding sequence on a substrate. TtAgo introduces one break in the phosphodiester backbone of the complementary substrate between positions 10 and 11 of the DNA guide.

Source: *Thermus thermophilus* argonaute (TtAgo) is purified from an *E. coli* strain that carries a cloned gene from the Gram-negative thermophilic bacterium *Thermus thermophilus* which is expressed as a recombinant N-terminal 6X His-tagged fusion.

Note: Visit www.neb.com/M0665 for usage guidelines.

Competent Cell Selection Chart for Cloning

	NEB® 5-alpha Competent *E. coli* NEB #C2987	NEB Turbo Competent *E. coli* NEB #C2984	NEB 5-alpha F'Iq Competent *E. coli* NEB #C2992	NEB 10-beta Competent *E. coli* NEB #C3019	*dam-/dcm-* Competent *E. coli* NEB #C2925	NEB® Stable Competent *E. coli* NEB #C3040
Features						
Versatile	•					•
Fast growth (< 8 hours)		•				
Toxic gene cloning			•			•
Large plasmid/BAC cloning				•		•
Dam/Dcm-free plasmid growth					•	
Retroviral/lentiviral vector cloning						•
recA⁻	•		•	•		•
endA⁻	•	•	•	•	•	•
Formats						
Chemically competent	•	•	•	•	•	•
Electrocompetent				•		
Subcloning	•					
96-well format*	•			•		
384-well format*	•					
12 x 8-tube strips*	•					

* Other strains are available upon request. For more information, contact custom@neb.com.

Monarch® Nucleic Acid Purification Kits

Monarch kits provide fast and reliable purification of high quality DNA and RNA from a variety of sources using best-in-class silica-column and innovative glass bead technology. DNA and RNA purified with Monarch kits is highly pure and suitable for use in a wide range of applications, including IVT RNA synthesis, sequencing, cloning, PCR and other enzymatic manipulations. Monarch kits are developed for performance and with sustainability in mind; they use significantly less plastic and are packaged in responsibly-sourced, recyclable material. For flexibility, all Monarch kit components are available separately. Learn more at **NEBmonarch.com**.

Product	NEB #	Size
Monarch Plasmid Miniprep Kit	T1010S T1010L	50 preps 250 preps
Monarch DNA Gel Extraction Kit	T1020S T1020L	50 preps 250 preps
Monarch PCR & DNA Cleanup Kit (5 µg)	T1030S T1030L	50 preps 250 preps
Monarch Genomic DNA Purification Kit	T3010S T3010L	50 preps 150 preps
Monarch HMW DNA Extraction Kit for Tissue	T3060S T3060L	5 preps 50 preps
Monarch HMW DNA Extraction Kit for Cells & Blood	T3050S T3050L	5 preps 50 preps
Monarch Total RNA Miniprep Kit	T2010S	50 preps
Monarch RNA Cleanup Kit (10 µg)	T2030S T2030L	10 preps 100 preps
Monarch RNA Cleanup Kit (50 µg)	T2040S T2040L	10 preps 100 preps
Monarch RNA Cleanup Kit (500 µg)	T2050S T2050L	10 preps 100 preps
Columns Available Separately		
Monarch Plasmid Miniprep Columns	T1017L	100 columns + tubes
Monarch DNA Cleanup Columns (5 µg)	T1034L	100 columns + tubes
Monarch RNA Purification Columns	T2007L	100 columns + tubes
Monarch gDNA Purification Columns	T3017L	100 columns + tubes
Monarch RNA Cleanup Columns (10 µg)	T2037L	100 columns + tubes
Monarch RNA Cleanup Columns (50 µg)	T2047L	100 columns + tubes
Monarch RNA Cleanup Columns (500 µg)	T2057L	100 columns + tubes

Monarch kit components are available separately. See Nucleic Acid Purification Chapter for details.

Endangered: the monarch butterfly

It is, perhaps, the most recognizable butterfly in the world. The monarch butterfly's distinctive orange, white and black wing pattern makes it easy to identify, but its nearly 3,000 mile annual migratory pattern makes it a true biological wonder. In 2022, the International Union for Conservation of Nature (IUCN) classified the monarch butterfly as endangered and threatened by climate change.

The World Wildlife Fund monitors areas of the forest occupied by monarch colonies overwintering in Mexico, and it reported that the eastern migratory monarch butterfly population has decreased by more than 80% over the last three decades. Since 1980, the western migratory monarch population, which overwinters in California, has dropped more than 99%, from 4.5 million to 1,914 monarchs. These decreases are likely driven by a combination of factors. This includes a decrease in the milkweed habitat, on which monarch butterflies lay their eggs, and which also serves as a food source for the caterpillars after hatching. Additionally, the change in overwintering habitat due to land use changes, deforestation in Mexico, poor management of overwintering groves in California, drought, and climate change, all leave monarch habitats threatened year-round.

There has been habitat improvement in recent years, but more is needed. World Wildlife Fund Mexico reported that the butterflies occupied approximately 35% more area during the winter of 2021-2022 compared to the previous year, and so debate over the necessity of the endangered species designation continues. Increasing milkweed habitats can help increase the reproduction of the summer population, which in turn, increases the size of the overwintering population, according to insect ecologist Orley Taylor of the University of Kansas, U.S. Taylor is also the founder of Monarch Watch, whose Bring Back the Monarch Program distributes milkweed seed for monarch habitat restoration. But Taylor states, "The scale of that needs to be much larger. Since 2010 when we started that program, we've distributed about a million milkweed plants for restoration. We're doing more of that sort of work than any other organization in the country, and we can do a lot more, but to do so requires underwriting."

Weather and long-term climate change also pose a risk to the monarch that is harder to address. A monarch butterfly's body temperature strongly regulates its flight muscles, and Monarchs knocked to the ground by raindrops become easy prey. "Since 2002, there have been four major winter kills at the overwintering sites. It doesn't usually rain in central Mexico in the wintertime, but it has rained significantly in those four events with devastating effects on the population, killing 70-80% of the butterflies at the overwintering sites. That is a real threat," says Taylor.

And though all are not convinced that the monarch butterfly's endangered species designation is warranted at this time, it seems inevitable. As Taylor explains, "Looking at this from a long-term perspective, climate change, these events that are coming off the Pacific, the butterfly is going to be endangered at some point in the future. You just can't predict it."

Monarch Butterflies in Michoacan, Mexico, millions are migrating every year and waking up with the sun.
Credit: reisegraf, Adobe Stock

Explore monarchs in flight in 3D.

Nucleic Acid Purification

Time for change.

Nucleic acid purification is an important step in molecular biology workflows and there are many commercially-available solutions from which to choose. Our Research and Development team spent time with customers to better understand what could be done to improve upon current nucleic acid purification kits. This feedback helped us develop our line of Monarch® Nucleic Acid Purification kits, which have been optimized for maximum performance and minimal environmental impact.

Monarch kits are available for DNA and RNA extraction and cleanup, plasmid purification, and gel extraction. Our novel glass bead-based solution for extraction of high molecular weight DNA supports long read sequencing. Our other Monarch kits utilize unique column designs, which enable the isolation of highly-pure nucleic acids, free from contaminants and often in low volumes. Monarch kits are supported by a variety of validated, user-friendly protocols to support multiple workflows and applications.

We know that it can be difficult to be environmentally friendly in the lab, where sterility and convenience are of utmost importance. At times, it may seem that sustainability and benchwork are at odds with each other. Although we can't completely solve this problem, we can make changes to our product design to help move toward the goal of a greener lab, and that's exactly what we did with the design of our Monarch kits. Wherever possible, these kits use less plastic, as well as responsibly-sourced and recyclable packaging. The columns have thinner walls, reducing total plastic usage without affecting performance. All bottles were carefully chosen to minimize plastic usage, and the kit boxes are made from 100% post consumer content and are designed to be reused.

Let's work together to clean up the world of nucleic acid purification, one prep at a time.

Featured Products

135 Monarch RNA Cleanup Kit

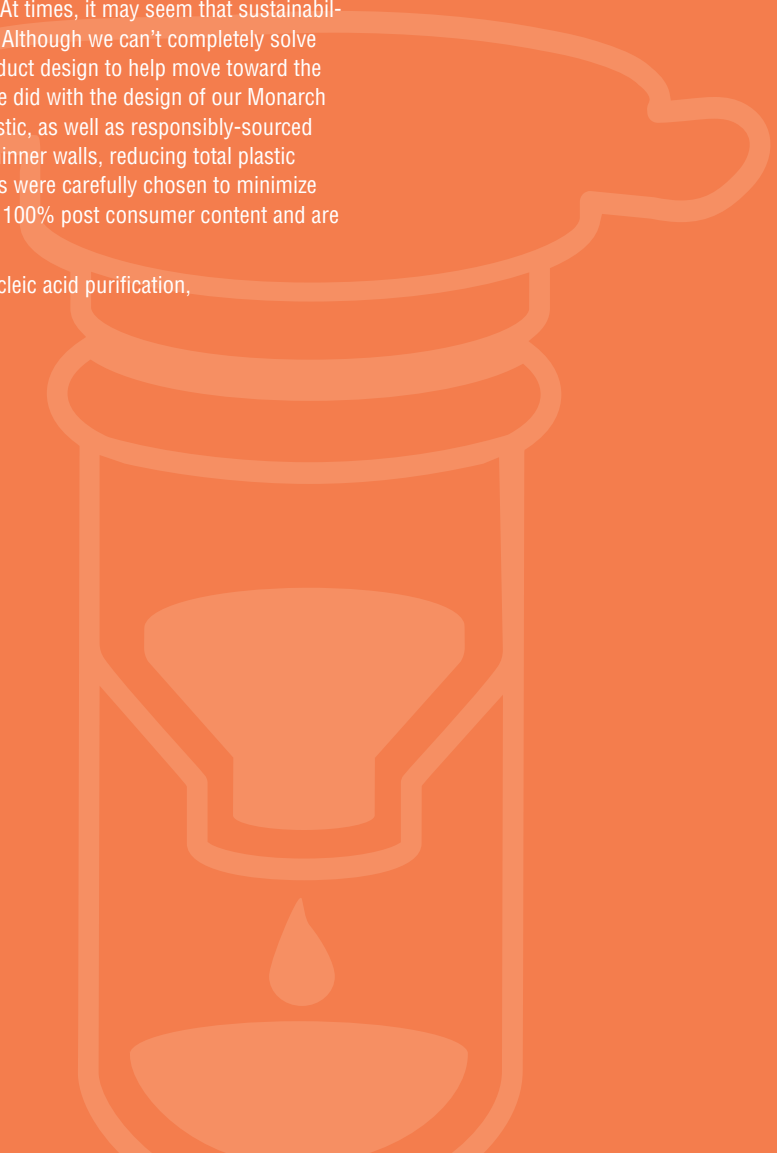
136 Monarch Total RNA Miniprep Kit

137 Monarch Genomic DNA Purification Kit

138 Monarch HMW DNA Extraction Kits

Featured Tools & Resources

Visit **NEBMonarch.com** to access our full line of products for nucleic acid purification as well as associated protocols, videos, FAQs and troubleshooting guides.



RNA Purification

Monarch RNA Cleanup Kit (10 µg) 135
Monarch RNA Cleanup Kit (50 µg) 135
Monarch RNA Cleanup Kit (500 µg) 135
Monarch Total RNA Miniprep Kit 136

Genomic DNA Purification

Monarch Genomic DNA Purification Kit 137

HMW DNA Extraction

Monarch HMW DNA Extraction Kit for Tissue 138
Monarch HMW DNA Extraction Kit for Cells & Blood 138

DNA Cleanup

Monarch PCR & DNA Cleanup Kit (5 µg) 139
Monarch DNA Gel Extraction Kit 140

Plasmid Purification

Monarch Plasmid Miniprep Kit 141

Monarch Kit Components

Available separately. See individual product pages for details.

Make the right choice and migrate to Monarch®

Workflows for detecting, analyzing, amplifying or manipulating DNA and RNA often require extraction and purification from a biological sample and/or enzymatic reactions. Monarch nucleic acid purification kits provide fast and reliable purification of high-quality DNA and RNA from a variety of sources using best-in-class silica column technology and a novel glass-bead based workflow for HMW DNA extraction. DNA and RNA purified with Monarch Kits is highly-pure and suitable for use in a wide variety of downstream applications including sequencing, cloning, PCR and other enzymatic manipulations. Monarch kits are all designed with sustainability in mind; they use less plastic whenever possible and are packaged in responsibly-sourced, recyclable material. For convenience, all Monarch kit components are also available separately.

Reduced lab waste

Significantly less plastic as compared to leading supplier

Monarch kits still deliver high yields, purity and performance

Thinner-walled columns

Reduction in total plastic without affecting performance

Buffer bottles

Carefully designed to minimize plastic usage

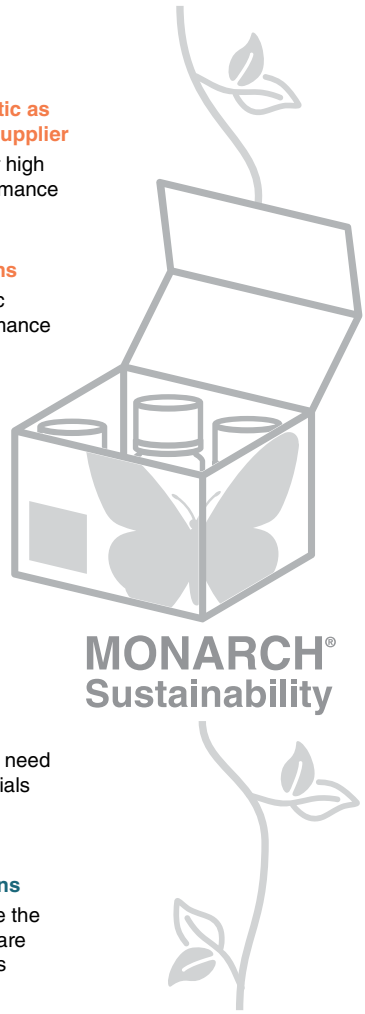
Flexible purchasing options

Buffers and columns sold separately

Purchase only what you need and avoid wasted materials

Same performance, design and formulations

Standalone products are the same components that are included in complete kits



No excessive packaging

Sturdy, reusable boxes at just the right size

Carefully designed to eliminate empty space, versatile Monarch boxes can be reused anywhere

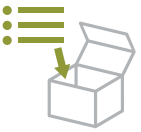
Concise protocol cards replace printed manuals

Both cards and manuals are available online as PDFs

Sustainable & recyclable packaging

Sourced for recyclability

All components are purposefully sourced for recyclability



Instructions for recycling kit components

Can be found on product packing or online

Recycled paper

Used to make the kit boxes, inserts and paper materials

Eco-friendly printing

Printing of boxes and packaging powered by green sustainable sources such as wind

To learn more, visit **neb.com/monarchsustainability**

Monarch® RNA Cleanup Kits

Monarch RNA Cleanup Kit (10 µg)
#T2030S 10 preps
#T2030L 100 preps

Monarch RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

Monarch RNA Cleanup Kit (500 µg)
#T2050S 10 preps
#T2050L 100 preps

Companion Products:

Monarch RNA Cleanup Columns (10 µg)
#T2037L 100 columns

Monarch RNA Cleanup Columns (50 µg)
#T2047L 100 columns

Monarch RNA Cleanup Columns (500 µg)
#T2057L 100 columns

Monarch Collection Tubes II
#T2018L 100 tubes

Monarch RNA Cleanup Binding Buffer
#T2041L 80 ml

Monarch RNA Cleanup Wash Buffer
#T2042L 40 ml

Nuclease-free Water
#B1500S 25 ml
#B1500L 100 ml

- *Choose from 3 different binding capacities and flexible elution volumes*
- *Quickly and easily purify large quantities of high quality RNA from in vitro transcription (IVT) reactions*
- *Efficiently remove unincorporated nucleotides from your RNA sample*
- *Compatible with Qiacube® and KingFisher™ Flex automation platforms*

Great for RNA cleanup following *in vitro* transcription with HiScribe® Kits.

Description: The Monarch RNA Cleanup Kits provide a fast and simple silica spin column-based solution for RNA cleanup and concentration after any enzymatic reaction (including *in vitro* transcription, DNase I treatment, capping and labeling) and after other purification methods such as phenol/chloroform extraction. These kits can also be used to extract total RNA from cells, saliva and swabs (buccal, nasopharyngeal, etc.). The Monarch RNA Cleanup Kits are available in 3 different binding capacities: 10 µg, 50 µg and 500 µg. Each kit contains unique columns, all designed to prevent buffer retention and ensure no carryover of contaminants, enabling low-volume elution of highly-pure RNA. Following the standard protocol, RNA ≥ 25 nt is purified with this kit; however, a modified protocol is available to enable the binding of RNA as small as 15 nt (including miRNAs).

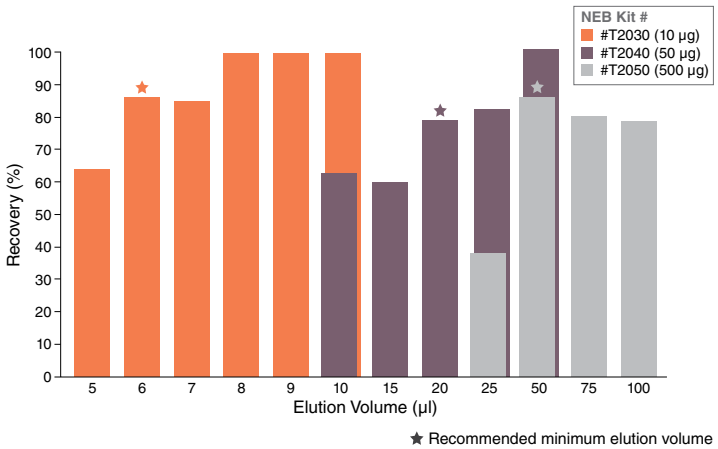
Applications:

- RNA cleanup and concentration (including from the TRIzol aqueous phase)
- Enzymatic reaction cleanup
- *In vitro* transcription cleanup
- Total RNA extraction from some samples
- RNA gel extraction
- RNA fractionation

The Monarch RNA Cleanup Kits Include:

- RNA Cleanup Columns (10, 50 or 500 µg)
- RNA Cleanup Binding Buffer
- RNA Cleanup Wash Buffer
- Collection Tubes II
- Nuclease-free Water

Monarch RNA Cleanup Kit	NEB #T2030 (10 µg)	NEB #T2040 (50 µg)	NEB #T2050 (500 µg)
Binding Capacity	10 µg	50 µg	500 µg
RNA Size Range	≥ 25 nt (≥ 15 nt with modified protocol)		
Typical Recovery	70–100%		
Elution Volume	6–20 µl	20–50 µl	50–100 µl
Purity	$A_{260/280}$ > 1.8 and $A_{260/230}$ > 1.8		
Protocol Time	5 minutes of spin and incubation time		10–15 minutes of spin and incubation time



Recovery of RNA from Monarch RNA Cleanup Kits with Varying Elution Volumes. *rRNA (10, 50 or 500 µg, respectively of 16S and 23S Ribosomal Standard from E. coli, Sigma) was purified using a Monarch RNA Cleanup Kit (10 µg, NEB #T2030) (50 µg, NEB #T2040) (500 µg, NEB #T2050). Nuclease-free water was used to elute the RNA. The percent recovery of the RNA was calculated from the resulting A260 as measured using a Trinean® DropSense® 16. ~80% of RNA can be efficiently recovered in 6 µl from the Monarch RNA Cleanup Kit (10 µg, NEB #T2030), 20 µl from the Monarch RNA Cleanup Kit (50 µg, NEB #T2040), and 50 µl from the Monarch RNA Cleanup Kit (500 µg, NEB #T2050).*

View tips for successful purification using the Monarch RNA Cleanup Kits.

Monarch® Total RNA Miniprep Kit

#T2010S 50 preps

Companion Products:

Monarch RNA Purification Columns
#T2007L 100 columns

Monarch gDNA Removal Columns
#T2017L 100 columns

Monarch Collection Tubes II
#T2018L 100 tubes

Monarch DNA/RNA Protection Reagent
#T2011L 56 ml

Monarch RNA Lysis Buffer
#T2012L 100 ml

Monarch Total RNA Miniprep Enzyme Pack
#T2019L 1 Pack

Monarch RNA Priming Buffer
#T2013L 56 ml

Monarch RNA Wash Buffer
#T2014L 50 ml

Nuclease-free Water
#B1500S 25 ml
#B1500L 100 ml

- *Use with a wide variety of sample types*
- *Purify RNA of all sizes, including miRNA & small RNAs >20 nucleotides*
- *Includes DNase I, gDNA removal columns, Proteinase K, and a stabilization reagent*
- *Efficiently remove contaminating genomic DNA*
- *Compatible with Qiacube and Kingfisher Flex automation platforms*
- *Protocols available for RNA fractionation and RNA cleanup*
- *Save money with value pricing for an all-in-one kit*

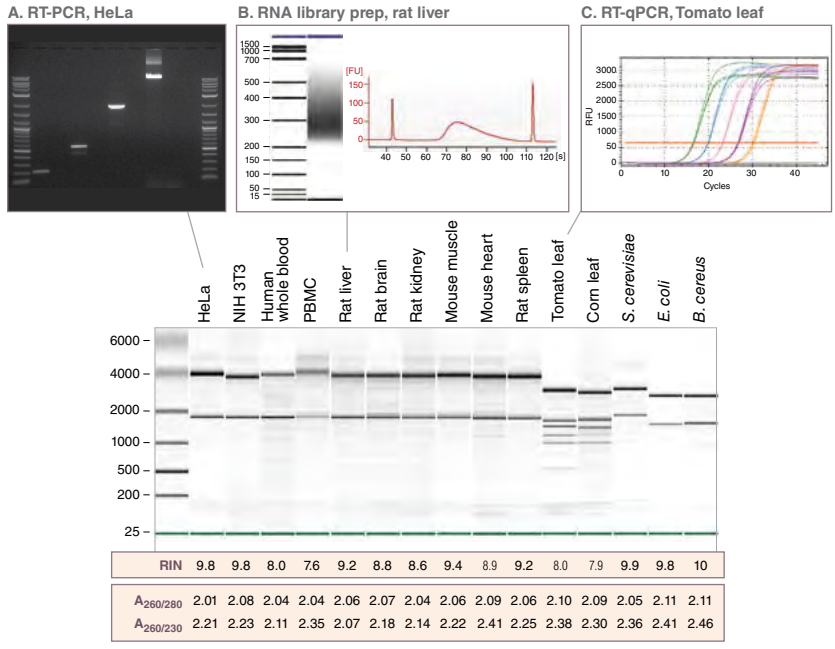
Description: The Monarch Total RNA Miniprep Kit is a comprehensive solution for sample preservation, cell lysis, gDNA removal, and purification of total RNA from a wide variety of biological samples, including cultured cells, blood, and mammalian tissues. Additionally, tough-to-lyse samples, such as bacteria, yeast, and plant, can be processed with additional steps that enhance lysis. Total RNA, including viral RNA, can also be extracted from clinically-relevant samples like saliva, buccal swabs and nasopharyngeal swabs. Cleanup of enzymatic reactions or purification of RNA from TRIzol® -extracted samples is also possible using this kit. Purified RNA has high quality metrics, including $A_{260/280}$ and $A_{260/230}$ ratios ≥ 1.8, high RIN scores, and minimal residual gDNA. Captured RNA ranges in size from full-length rRNAs down to intact miRNAs. Additionally, differential binding conditions allow selective capture or exclusion of the sub-200 nucleotide RNA pool that includes miRNA, 5S rRNA, and tRNA. Purified RNA is suitable for downstream applications, such as RT-qPCR, cDNA synthesis, RNA-seq, Northern blot analysis, etc.

Kit Includes:

- gDNA Removal Columns
- RNA Purification Columns
- Collection Tubes II
- DNA/RNA Protection Reagent
- RNA Lysis Buffer
- Proteinase K Reaction Buffer
- DNase I Reaction Buffer
- RNA Priming Buffer
- RNA Wash Buffer
- Nuclease-free Water
- Proteinase K
- Proteinase K Resuspension Buffer
- DNase I

Specifications	
Binding Capacity	100 µg RNA
RNA Size	≥ 20 nt
Purity	$A_{260/280}$ and $A_{260/230}$ usually ≥ 1.8
Input Amount	up to 10^7 cells or 50 mg tissue*
Elution Volume	30–100 µl
Yield	varies depending on sample type
Compatible downstream applications	RNA Library prep for NGS, RT-PCR, RT-qPCR, Northern blots

*See "Guidelines for Choosing Sample Input Amounts" in the technical reference section or at **www.neb.com/MonarchRNAInputs.**



Monarch-purified RNA is high-quality and compatible with a wide variety of downstream applications. *Total RNA from a broad array of sample types was purified using the Monarch Total RNA Miniprep Kit. Aliquots were run on an Agilent Bioanalyzer® 2100 using the Nano 6000 RNA chip (S. cerevisiae RNA was run using a plant Nano assay). RIN values and O.D. ratios confirm the overall integrity and purity of the RNA. To demonstrate compatibility with downstream applications, samples were subsequently used for RT-PCR (+/– RT) (A) for detection of 4 different RNA species using ProtoScript® II Reverse Transcriptase/LongAmp® Taq DNA Polymerase, NGS library prep (B) using NEBNext® Ultra™ II RNA Library Prep Kit and RT-qPCR (C) using Luna® One-Step RT-qPCR Reagents.*

View tips for successful RNA purification using Monarch Total RNA Miniprep Kit.

Monarch® Genomic DNA Purification Kit

#T3010S 50 preps
#T3010L 150 preps

Companion Products:

Monarch gDNA Purification Columns
#T3017L 100 preps

Monarch gDNA Elution Buffer
#T3016L 34 ml

Monarch gDNA Tissue Lysis Buffer
#T3011L 34 ml

Monarch gDNA Cell Lysis Buffer
#T3012L 20 ml

Monarch gDNA Blood Lysis Buffer
#T3013L 20 ml

Monarch gDNA Binding Buffer
#T3014L 65 ml

Monarch gDNA Wash Buffer
#T3015L 60 ml

Monarch HMW DNA Extraction Kit for Tissue
#T3060S 5 preps
#T3060L 50 preps

Monarch HMW DNA Extraction Kit for Cells & Blood
#T3050S 5 preps
#T3050L 50 preps

Monarch Collection Tubes II
#T2018L 100 tubes

Proteinase K, Molecular Biology Grade
#P8107S 2 ml

Monarch RNase A
#T3018L 1 ml

- *Purify high quality gDNA from a wide variety of sample types (cells, blood, tissues and more)*
- *Experience extremely low residual RNA contamination (typically <1%)*
- *Isolate high molecular weight gDNA (peak size typically ≥ 50 kb)*
- *Take advantage of user-friendly protocols with fast and efficient lysis steps*
- *Additionally, use the kit to clean up genomic DNA*
- *Enjoy the flexibility to purchase kit components separately*

The Monarch Genomic DNA Purification Kit is an excellent complement to the NEBNext Library Preparation products for NGS.

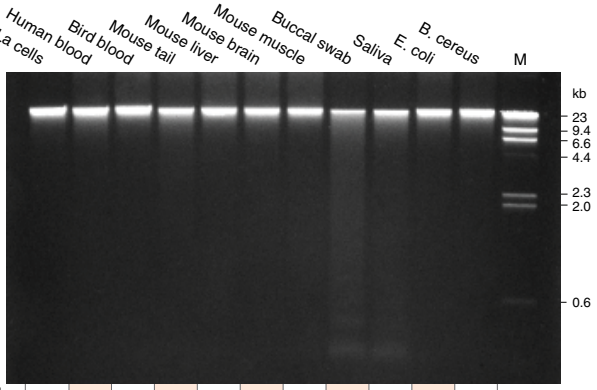
Description: The Monarch Genomic DNA Purification Kit is a comprehensive solution for cell lysis, RNA removal, and purification of intact genomic DNA (gDNA) from a wide variety of biological samples, including cultured cells, blood, and mammalian tissues. Additionally, bacteria and yeast can be processed with extra steps to enhance lysis in these tough-to-lyse samples. Protocols are also included to enable purification from clinically-relevant samples, such as saliva and cheek swabs, as well as rapid cleanup of previously extracted gDNA. Purified gDNA has high quality metrics, including $A_{260/280}$ > 1.8 and $A_{260/230}$ > 2.0, high DIN scores and minimal residual RNA. The purified gDNA is suitable for downstream applications, such as endpoint PCR, qPCR and library prep for next generation sequencing (NGS). Typical peak size is 50–70 kb, making this kit an excellent choice upstream of next generation sequencing (NGS) platforms.

Kit Includes:

- gDNA Tissue Lysis Buffer
- gDNA Cell Lysis Buffer
- gDNA Blood Lysis Buffer
- gDNA Binding Buffer
- gDNA Wash Buffer
- gDNA Elution Buffer
- gDNA Purification Columns
- Collection Tubes II
- Proteinase K, Molecular Biology Grade
- RNase A

Specifications	
Input	• Cultured mammalian cells: up to 5 x 10^6 cells • Mammalian whole blood: 100 µl • Tissue: up to 25 mg, depending on tissue type • Bacteria: up to 2 x 10^9 • Yeast: up to 5 x 10^7 • Saliva: up to 500 µl • Buccal swabs • Genomic DNA requiring cleanup
Binding Capacity	30 µg genomic DNA
Yield	Varies depending on sample type*
Genomic DNA Size	Peak size > 50 kb for most sample types; may be lower for saliva and buccal swabs
RNA Content	< 1% (with included RNase A treatment)
Purity	$A_{260/280}$ ≥ 1.8, $A_{260/230}$ ≥ 2.0

*See "Guidelines for Choosing Sample Inputs" in the technical reference section or at **www.neb.com/MonarchgDNAInputs**.



	HeLa cells	Human blood	Bird blood	Mouse tail	Mouse liver	Mouse brain	Mouse muscle	Buccal swab	Saliva	E. coli	B. cereus
Peak size (kb)	> 60	54	> 60	> 60	> 60	> 60	> 60	39	45	53	> 60
DIN	9.3	9.0	9.2	8.7	9.4	9.3	9.2	6.6	7.4	8.7	8.9
A_{260}/A_{280}	1.86	1.85	1.87	1.85	1.85	1.87	1.87	1.85	1.86	1.86	1.88
A_{260}/A_{230}	2.30	2.20	2.40	2.36	2.34	2.47	2.40	2.00	2.17	2.33	2.04

The Monarch Genomic DNA Purification Kit efficiently purifies high-quality, high molecular weight gDNA from a variety of sample types. *100 ng of genomic DNA from each sample was loaded on a 0.75% agarose gel. gDNA was isolated following the standard protocols for blood, cultured cells and tissue, and the supplemental protocols for buccal swabs, saliva, Gram- and Gram+ bacteria. Starting material used: 1 x 10^6 HeLa cells, 100 µl human blood, 10 µl bird blood, 10 mg frozen tissue powder, 1 buccal swab, 500 µl saliva and ~1 x 10^9 bacterial cells. Lambda DNA-Hind III digest (NEB #N3012) was used as a marker in the last lane (M). Purified gDNA samples were analyzed using a Genomic DNA ScreenTape® on an Agilent Technologies® 4200 TapeStation®. Samples typically yield peak sizes 50–70 kb and DINs of ~9. The cell fractions processed in the buccal swab and saliva preps contain dead cells, as expected, causing a smear like pattern with typical low molecular weight apoptotic bands.*

Monarch® HMW DNA Extraction Kits

Monarch HMW DNA Extraction Kit for Tissue
#T3060S 5 preps
#T3060L 50 preps

Monarch HMW DNA Extraction Kit for Cells & Blood
#T3050S 5 preps
#T3050L 50 preps

Companion Products:

Monarch Pestle Set
#T3000L 100 sets

Monarch 2 ml Tubes
#T3003L 100 tubes

Monarch DNA Capture Beads
#T3005L 200 beads

Monarch Bead Retainers
#T3004L 100 sets

Monarch gDNA Nuclei Prep & Lysis Buffer Pack
#T3054L 1 Pack

Monarch RBC Lysis Buffer
#T3051L 160 ml

Monarch gDNA Elution Buffer II
#T3056L 24 ml

Monarch HMW gDNA Tissue Lysis Buffer
#T3061L 62 ml

Monarch Protein Separation Solution
#T3062L 36 ml

Monarch Precipitation Enhancer
#T3055L 10 ml

- *Fast workflow (cells: 30 min, blood: 60 min, tissue/bacteria: 90 min)*
- *Extract DNA into the megabase (Mb size range with cells, blood, soft organ tissues and bacteria*
- *Tune DNA size based on agitation speed during lysis*
- *Achieve best-in-class yields and purity*
- *Consistently achieve reproducible results*
- *Effectively remove RNA*
- *Elute DNA easily and completely*

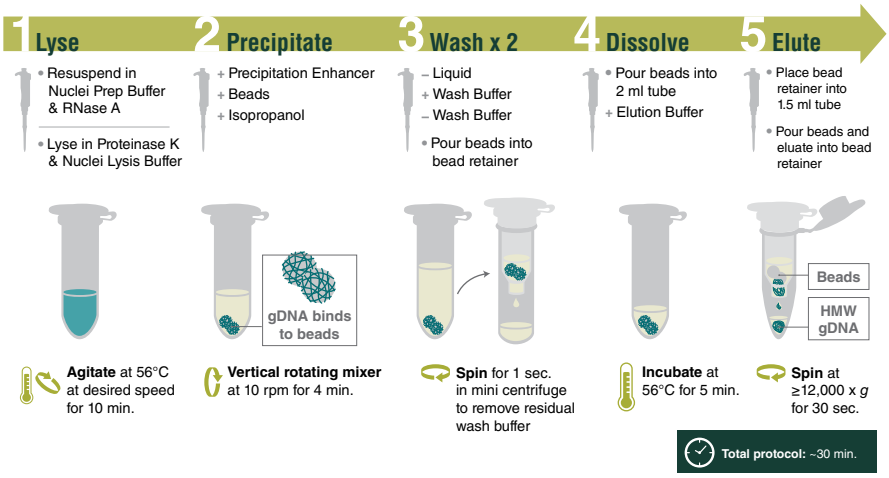
Try the NEBNext® Companion Module for Oxford Nanopore Technologies Ligation Sequencing (NEB #E7180) for library prep after extraction.

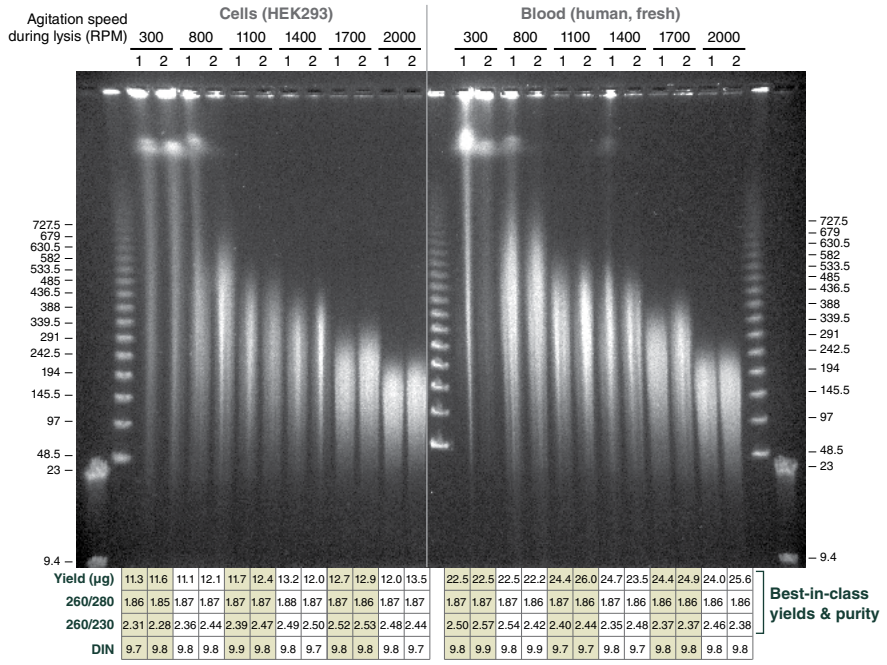
Description: Monarch HMW DNA Extraction Kits provide a rapid and reliable process for extracting high molecular weight DNA (HMW DNA) from biological samples including cells, blood, tissue, bacteria and other sample types. Utilizing an optimized process that combines gentle cell lysis with a tunable fragment length generation, followed by precipitation of the extracted DNA onto the surface of large glass beads, the prep proceeds rapidly. DNA size ranges from 50-250 kb for the standard protocol and into the Mb range on several sample types when the lowest agitation speeds are used. Purified DNA is recovered in high yield with excellent purity, including nearly complete removal of RNA. Purified HMW gDNA is easy to dissolve and is suitable for a variety of downstream applications including long-read sequencing (e.g., PacBio® and Oxford Nanopore Technologies®).

The Monarch HMW DNA Extraction Kits Include:

- DNA Capture Beads & Bead Retainers
- 2 ml Tubes & Collection Tubes II
- RNase A
- Proteinase K, Molecular Biology Grade
- RBC Lysis Buffer (NEB #T3050 only)
- gDNA Nuclei Prep & Nuclei Lysis Buffers (NEB #T3050 only)
- Precipitation Enhancer (NEB #T3050 only)
- Protein Separation Solution (NEB #T3060 only)
- Pestles & Pestle Tubes (NEB #T3060 only)
- HMW gDNA Tissue Lysis Buffer (NEB #T3060 only)
- gDNA Wash Buffer & Elution Buffer II

Workflow for cells.





DNA fragment size is tunable based on agitation speed during lysis. *Preps were performed on duplicate aliquots of 1 x 10^6 HEK 293 cells and 500 µl fresh human blood. Samples were agitated at the indicated speed during the lysis step to control the fragmentation of the DNA. Equal amounts of DNA from the replicates (cells: 500 ng; blood: 650 ng) were resolved by PFGE (1% agarose gel, 6 V/cm, 13°C for 20 hours, switch times ramped from 0.5–94 seconds on a BioRad® CHEF-DR III System). Yield and purity ratios of the individual preps are shown in the accompanying tables. Lambda PFG Ladder and Lambda DNA-Hind III Digest (NEB #N0341 and #N3012) were used as molecular weight standards. Yield, purity ratios and DINs of the individual preps are shown in the accompanying tables.*

Monarch® PCR & DNA Cleanup Kit (5 µg)

#T1030S 50 preps
#T1030L 250 preps

Companion Products:

Monarch DNA Cleanup Columns (5 µg)
#T1034L 100 columns

Monarch DNA Wash Buffer
#T1032L 25 ml

Monarch Plasmid Miniprep Kit
#T1010S 50 preps
#T1010L 250 preps

Monarch DNA Cleanup Binding Buffer
#T1031L 175 ml

Monarch DNA Gel Extraction Kit
#T1020S 50 preps
#T1020L 250 preps

Monarch DNA Elution Buffer
#T1016L 25 ml

- *Elute in as little as 6 µl*
- *Prevent buffer retention and salt carry-over with optimized column design*
- *Purify small DNA and oligos with a slight protocol modification*
- *Save time with fast, user-friendly protocol*
- *Purchase optimized kit formats or buffers & columns separately for your convenience*

With the Monarch PCR & DNA Cleanup Kit, you can purify your DNA in as little as 5 minutes.

Prefer an enzymatic cleanup approach? Check out the Exo-CIP Rapid PCR Cleanup Kit.

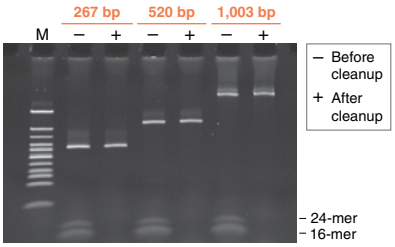
Description: The Monarch PCR & DNA Cleanup Kit (5 µg) is a rapid and reliable method for the purification and concentration of up to 5 µg of high-quality, double-stranded DNA from enzymatic reactions such as PCR, restriction digestion, ligation and reverse transcription. This method employs a bind/wash/elute workflow with minimal incubation and spin times, resulting in purification in less than 5 minutes. DNA Cleanup Binding Buffer is used to dilute the samples and ensure they are compatible for loading onto the proprietary silica matrix under high salt conditions. The DNA Wash Buffer ensures enzymes, short primers (≤ 40 nt), detergents and other low-molecular weight reaction components (e.g., nucleotides, DMSO, betaine) are removed, thereby allowing low-volume elution of concentrated, high-purity DNA. Eluted DNA is ready for use in restriction digests, DNA sequencing, ligation and other enzymatic manipulations. The unique column design ensures no buffer retention and no carryover of contaminants, allowing elution of sample in volumes as low as 6 µl. A slight protocol modification enables purification of small DNA and oligonucleotides.

Applications:

- PCR cleanup
- Enzymatic reaction cleanup
- cDNA cleanup
- Labeling cleanup
- Plasmid cleanup
- Oligonucleotide cleanup

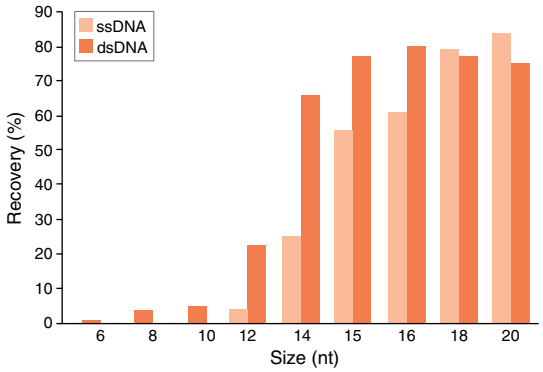
Kit Includes:

- DNA Elution Buffer
- DNA Cleanup Columns (5 µg)
- Collection Tubes (2 ml)
- DNA Wash Buffer
- DNA Cleanup Binding Buffer



Monarch PCR & DNA Cleanup Kit (5 µg) removes low molecular weight primers from dsDNA samples. *Three independent amplicons (267 bp, 520 bp, 1003 bp) were spiked with two oligonucleotides (16-mer, 24-mer) to a final concentration of 1 µg. Half of each mix was purified with the Monarch PCR & DNA Cleanup Kit (5 µg) following the included protocol. Equivalent fractions of the original mixture and the eluted material were resolved on a 20% TBE acrylamide gel at 100V for one hour and stained with SYBR® Green II.*

Specifications	
Binding Capacity	5 µg
DNA Size Range	~50 bp–25 kb DNA ≥ 15 bp to 25 kb (dsDNA) and DNA ≥ 18 nt to 10 kb (ssDNA) can also be purified using the Oligonucleotide Cleanup Protocol
Elution Volume	≥ 6 µl
Typical Recovery	DNA 50 bp–10 kb 70–90% DNA 11–23 kb 50–70% ssDNA ≥ 18 nt and dsDNA ≥ 15 bp 70–85%
Protocol Time:	5 minutes



Recovery of ssDNA and dsDNA oligonucleotides (1 µg) using the Oligonucleotide Cleanup Protocol and the Monarch PCR & DNA Cleanup Kit. *Synthesized ssDNA and dsDNA oligonucleotides (1 µg in 50 µl H_2O) of varying lengths (6-20 nt) were purified using the Oligonucleotide Cleanup Protocol and the Monarch PCR & DNA Cleanup Kit (NEB #T1030) and were eluted in 50 µl water. The average percent recovery (n=3) of the oligonucleotides was calculated from the resulting A260 as measured using a Trinean DropSense™ 16. Use of the Oligonucleotide Cleanup Protocol and the Monarch PCR & DNA Cleanup Kit (NEB #T1030) results in the efficient removal of small oligonucleotides (6-12 nt) and > 70% recovery and cleanup of oligonucleotides ≥ 15 bp (dsDNA) or ≥ 18 nt (ssDNA).*

View tips for using the Monarch PCR & DNA Cleanup Kit.

Monarch® DNA Gel Extraction Kit

#T1020S 50 preps
#T1020L 250 preps

Companion Products:

Monarch Gel Dissolving Buffer
#T1021L 235 ml

Monarch Plasmid Miniprep Kit
#T1010S 50 preps
#T1010L 250 preps

Monarch PCR & DNA Cleanup Kit (5 µg)
#T1030S 50 preps
#T1030L 250 preps

Monarch DNA Wash Buffer
#T1032L 25 ml

Monarch DNA Cleanup Columns (5 µg)
#T1034L 100 columns

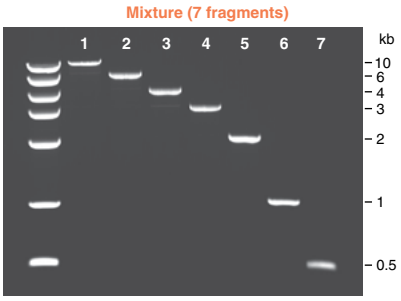
β-Agarase I
#M0392S 100 units
#M0392L 500 units

Monarch DNA Elution Buffer
#T1016L 25 ml

Monarch Genomic DNA Purification Kit
#T3010S 50 preps
#T3010L 150 preps

- *Elute in as little as 6 µl*
- *Prevent buffer retention and salt carry-over with optimized column design*
- *Save time with fast, user-friendly protocol*
- *Purchase optimized kit formats or buffers & columns separately for your convenience*

Description: The Monarch DNA Gel Extraction Kit rapidly and reliably purifies up to 5 µg of concentrated high-quality, double-stranded DNA from agarose gels. This method employs a bind/wash/elute workflow with minimal incubation and spin times, resulting in purification in less than 15 minutes. The Monarch Gel Dissolving Buffer is used to digest the agarose gel slice and ensure the sample is compatible for loading the DNA onto the proprietary silica matrix under high salt conditions. The wash buffer ensures trace amounts of DNA binding dyes, electrophoresis buffer salts and gel loading buffer components are removed. Low-volume elution produces concentrated, highly pure DNA ready for use in restriction digests, DNA sequencing, ligation, and other enzymatic manipulations. The unique column design ensures no buffer retention and no carryover of contaminants, allowing elution of sample in volumes as low as 6 µl.

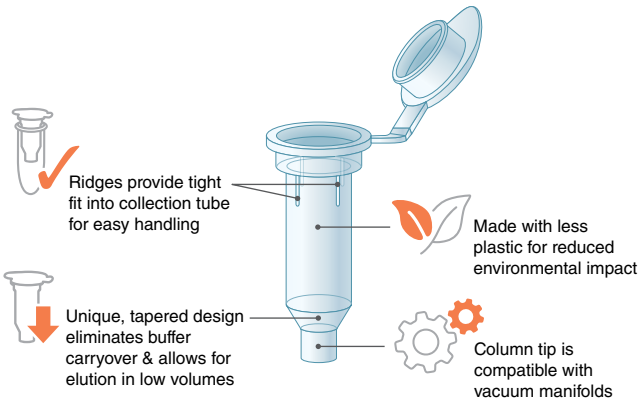


Monarch DNA Gel Extraction Kit reproducibly recovers DNA over a broad range of molecular weights. *A mixture of 7 DNA fragments ranging from 10 kb down to 0.5 kb was prepared and one-half of the mixture was resolved on a 1% gel. Each fragment was manually excised from the agarose gel and processed using the Monarch DNA Gel Extraction Kit. The entire elution of each fragment was resolved on a new gel with the remainder of the original mixture for comparison.*

Kit Includes:

- DNA Elution Buffer
- DNA Cleanup Columns (5 µg)
- Collection Tubes (2 ml)
- DNA Wash Buffer
- Gel Dissolving Buffer

Specifications	
Binding Capacity	5 µg
DNA Size Range	50 bp–25 kb
Elution Volume	≥ 6 µl
Typical Recovery	DNA 50 bp–10 kb 70–90% DNA 11–25 kb 50–70%
Protocol Time:	15 minutes



Our optimized column design supplied with the Monarch DNA Gel Extraction and PCR & DNA Cleanup Kits eliminates buffer retention.

View tips for using the Monarch Gel Extraction Kit.

Monarch® Plasmid Miniprep Kit

#T1010S 50 preps
#T1010L 250 preps

Companion Products:

Exonuclease V (RecBCD)
#M0345S 1,000 units
#M0345L 5,000 units

Monarch DNA Gel Extraction Kit
#T1020S 50 preps
#T1020L 250 preps

Monarch PCR & DNA Cleanup Kit (5 µg)
#T1030S 50 preps
#T1030L 250 preps

Monarch Genomic DNA Purification Kit
#T3010S 50 preps
#T3010L 150 preps

Monarch Plasmid Miniprep Columns
#T1017L 100 columns

Monarch Plasmid Resuspension Buffer (B1)
#T1011L 55 ml

Monarch Plasmid Lysis Buffer (B2)
#T1012L 54 ml

Monarch Plasmid Neutralization Buffer (B3)
#T1013L 110 ml

Monarch Plasmid Wash Buffer 1
#T1014L 54 ml

Monarch Plasmid Wash Buffer 2
#T1015L 30 ml

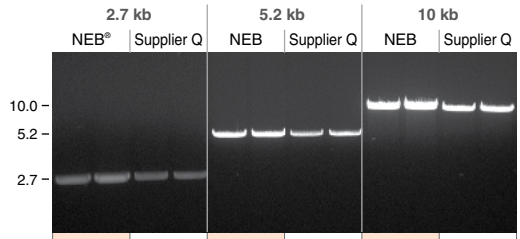
Monarch DNA Elution Buffer
#T1016L 25 ml

- *Prevent buffer retention and salt carry-over with optimized column design*
- *Monitor completeness of certain steps using colored buffer system*
- *No need to add RNase before starting*
- *Elute in low volumes*
- *Purchase optimized kit formats or buffers & columns separately for your convenience*
- *Easily label columns using tab and frosted surfaces*

Description: The Monarch Plasmid Miniprep Kit is a rapid and reliable method for the purification of high-quality plasmid DNA. This method employs standard cell resuspension, alkaline lysis and neutralization steps, with the additional benefit of color indicators at certain steps to easily monitor completion. After clarification of the lysate by centrifugation, the DNA is bound to the proprietary silica matrix under high salt conditions. Unique wash buffers ensure salts, proteins, RNA and other cellular components (endotoxins) are removed, allowing low-volume elution of concentrated, highly pure DNA, ready for use in restriction digests, DNA sequencing, PCR and other enzymatic manipulations.

Kit Includes:

- Plasmid Miniprep Columns
- DNA Elution Buffer
- Plasmid Wash Buffer 2
- Plasmid Wash Buffer 1
- Plasmid Neutralization Buffer (B3)
- Plasmid Lysis Buffer (B2)
- Plasmid Resuspension Buffer (B1)



	2.7 kb		5.2 kb		10 kb	
	NEB®	Supplier Q	NEB	Supplier Q	NEB	Supplier Q
Conc. (ng/µl)	334.9	202.2	384.2	216.4	607.6	351.0
Total yield (µg)	10.0	10.1	11.5	10.8	18.2	17.6
A_{260}/A_{280}	1.9	1.9	1.9	1.9	1.9	1.9
A_{260}/A_{230}	2.4	2.3	2.4	2.4	2.3	2.4

Monarch Plasmid Miniprep Kits consistently yield more concentrated plasmid DNA with equivalent purity and functionality as the leading supplier. *Preps were performed according to recommended protocols using 1.5 ml aliquots of the same overnight culture. One microliter of each prep was digested with HindIII-HF (NEB #R3104) to linearize the vector and the digests were resolved on a 1% w/v agarose gel.*

Specifications	
Culture Volume	1-5 ml, not to exceed 15 OD units
Binding Capacity	up to 20 µg
Plasmid Size	up to 25 kb
Typical Recovery	up to 20 µg. Yield depends on plasmid copy number, host strain, culture volume, and growth conditions.
Elution Volume	≥ 30 µl
Purity	$A_{260/280}$ and $A_{260/230}$ ≥ 1.8
Protocol Time	10½ minutes of spin and incubation time
Compatible Downstream Applications	restriction digestion and other enzymatic manipulations, transformation, transfection, DNA sequencing, PCR, labeling, cell-free protein synthesis, etc.

View tips for using the Monarch Plasmid Miniprep Kit.

Can we stop global food insecurity?

Food insecurity is the lack of a consistent supply of nutritious food necessary for human development, activity and health. Alarmingly, about 800 million people face food insecurity in the world today. Climate change is expected to add over 180 million more by 2050. Farms and fisheries are threatened, especially in hotter climates with poorer communities. Nonetheless there is cause for optimism.

Geographical disparities in seasonal temperatures and water cycles can affect food production. Water evaporation increases as greenhouse gases trap heat in the Earth's atmosphere. Weather patterns can change as oceans absorb heat. Dryer landscapes can experience more droughts, and flood-prone landscapes can experience more storms. These are the breaking points.

Climate change is likely to worsen crop failures in regions of extreme poverty. Weather extremes have been the primary drivers of food insecurity in African countries. Madagascar is drought prone and relies on subsistence agriculture. In 2021, both Madagascar's Grand Sud and Southwestern Angola experienced the worst drought in forty years, pushing 14,000 people into food insecurity.

Paradoxically, the warmer weather and higher CO_2 levels that increase crop growth at higher latitudes can also hinder food security. Grains grow starchier, which may dilute protein, lipid, vitamin, and mineral nutrients. Crop disease outbreaks occur at uncharacteristic times and locations such that temperature-dependent infection risk for common pathogens has been proposed to track crop yields under climate change. Agriculture in milder climates contends with both imbalanced plant stoichiometry and changes in pathogen range and severity.

Fisheries have a critical role in food security that is also hit harder in socioeconomically vulnerable regions. Seafood production is declining worldwide. Fish species are moving poleward, away from the tropics. Fish population distribution changes are tied to seafood shortages and higher costs.

Encouragingly, communities facing regional stresses to food security have been empowered with practical knowledge and support. The Food and Agriculture Organization of the United Nations (FAO) works in developing countries to improve production and post-harvest processing, while focusing on downstream consumption and redistributing surplus. The FAO Global Partnership Initiative for Plant Breeding Capacity Building promotes the availability of genetic resources, technology and training for food and agriculture in vulnerable countries. As examples, genetically modified, drought tolerant sugarcane and maize are grown in Indonesia and alfalfa with reduced lignin improves livestock milk production and reduces soil damage in Argentina. Micronutrient deficiencies are addressed using biofortification – a process of selective breeding or genetic engineering that increases essential nutrients. Zimbabwe communities have adopted biofortified Orange Maize, Orange Fleshed Sweet Potato, and zinc- and iron-enriched beans suited to their farming conditions. At the same time, the FAO has provided data collection resources to industrial nations to target food waste. South Korea has used technical food waste measurement to develop the most advanced food waste reduction infrastructure in the world. Instead of landfill or incineration, food waste is recycled into biomass, biofuel, compost, and even livestock feed (once processed and sterilized).

Global food insecurity is an urgent issue exacerbated by climate change that can be counteracted. Conditions are expected to worsen for the poorest populations and world food prices have never been higher. Yet, higher food productivity can enable price stability. Communities that adopt agroecological management and sustainable fishing practices have become more resilient. Matching the pace of these efforts to climate change is vital to the common good.

Learn more about the Food and Agriculture Organization.

Fungal diseases on the ear of brewing barley.
Credit: Oleksandr. Adobe Stock

NEBNext® Reagents for Next Generation Sequencing

Leading the way in library preparation for next generation sequencing.

Library preparation is a critical part of the next generation sequencing workflow; successful sequencing requires the generation of high quality libraries of sufficient yield and quality.

As sequencing technologies continue to improve and capacities expand, the need for high performance sample prep is greater than ever, from decreasing input quantities and samples of lower quality, for an expanding range of applications.

To meet these growing challenges, the NEBNext suite of products continues to evolve to support next generation sequencing with sample preparation tools that expand applications, streamline workflows, minimize inputs, and improve library quality and yields.

NEBNext reagents are available for sample preparation for DNA, RNA, ChIP, FFPE, small RNA, single cell and microbiome samples, for use with Illumina®, Oxford Nanopore Technologies®, Ion Torrent™ and other sequencing platforms. Kits for specialized applications including methylome analysis, virus sequencing and immune repertoire sequencing are also included in the NEBNext line.

Products are in user-friendly formats including kits and modules. A fast-growing range of adaptors and primers is available separately, for maximized flexibility. Use of NEBNext products has been cited in tens of thousands of peer-reviewed publications.

For additional convenience and cost-effectiveness in high-throughput workflows, NEBNext reagents are also available in bulk and customized formats. For more information, contact Custom@neb.com.

Find an overview of NGS library preparation.

Featured Products

150 NEBNext Ultra II FS DNA Library Prep Kits

152 NEBNext Enzymatic Methyl-seq

158 NEBNext Ultra II RNA Library Prep Kits

160 NEBNext rRNA Depletion for Human/Mouse/Rat v2

160 NEBNext rRNA Depletion for Bacteria

161 Customizable RNA Depletion – NEBNext RNA Depletion Core Reagent Set

165 NEBNext Adaptors & Primers

Featured Tools & Resources

Visit **NEBNextSelector.neb.com** for help with selecting products.

Visit the **NEBNext Custom RNA Depletion Design Tool** to obtain custom probe sequences.

Visit **NEBNext.com** to keep up to date on everything NEBNext.

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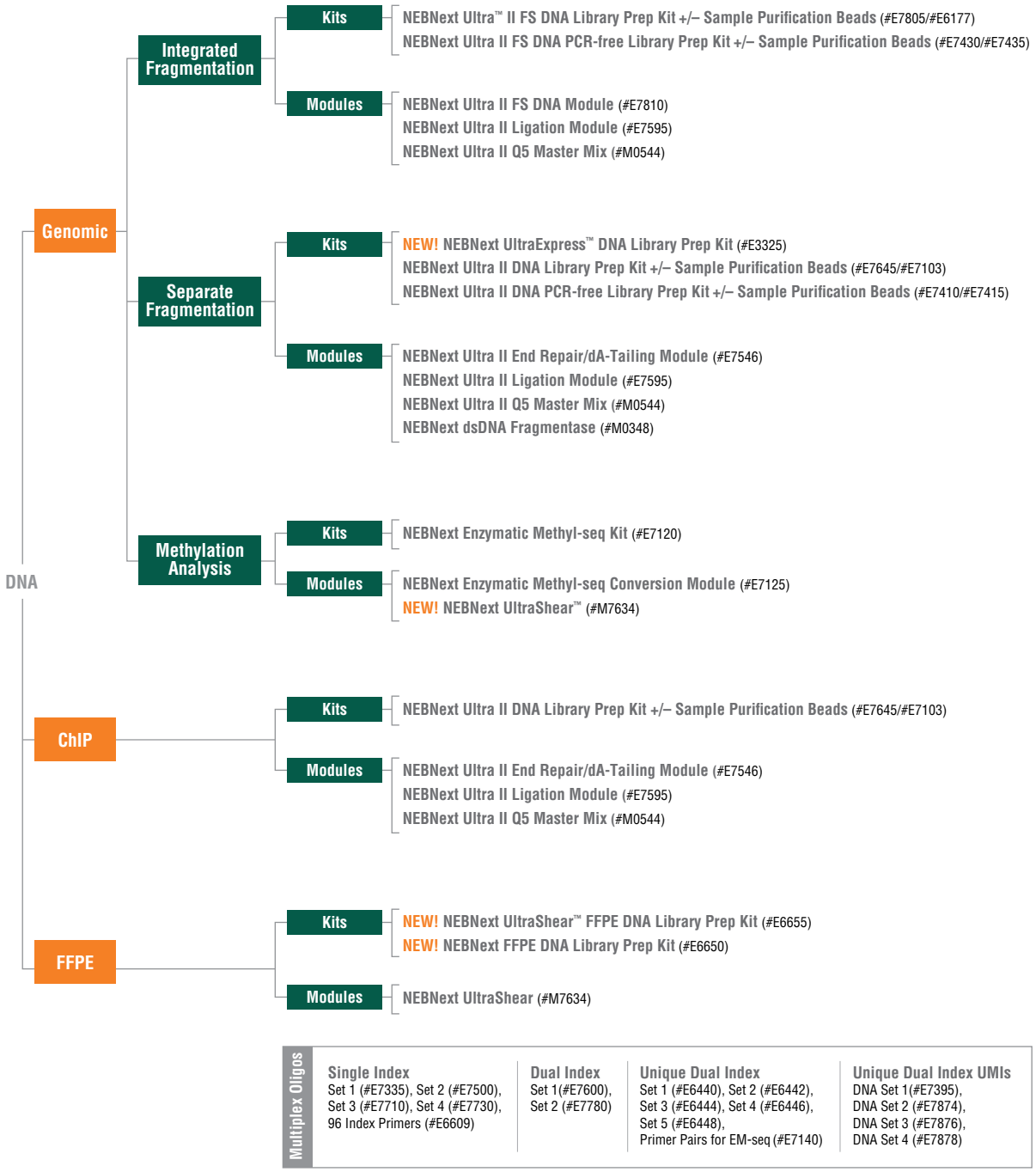
Illumina® DNA Product Selection Chart

Use the following chart to determine the best NEBNext® products for your Illumina DNA library prep needs. For the most up-to-date product and pricing information, visit **NEBNext.com**.



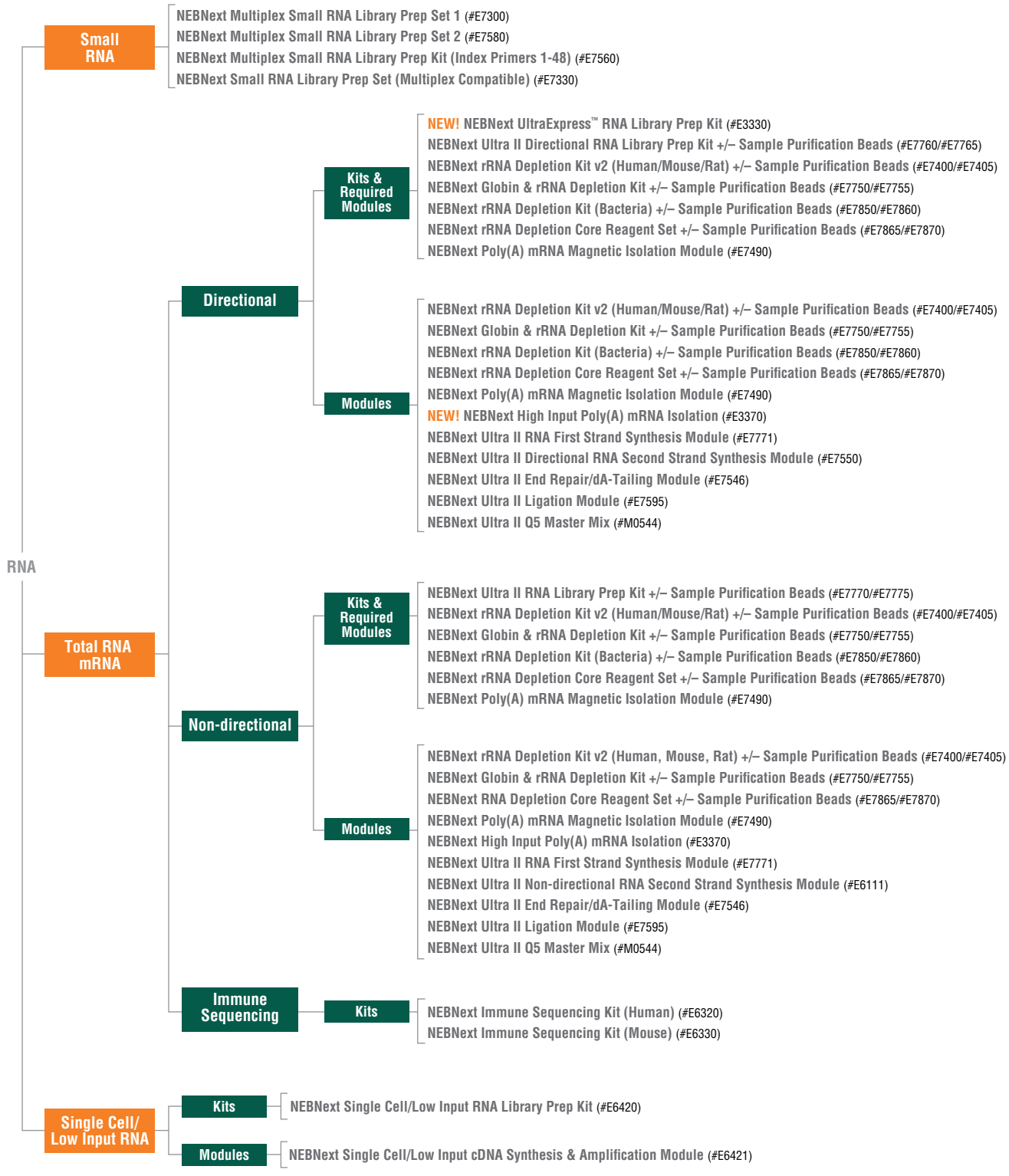
Reagents for the original Ultra workflow are also available.

NEBNext® Selector v1.0

For help selecting products, try our online product selection tool at **NEBNextSelector.neb.com**

Illumina® RNA Product Selection Chart

Use the following chart to determine the best NEBNext products for your Illumina RNA sequencing needs. For the most up-to-date product and pricing information, visit **NEBNext.com**.



Multiplex Oligos

Single Index	Dual Index	Unique Dual Index	Unique Dual Index UMIs
Set 1 (#E7335), Set 2 (#E7500), Set 3 (#E7710), Set 4 (#E7730), 96 Index Primers (#E6609)	Set 1(#E7600), Set 2 (#E7780)	Set 1 (#E6440), Set 2 (#E6442), Set 3 (#E6444), Set 4 (#E6446), Set 5 (#E6448), Primer Pairs for EM-seq (#E7140)	RNA Set 1 (#E7416)

Reagents for the original Ultra workflow are also available.

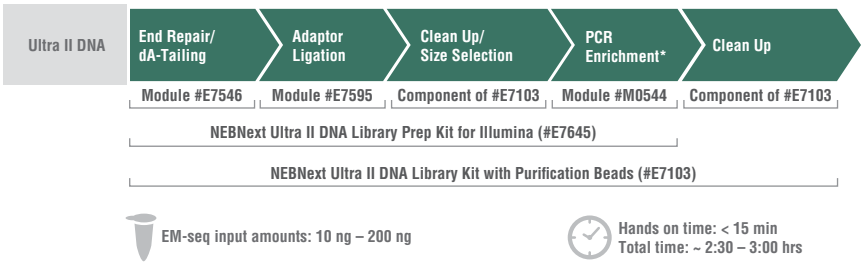
The heart of the matter – NEBNext® Ultra™ II Workflow

As sequencing technologies continue to improve and applications expand, the need for compatibility with ever-decreasing input amounts and sub-optimal sample quality grows. Reliability and high performance are critical, along with faster turnaround, higher throughput and automation compatibility.

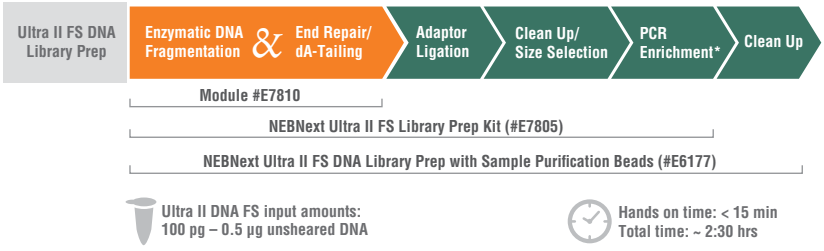
The NEBNext Ultra II workflow lies at the heart of NEB's portfolio for next generation sequencing library preparation. NEBNext Ultra II kits and modules for Illumina are the perfect combination of reagents, optimized formulations and simplified workflows, enabling you to generate DNA or RNA libraries of the highest quality and yield, even when starting from extremely low input amounts.

- *Learn one central workflow and apply it to a suite of different applications*
- *Save time with streamlined modular workflows, reduced hands-on time and automation compatibility*
- *Benefit from low input amount requirements, fewer PCR cycles and uniform GC coverage in all applications*

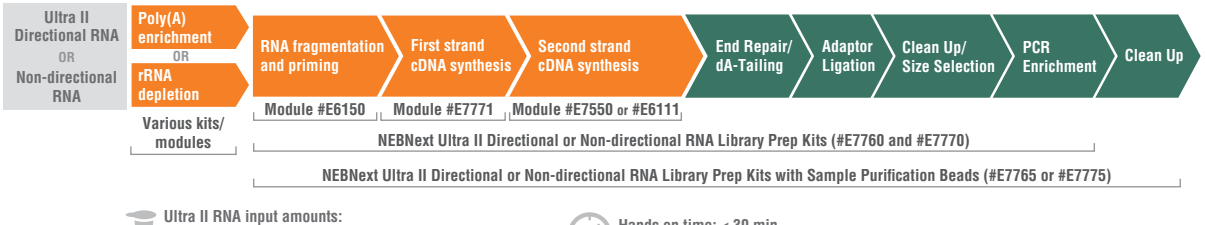
The Ultra II workflow is central to many NEBNext solutions, including:

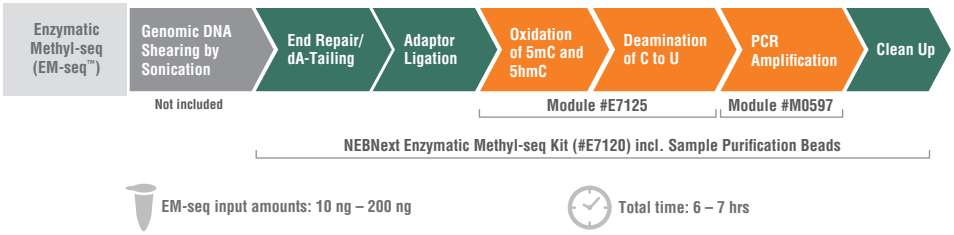


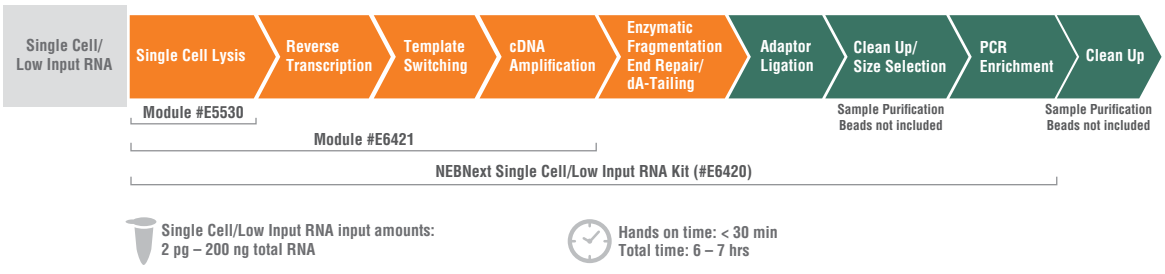
**PCR-free workflows are also available*



**PCR-free workflows are also available*







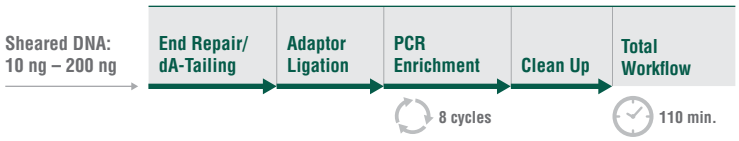
NEW

NEBNext UltraExpress™ DNA Library Prep Kit

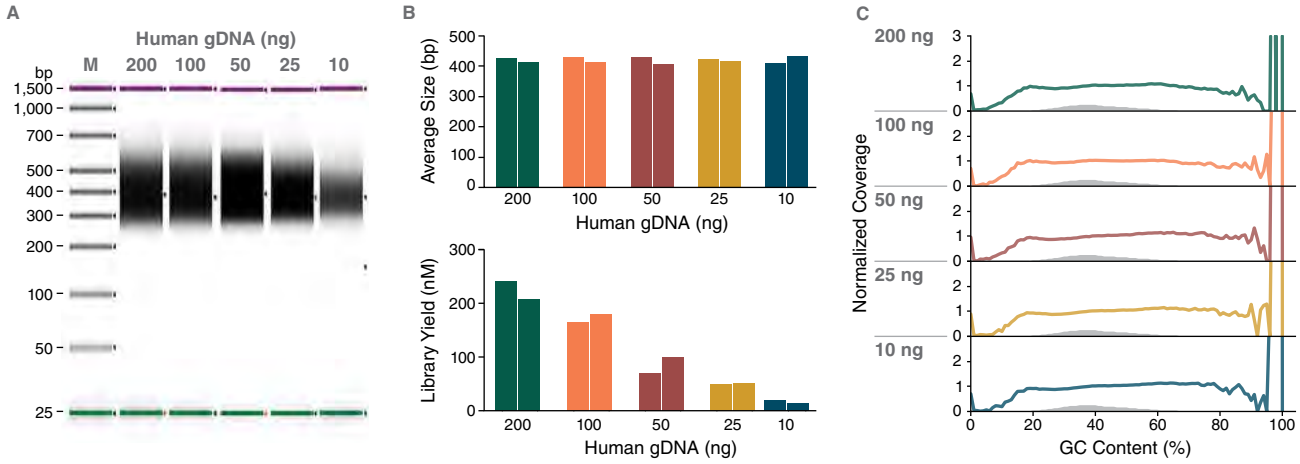
#E3325S	24 reactions
#E3325L	96 reactions

- *Fast workflow (< 2 hours)*
- *Fewer steps and consumables*
- *Fewer cleanups*
- *Wide input range (10-200 ng pre-sheared DNA)*
- *Single protocol for all inputs*
- *Automation friendly*

The NEBNext UltraExpress DNA Library Prep Kit is the latest generation of NEBNext DNA library prep, with a fast, streamlined workflow to generate high yields of high-quality libraries. The workflow allows processing of samples with a wide range of input amounts of pre-sheared DNA using a single protocol, without adjustment of reaction conditions.



NEBNext UltraExpress DNA Workflow



NEBNext UltraExpress DNA generates high yields of high quality libraries, across a broad input range.
A-B. *Libraries were made using 10-200 ng Covaris®-sheared Human NA19240 genomic DNA and the NEBNext UltraExpress™ DNA Library Prep Kit, with the same amount of adaptor and the same PCR conditions (8 cycles) for each. Libraries were pooled and sequenced on the Illumina® MiSeq®.*
C. *140,000 paired end reads were sampled (seqtk v1.3), adapter-trimmed (seqprep v0.1) and aligned to GRCh38 reference genome (bowtie2 v2.4.5). Libraries had high yields, uniform library profiles and even GC coverage.*

Polly and Harry are members of NEB's OEM and Customized Solutions Department. Polly initially joined the NEB Production Department in 2018, and later moved to her current role as a Program Manager. Harry joined NEB in 2020 as a Program Manager.

NEBNext® Ultra™ II DNA, FS and PCR-free DNA Library Prep Kits for Illumina®

NEBNext Ultra II DNA Library Prep Kit for Illumina
#E7645S 24 reactions
#E7645L 96 reactions

NEBNext Ultra II DNA Library Prep with Sample Purification Beads
#E7103S 24 reactions
#E7103L 96 reactions

NEBNext Ultra II DNA PCR-free Library Prep Kit for Illumina
#E7410S 24 reactions
#E7410L 96 reactions

NEBNext Ultra II DNA PCR-free Library Prep with Sample Purification Beads
#E7415S 24 reactions
#E7415L 96 reactions

NEBNext Ultra II FS DNA Library Prep Kit for Illumina
#E7805S 24 reactions
#E7805L 96 reactions

NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads
#E6177S 24 reactions
#E6177L 96 reactions

NEBNext Ultra II FS DNA PCR-free Library Prep Kit for Illumina
#E7430S 24 reactions
#E7430L 96 reactions

NEBNext Ultra II FS DNA PCR-free Library Prep with Sample Purification Beads
#E7435S 24 reactions
#E7435L 96 reactions

- *Get more of what you need, with the highest library yields*
- *Generate high quality libraries even with limited amounts of DNA, as low as 500 pg*
- *Prepare libraries from ALL of your samples, including GC-rich targets and FFPE DNA samples*
- *Save time with streamlined workflows, reduced hands-on time, and automation compatibility*
- *Access reliable and easy-to-use, scalable enzymatic DNA fragmentation, integrated into the workflow with the FS kit*
- *Enjoy the flexibility and reliability of the gold standard SPRIselect size selection and clean-up beads, supplied in just the amounts you need*

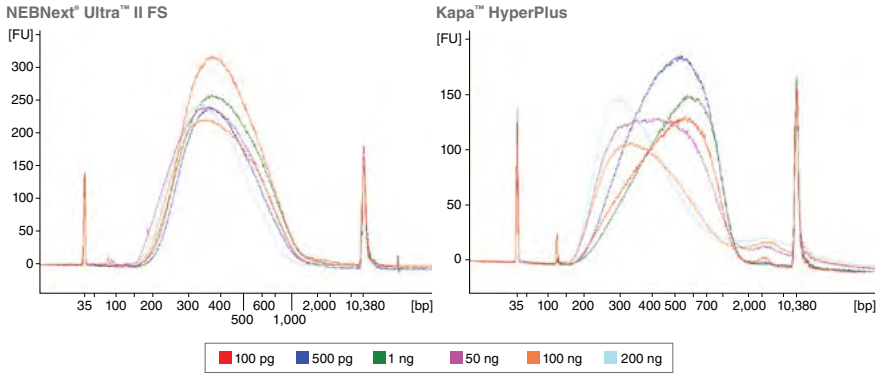
Visit **NEBNextUltraII.com** for more information, including our technical notes and protocol videos

Description: NEBNext Ultra II DNA Library Prep Kits for Illumina meet the challenge of constructing high quality libraries from ever-decreasing input quantities, enabling high yield preparation of high quality libraries from 500 picograms to 1 microgram of input DNA. Ultra II kits use a fast, streamlined, automatable workflow and enable use of fewer PCR cycles while also improving GC coverage. The kit is also effective with challenging samples such as FFPE DNA.

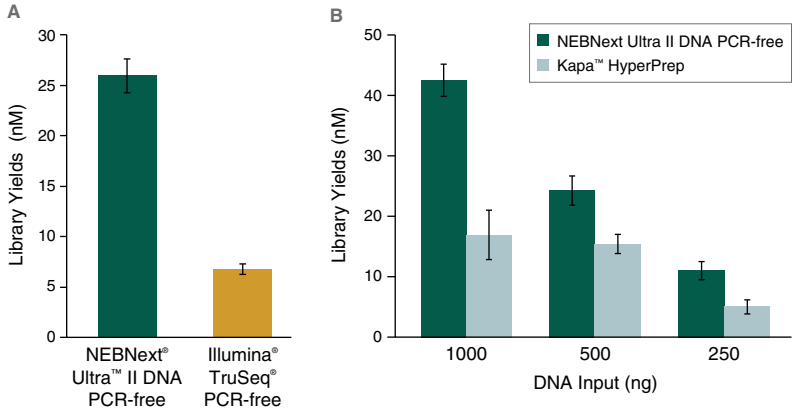
The Ultra II FS DNA Library Prep Kit combines robust enzymatic DNA fragmentation with end repair and dA-tailing, integrated into a streamlined library prep workflow.

PCR-free kits are now available for both the Ultra II DNA and Ultra II FS DNA workflows.

All Ultra II kits are available with or without SPRIselect® beads.



NEBNext Ultra II FS DNA provides consistent fragmentation regardless of input amount. *Libraries were prepared from Human NA19240 genomic DNA using the input amounts shown. NEBNext Ultra II FS libraries (left) were prepared using a 20-minute fragmentation time. For Kapa™ HyperPlus (right), input DNA was cleaned up with 3X beads prior to library construction, as recommended, and a 20-minute fragmentation time. Library size was assessed using the Agilent® Bioanalyzer®. Low input (1 ng and below) libraries were loaded on the Bioanalyzer without a dilution. High input libraries were loaded with a 1:5 dilution in 0.1X TE.*

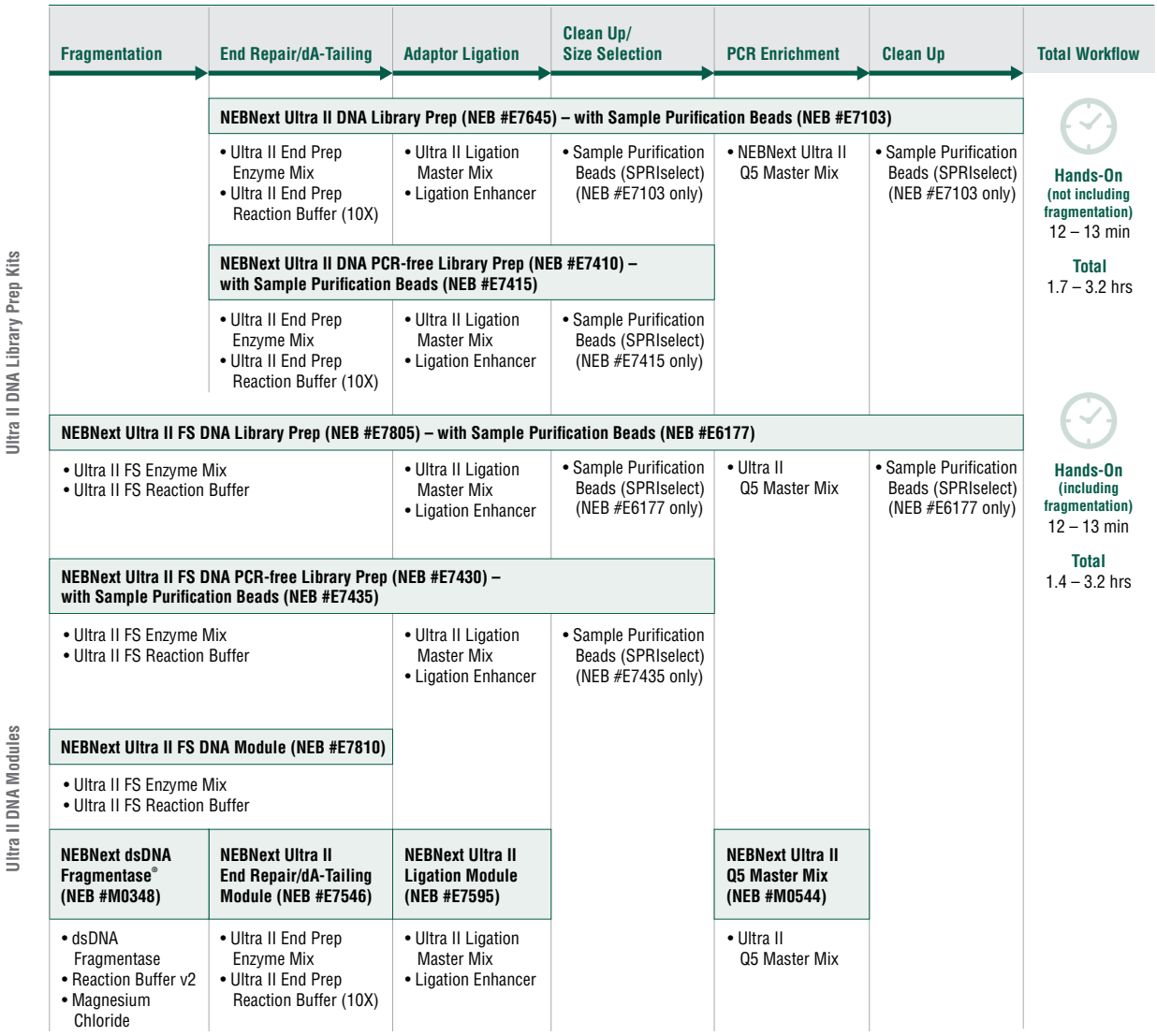


NEBNext Ultra II DNA PCR-free Library Prep Kit generates libraries with higher yields.
A. *PCR-free libraries were prepared with NA19240 genomic DNA (Coriell Institute) using NEBNext Ultra II DNA PCR-free and Illumina TruSeq® PCR-free library prep kits and size selected for 350 bp inserts. DNA inputs were 1 µg.*
B. *Libraries of 150-200 bp inserts were prepared using NEBNext Ultra II DNA PCR-free and Roche Sequencing Kapa HyperPrep library prep kits coupled with Covaris shearing without size selection. NEBNext Unique Dual Index UMI Adaptors DNA Set 1, IDT for Illumina (TruSeq DNA UD Indexes) and Kapa Dual-Indexed Adaptors were used for the NEBNext, Illumina, and Kapa kits, respectively, following manufacturers' recommendations.*

NEBNext® Ultra™ II DNA Reagents for Illumina® Sequencing

NEBNext Ultra II Kits for DNA are available with or without integrated enzymatic DNA fragmentation. Note that adaptors and primers for indexing are supplied separately. In addition to stringent QCs on individual components, the NEBNext DNA kits are also functionally validated by preparation of a library, followed by Illumina sequencing.

Input Ultra II DNA Workflow: 500 pg – 1 µg (PCR); 250 ng – 1,000 ng (PCR-free) Ultra II FS DNA Workflow: 500 pg – 1 µg (PCR); 50 ng – 500 ng (PCR-free)



NEBNext® Enzymatic Methyl-seq (EM-seq™)

NEBNext Enzymatic Methyl-seq Kit
#E7120S 24 reactions
#E7120L 96 reactions

NEBNext Enzymatic Methyl-seq Conversion Module
#E7125S 24 reactions
#E7125L 96 reactions

NEBNext Q5U Master Mix
#M0597S 50 reactions
#M0597L 250 reactions

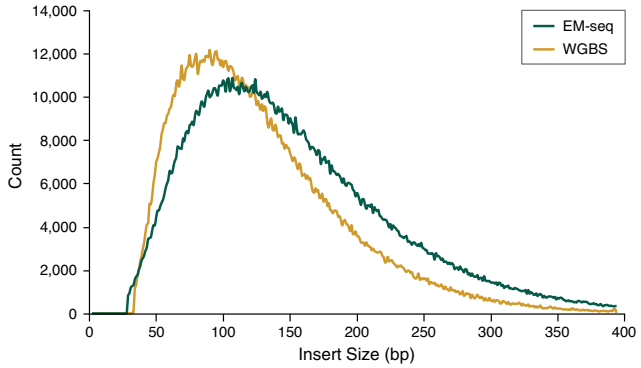
NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)
#E7140S 24 reactions
#E7140L 96 reactions

- *Superior sensitivity of 5mC and 5hmC detection*
- *Larger library insert sizes*
- *More uniform GC coverage*
- *Greater mapping efficiency*
- *High-efficiency library preparation*

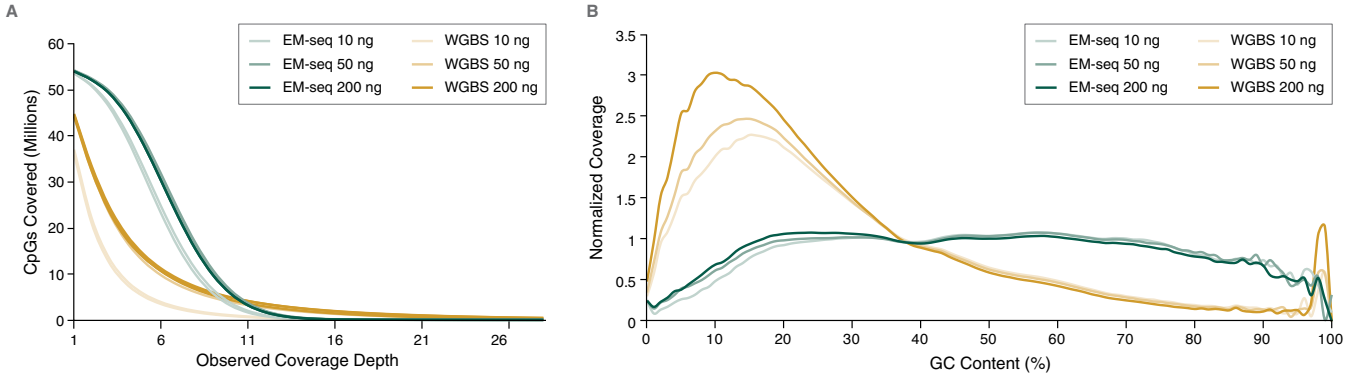
NEBNext Enzymatic Methyl-seq is an enzymatic alternative to bisulfite conversion with superior performance. For more information, including extensive performance data, visit **NEBNext.com.**

Description: While bisulfite sequencing has been the gold standard for the study of DNA methylation, this conversion treatment is damaging to DNA, resulting in DNA fragmentation, loss and GC bias. The NEBNext Enzymatic Methyl-seq Kit (EM-seq™) provides an enzymatic alternative to whole genome bisulfite sequencing (WGBS), combined with high efficiency streamlined library preparation suitable for Illumina sequencing.

The highly effective EM-seq enzymatic conversion minimizes damage to DNA and, in combination with the supplied NEBNext Ultra II library preparation workflow reagents, results in high quality libraries that enable superior detection of 5mC and 5hmC from fewer sequencing reads.



NEBNext Enzymatic Methyl-seq libraries have larger insert sizes. *50 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold™ kit for bisulfite conversion. Libraries were sequenced on an Illumina MiSeq (2 x 76 bases) and insert sizes were determined using Picard 2.18.14. The normalized frequency of each insert size was plotted, illustrating that library insert sizes are larger for EM-seq than for WGBS, and indicating that EM-seq does not damage DNA as bisulfite treatment does in WGBS.*



EM-seq identifies more CpGs than WGBS, at lower sequencing coverage depth with superior uniformity of GC coverage. *10, 50 and 200 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold Kit for bisulfite conversion. Libraries were sequenced on an Illumina NovaSeq® 6000 (2 x 100 bases). Reads were aligned to hg38 using bwa-meth 0.2.2. A: Coverage of CpGs with EM-seq and WGBS libraries was analyzed using 324 million paired end reads, and each top and bottom strand CpGs were counted independently, yielding a maximum of 56 million possible CpG sites. EM-seq identifies more CpGs at lower depth of sequencing. B: GC coverage was analyzed using Picard 2.17.2 and the distribution of normalized coverage across different GC contents of the genome (0-100%) was plotted. EM-seq libraries have significantly more uniform GC coverage, and lack the AT over-representation and GC under-representation typical of WGBS libraries.*

View the EM-seq workflow.

NEW

NEBNext® Enzymatic 5hmC-seq (E5hmC-seq™)

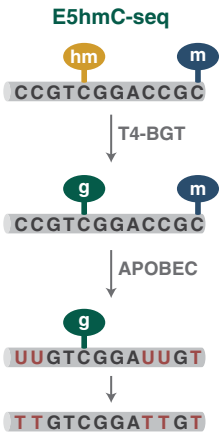
NEBNext Enzymatic 5hmC-seq Kit
#E3350S 24 reactions
#E3350L 96 reactions

NEBNext Enzymatic 5hmC-seq Conversion Module
#E3365S 24 reactions
#E3365L 96 reactions

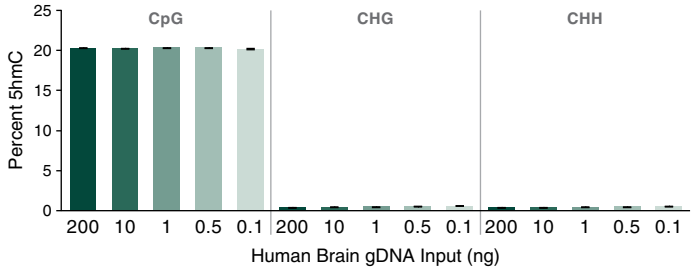
NEBNext Oligos for Enzymatic 5hmC-seq
#E3360S 24 reactions
#E3360L 96 reactions

- *Enzyme-based workflow enables high yields and high-quality data*
- *0.1 ng – 200 ng inputs*
- *Minimal GC bias*
- *E5hmC-seq and EM-seq data can be combined*
- *Conversion module also available separately*

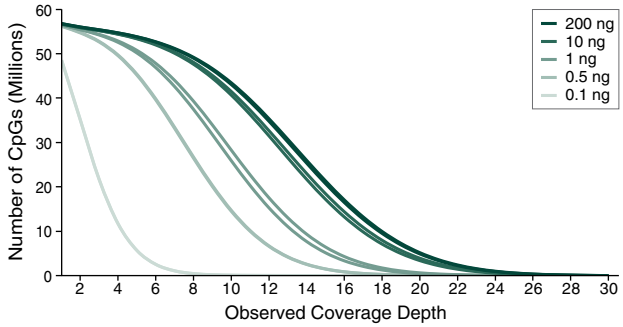
While NEBNext Enzymatic Methyl-seq (EM-seq) detects both 5mC and 5hmC, it does not distinguish between them. Specific detection of 5hmC sites is now enabled by the NEBNext Enzymatic 5hmC-seq Kit (E5hmC-seq™). The kit includes NEBNext Ultra II library prep reagents, and 5hmC is detected using a two-step enzymatic conversion workflow (Figure 1), that minimizes damage to DNA and allows discrimination of 5hmC from both cytosine and 5mC after Illumina sequencing. E5hmC-seq data can also be subtracted from EM-seq data, allowing determination of the precise location of individual 5mC and 5hmC sites.



E5hmC-seq conversion method. *To enable specific 5hmC detection, 5hmC is first glucosylated using T4-BGT. 5mC and unmodified cytosine are then deaminated by APOBEC to thymine and uracil, respectively, while the protected 5hmC is unconverted. During Illumina sequencing 5hmCs are represented as cytosine, while cytosine and 5mCs are represented as thymine.*



E5hmC provides consistent total 5hmC detection across a range of inputs. *0.1 ng to 200 ng of human brain genomic DNA was sheared to 350 bp using the Covaris ME220 instrument and used as input into the E5hmC-seq workflow. Libraries were sequenced on an Illumina NovaSeq 6000 (2 x 150 bases). Approximately 715 million reads for each library were aligned to the T2T version of the human genome using bwa-meth, and methylation information was extracted from the alignments using MethylDackel. Detected 5hmC levels are similar between all inputs in the CpG, CHG and CHH contexts. Values shown are the average of two technical replicates and error bars are +/– standard deviation.*



E5hmC-seq exhibits high CpG coverage for all inputs. *Reads were aligned to the T2T version of the human genome using bwa-meth. Coverage of CpGs in the human genome with E5hmC-seq was analyzed using 715 million reads. Top and bottom strand CpGs were counted independently, yielding a maximum of 67.8 million possible CpG sites in the T2T genome. E5hmC-seq consistently covers over 56 million CpG sites for 0.5 ng to 200 ng inputs and roughly 48 million CpG sites for 0.1 ng input libraries.*

NEW

NEBNext® FFPE DNA Library Prep & NEBNext UltraShear™ FFPE DNA Library Prep Kits

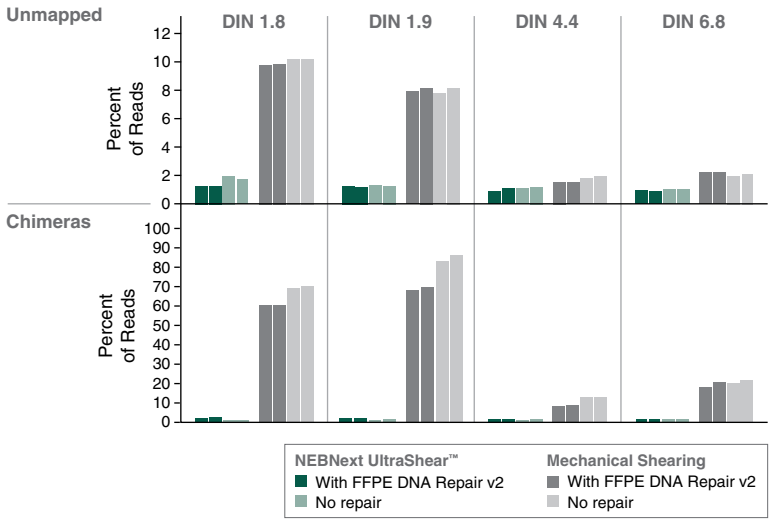
NEBNext FFPE DNA Library Prep Kit
#E6650S 24 reactions
#E6650L 96 Reactions

NEBNext UltraShear FFPE DNA Library Prep Kit
#E6655S 24 reactions
#E6655L 96 Reactions

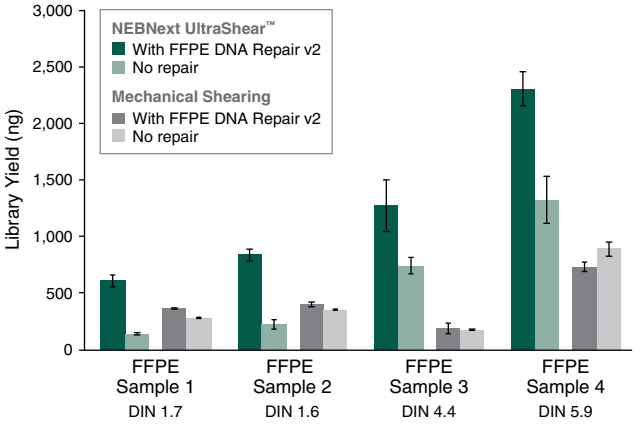
- *Includes FFPE DNA repair reagents plus optimized library prep reagents and protocol*
- *Optional NEBNext UltraShear enzymatic fragmentation*
- *Increased library yields*
- *Improved sequencing metrics*
- *Greater sensitivity of somatic variant calling*
- *Automation-friendly workflows*

FFPE DNA poses many challenges for library preparation, including characteristically low input amounts and highly variable damage from fixation, storage, and extraction methods. Regions of interest are often enriched using hybrid capture-based approaches – these workflows require a high input of diverse, uniform DNA library.

The NEBNext FFPE DNA Library Prep Kit includes the NEBNext FFPE DNA Repair v2 Mix, an optimized cocktail of enzymes designed to repair FFPE DNA, library prep reagents featuring a new polymerase master mix, and a protocol optimized for FFPE DNA. The NEBNext UltraShear FFPE DNA Library Prep Kit also includes NEBNext UltraShear, a new solution designed for enzymatic fragmentation of challenging samples (e.g., FFPE DNA). This enzymatic shearing solution further increases library yields and quality, while improving scalability and ease of use.



The NEBNext UltraShear FFPE DNA Library Prep Kit improves the quality of data from low and high quality FFPE DNA samples. *50 ng of FFPE DNAs with the DNA Integrity Numbers (DIN) shown were prepared using either the NEBNext UltraShear FFPE DNA Library Prep Kit (NEB #E6655), the NEBNext UltraShear Module (NEB #M7634), the NEBNext FFPE DNA Library Prep Kit (NEB #E6650) with Covaris-sheared DNA, or the NEBNext Ultra II DNA Library Prep Kit with Covaris-sheared DNA. Libraries were prepared using the NEBNext Multiplex Oligos Unique Dual Index Primer Pairs (NEB #E6440) with 10 PCR cycles, and sequenced on the Illumina NextSeq 500. Data was analyzed using 2 million paired-end reads, mapped using Bowtie 2 v2.3.2.2 end-to-end mapping, and analyzed using Picard Collect Alignment Summary Metrics v2.18.2.1. The NEBNext UltraShear FFPE DNA Library Prep Kit and the NEBNext UltraShear module increase the mapping rate and decrease the rate of chimeras.*



NEBNext UltraShear FFPE DNA Library Prep Kit improves yields across a range of sample quality. *50 ng of FFPE DNAs with the DNA Integrity Numbers (DIN) shown were prepared using either the NEBNext UltraShear FFPE DNA Library Prep Kit (NEB #E6655), the NEBNext UltraShear Module (NEB #M7634), the NEBNext FFPE DNA Library Prep Kit (NEB #E6650) with Covaris-sheared DNA, or the NEBNext Ultra II DNA Library Prep Kit with Covaris-sheared DNA. Libraries were prepared using the NEBNext Multiplex Oligos Unique Dual Index Primer Pairs (NEB #E6440) with 10 PCR cycles and libraries were quantified using the Agilent Tapestation® High Sensitivity D1000 assay. The highest library yields are obtained when using the NEBNext UltraShear FFPE DNA Library Prep Kit.*

TAPESTATION® is a registered trademark of Agilent Technologies, Inc.

NEW

NEBNext® FFPE DNA Repair v2 Module

#E7360S 24 reactions
#E7360L 96 reactions

Companion Product:

NEBNext FFPE DNA Repair Mix
#M6630S 24 reactions
#M6630L 96 reactions

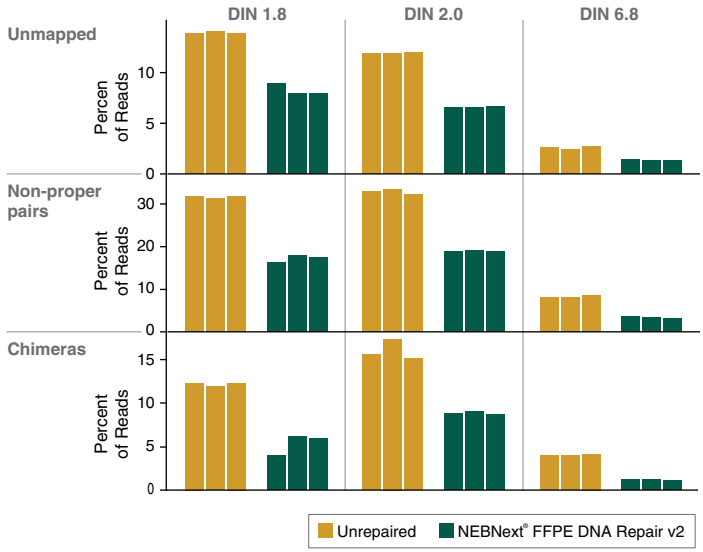
- *Higher repair efficiency with FFPE DNA*
- *A more convenient reaction buffer containing all the required buffer components for both efficient FFPE DNA repair and downstream end repair and dA-tailing*
- *No cleanup is required between repair and library prep, through the use of Thermolabile Proteinase K*

FFPE Damage Type	Repaired by the FFPE DNA Repair v2 Module?
Deamination of cytosine to uracil	Yes
Nicks and gaps	Yes
Oxidized bases	Yes
Blocked 3´ ends	Yes
DNA fragmentation	No
DNA-protein crosslinks	No

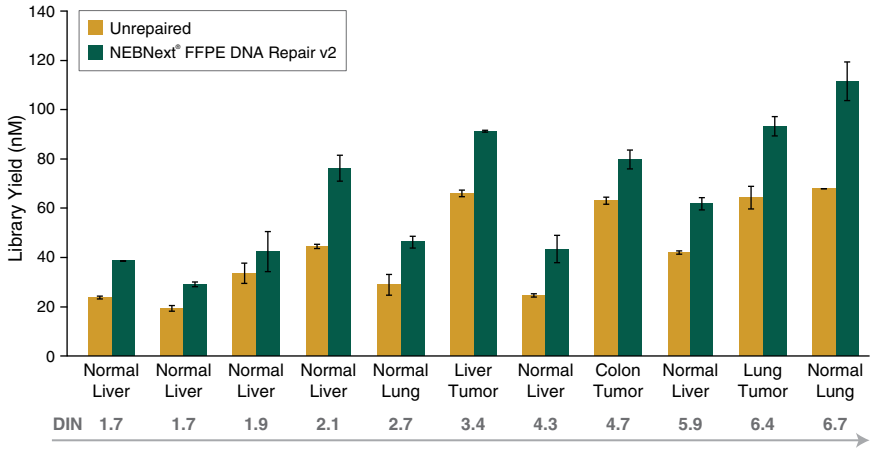
Types of FFPE DNA damage and their ability to be repaired by the NEBNext FFPE DNA Repair v2 Module.

The methods used for fixation and storage of Formalin-Fixed, Paraffin-Embedded (FFPE) DNA samples cause significant damage, making it challenging to obtain high quality sequence data. The NEBNext FFPE DNA Repair v2 Module is an optimized cocktail of enzymes designed to repair FFPE DNA, and supplied with optimized reagents to enable a streamlined workflow for NGS library preparation.

The NEBNext FFPE DNA Repair v2 Module improves upon the performance of the original NEBNext FFPE DNA Repair Mix, and offers higher efficiency, a more streamlined workflow, a more convenient reaction buffer and no cleanup is required between repair and library prep.



The NEBNext FFPE DNA Repair v2 Module improves library quality metrics including mapping rate, properly paired reads, and chimeric reads. *Libraries were prepared with 50 ng of three different quality normal liver FFPE DNA samples in triplicate, either untreated or treated with the NEBNext FFPE DNA Repair v2 Module (NEB #E7360) before library preparation using the NEBNext Ultra II DNA Library Prep Kit (NEB #E7645). Libraries were sequenced on the Illumina NextSeq 500. Paired-end reads were downsampled to 1 million reads and mapped to the GRCh38 human reference (RefSeq 884148) using Bowtie2 (v2.3.2). Mapped reads were analyzed with MarkDuplicates (v1.56.0) and Picard SAM/BAM alignment summary metrics (v1.56.0). Treatment with the NEBNext FFPE DNA Repair v2 Module increases the mapping rate and decreases the level of non-properly paired and chimeric reads.*



The NEBNext FFPE DNA Repair v2 Module enables robust library preparation from a broad range of FFPE DNA sample qualities. *Libraries were prepared with 25 ng of Covaris acoustic-sheared FFPE DNA samples of different qualities and tissue sources. The NEBNext FFPE DNA Repair v2 Module (NEB #E7360) was used followed by NEBNext Ultra II DNA library preparation (NEB #E7645) with 9 PCR cycles. Libraries were quantified using the Agilent HS D1000 TapeStation. The NEBNext FFPE DNA Repair v2 Module improves the yield of FFPE libraries by varying degrees depending on the quality and damage types present in the input DNA. Error bars indicate the standard deviation of two replicates for each library sample.*

NEW

NEBNext UltraShear™

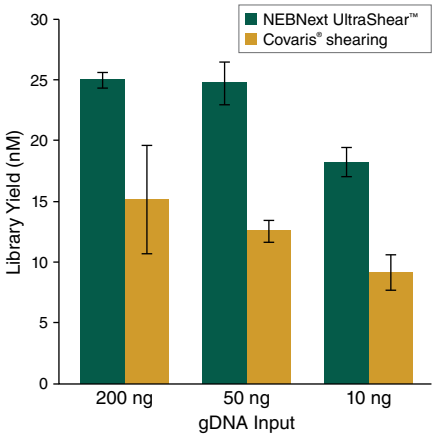
#M7634S 24 reactions
#M7634L 96 reactions

- *Compatible with methylation analysis workflows, including NEBNext® Enzymatic Methyl-seq (EM-seq™)*
- *Compatible with FFPE DNA*
- *Fast workflow with minimal hands-on time*
- *For methylation analysis, improves CpG coverage and sequencing metrics*
- *For FFPE DNA, increases usable reads and coverage uniformity*

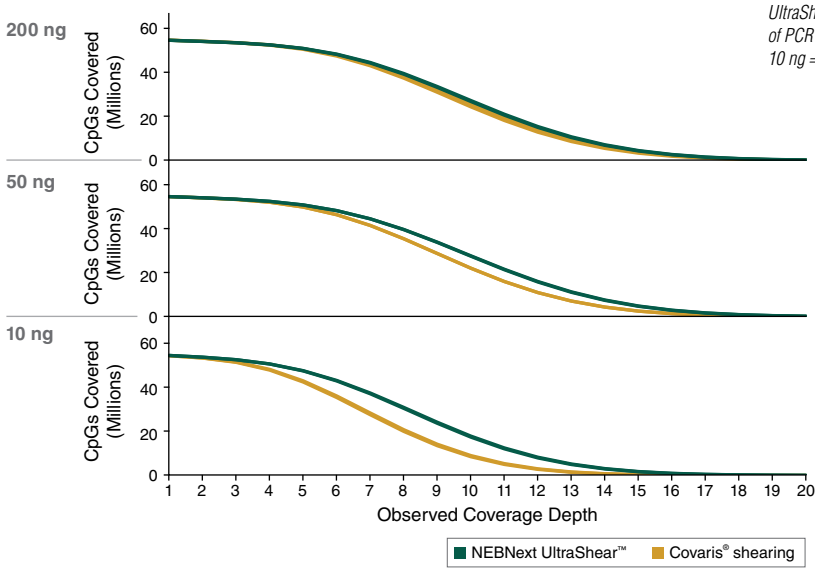
Note that the NEBNext Ultra II FS DNA Library Kit for Illumina (NEB #E7805, #E6177) is recommended for Illumina library prep with high quality genomic DNA, and provides a streamlined workflow.

Enzymatic fragmentation of DNA as part of the library prep workflow provides many advantages compared to mechanical shearing. However, specialized fragmentation reagents are required for enzymatic shearing of challenging samples such as FFPE DNA, and in order to maintain methylation marks on samples for methylome analysis.

NEBNext UltraShear™ is a mix of enzymes that has been designed and optimized to fragment these sample types upstream of library preparation. This improves library yields and diversity, and allows retention of methylation marks.



NEBNext UltraShear increases EM-seq library yields. *200 ng, 50 ng and 10 ng of NA12878 DNA spiked with control DNA used in the EM-seq workflow (CpG-methylated pUC19 DNA and unmethylated lambda DNA) were fragmented by either NEBNext UltraShear (20 minutes at 37°C) or Covaris ME220 (350 bp protocol) followed by EM-seq library preparation. Library yields were quantified using Agilent TapeStation with the High Sensitivity D1000 ScreenTape. EM-seq libraries fragmented by NEBNext UltraShear have higher yields than Covaris for the same number of PCR cycles for each input (200 ng = 4 cycles; 50 ng = 6 cycles; 10 ng = 8 cycles).*



Improved CpG coverage in EM-seq libraries produced using NEBNext UltraShear. *200 ng, 50 ng and 10 ng of NA12878 DNA spiked with control DNA (CpG-methylated pUC19 DNA and unmethylated lambda DNA) used in the NEBNext EM-seq workflow was fragmented by either NEBNext UltraShear (20 minutes at 37°C) or Covaris ME220 (350 bp protocol) followed by EM-seq library preparation. Technical replicates were generated for each input amount. All libraries were sequenced on the same flowcell of an Illumina NovaSeq 6000 (2x100 bases). 725 million reads were sampled (seqtk) from each library for methylation analysis. Reads were adaptor trimmed (fastp), aligned to the GRCh38 reference (bwa-meth), and duplicate marked (Picard MarkDuplicates) before calling methylation using MethylDackel. NEBNext UltraShear and Covaris fragmentation used ahead of the NEBNext EM-seq workflow yielded a similar number of CpGs (~54 million) at minimum 1X coverage. At minimum 10X coverage, more CpGs are identified when NEBNext UltraShear is used, due to improved library diversity and coverage evenness.*

NEBNext® dsDNA Fragmentase®

#M0348S 50 reactions
#M0348L 250 reactions

Companion Product:

NEBNext dsDNA Fragmentase Reaction Buffer v2
#B0349S 6 ml

- *Generation of dsDNA fragments for sequencing on next generation sequencing platforms*
- *Generation of dsDNA fragments for libraries*

Description: NEBNext dsDNA Fragmentase generates dsDNA breaks in a time-dependent manner to yield 50–1,000 bp DNA fragments depending on reaction time. NEBNext dsDNA Fragmentase contains two enzymes, one randomly generates nicks on dsDNA and the other recognizes the nicked site and cuts the opposite DNA strand across from the nick, producing dsDNA breaks. The resulting DNA fragments contain short overhangs, 5´-phosphates, and 3´-hydroxyl groups. The random nicking activity of NEBNext dsDNA Fragmentase has been confirmed by preparing libraries for next-generation sequencing. A comparison of the sequencing results between gDNA prepared with NEBNext dsDNA fragmentase and with mechanical shearing demonstrates that the NEBNext dsDNA Fragmentase does not introduce any detectable bias during the sequencing library preparation and no difference in sequence coverage is observed using the two methods.

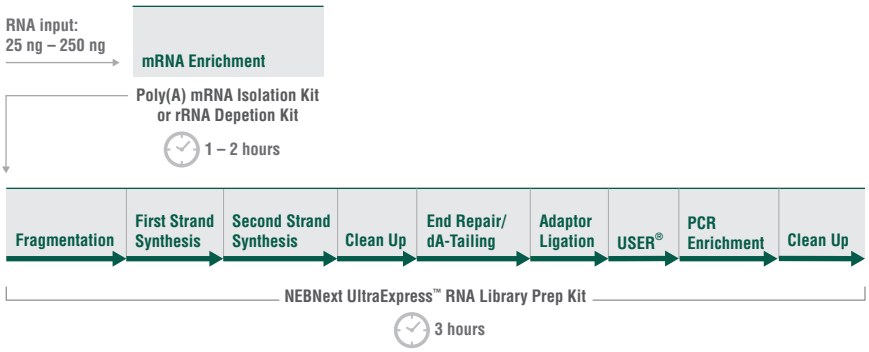
NEW

NEBNext UltraExpress™ RNA Library Prep Kit

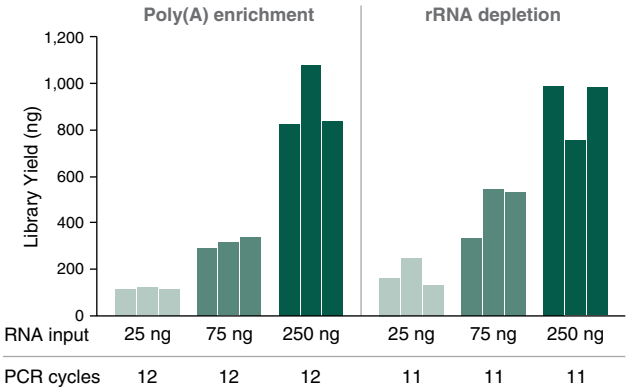
#E3330S	24 reactions
#E3330L	96 reactions

- *Fast workflow (3 hours)*
- *Fewer steps and consumables*
- *Fewer cleanups*
- *Single protocol for all inputs*
- *Compatible with a range of sample types including bacterial RNA, human whole blood and FFPE RNA*
- *Automation friendly*

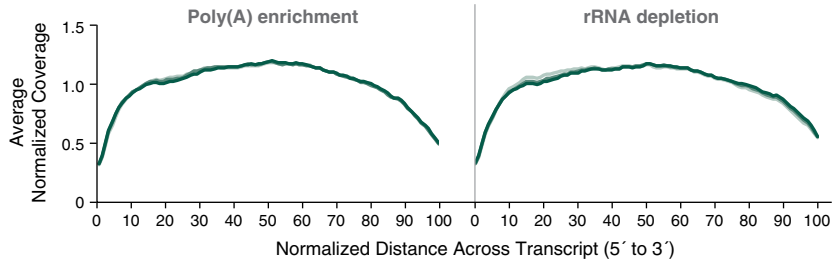
The NEBNext UltraExpress RNA Library Prep Kit is the latest generation of NEBNext RNA library prep, with a fast, streamlined workflow. The kit is compatible with mRNA isolation and rRNA depletion workflows and a wide range of sample types. With a 3-hour library prep protocol, the kit enables creation of high-quality RNA libraries in a single day, in conjunction with mRNA or rRNA depletion kits.



High quality RNA-Seq libraries in a day.



The NEBNext UltraExpress RNA Library Prep Kit produces high library yields for a range of inputs, in poly(A) enrichment and rRNA depletion workflows. *Universal human reference RNA with the indicated input amounts was (A) enriched for poly(A) mRNA (NEB #E7490) or (B) depleted of ribosomal RNA (NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB #E7400), followed by creation of strand-specific libraries using the NEBNext UltraExpress RNA Library Preparation Kit. Library yields were assessed using TapeStation® 4200 and values shown are for three replicates for each input amount.*



NEBNext UltraExpress RNA provides even transcript coverage with a range of input amounts. *Universal human reference RNA with the indicated input amounts was (A) enriched for poly(A) mRNA (NEB #E7490) or (B) depleted of ribosomal RNA (NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) (NEB #E7400), followed by creation of strand-specific libraries using the NEBNext UltraExpress™ RNA Library Preparation Kit. Libraries were sequenced on an Illumina NextSeq® 500 (2x75 bp). 9M reads were sample per library. Reads were mapped to the hg38 reference genome using RNA STAR v2.7.8a and 5´ to 3´ transcript coverage was calculated from the first 1000 highest abundance transcripts using the CollectRnaSeqMetrics (Picard) tool v2.18.2.2.*

NEBNext® Ultra™ II Library Prep Kits for RNA

NEBNext Ultra II Directional RNA Library Prep Kit for Illumina
#E7760S 24 reactions
#E7760L 96 reactions

NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads
#E7765S 24 reactions
#E7765L 96 reactions

NEBNext Ultra II RNA Library Prep Kit for Illumina
#E7770S 24 reactions
#E7770L 96 reactions

NEBNext Ultra II RNA Library Prep with Sample Purification Beads
#E7775S 24 reactions
#E7775L 96 reactions

- *Get more of what you need, with the highest library yields*
- *Generate high quality libraries with limited amounts of RNA: 10 ng–1 µg Total RNA (polyA mRNA workflow); 10 ng–1 µg (rRNA depletion workflow)*
- *Minimize bias, with fewer PCR cycles required*
- *Maximize the flexibility to order reagents for your specific workflow needs*
- *Directional (strand-specific, using the "dUTP method") and non-directional workflow options available*
- *rRNA Depletion and poly(A) mRNA isolation reagents are available separately*
- *Save time with streamlined workflows, reduced hands-on time, and automation compatibility*
- *Enjoy the reliability of the gold standard SPRIselect size selection and clean-up beads, supplied in just the amounts you need*
- *Rely on robust performance, even with low quality RNA*

Do you need increased sensitivity and specificity from your RNA-seq experiments? Do you have ever-decreasing amounts of input RNA? To address these challenges, our latest generation of RNA library prep kits generate several fold higher yields of high quality libraries and enable use of lower input amounts and fewer PCR cycles. The kits have streamlined, automatable workflows and are available for directional (strand-specific, using the "dUTP method") and non-directional library prep, with the option of SPRISelect beads for size-selection and clean-up steps.

NEBNext Ultra II Directional RNA Library Prep Kit workflow

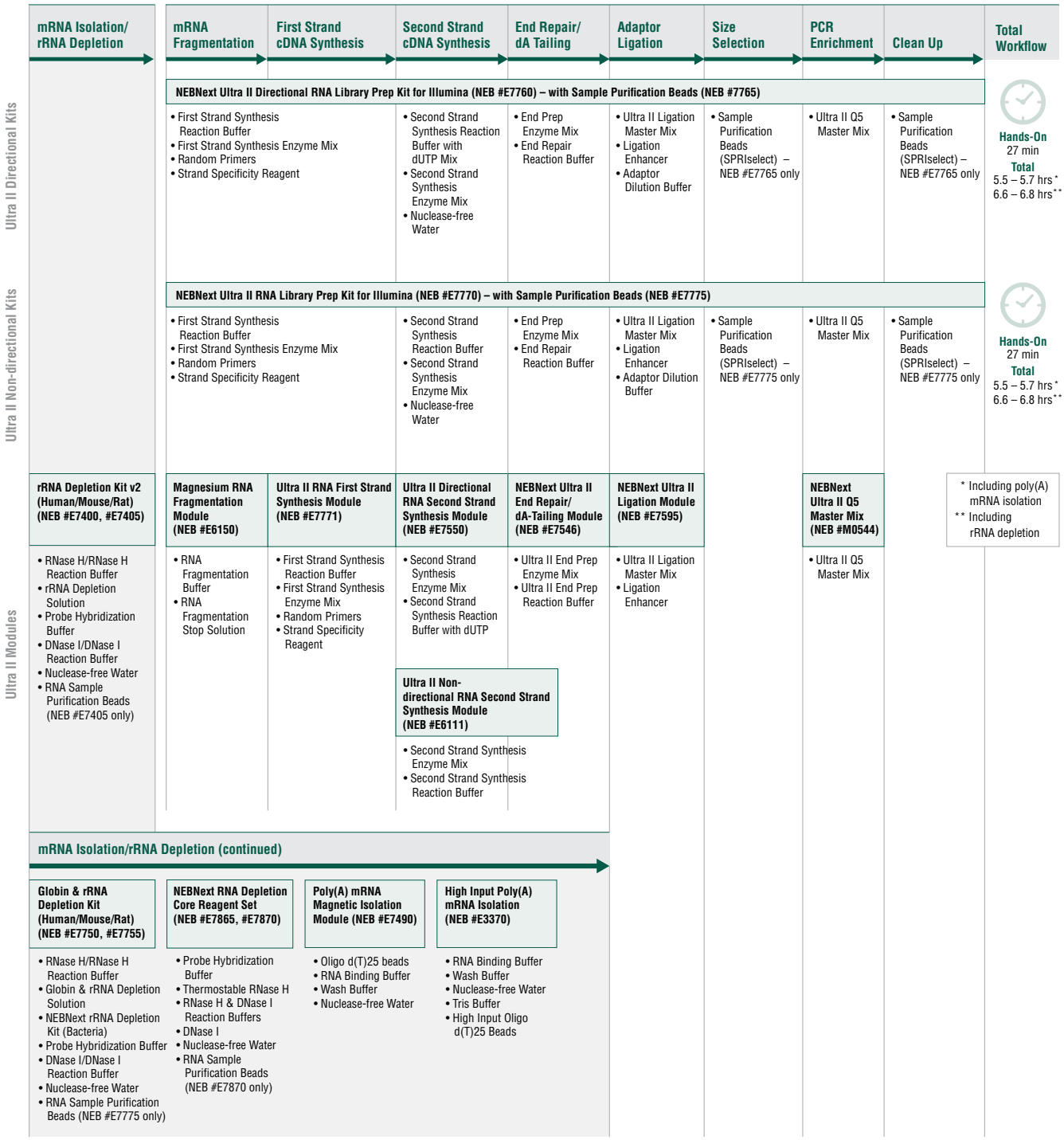
Visit **UltraIIRNA.com** to learn more and to view performance data

View the NEBNext Ultra II Directional RNA Workflow.

NEBNext® Ultra™ II RNA Reagents for Illumina® Sequencing

NEBNext Ultra II RNA Kits are available for directional (strand-specific) and non-directional library preparation, and for bulk RNA and single cell samples. These kits utilize streamlined workflows and have been designed for performance with input amounts as low as 5 ng. Note that reagents for rRNA depletion and poly(A) mRNA enrichment are supplied separately, as are adaptors and primers. In addition to stringent QCs on individual components, the NEBNext RNA kits are functionally validated by preparation of a library, followed by Illumina sequencing.

Input Poly(A) mRNA Workflow: 10 ng – 1 µg rRNA Depletion Workflow: 10 ng – 1 µg



NEBNext® rRNA Depletion Kits (Human/Mouse/Rat and Bacteria)

NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)

#E7400S	6 reactions
#E7400L	24 reactions
#E7400X	96 reactions

NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads

#E7405S	6 reactions
#E7405L	24 reactions
#E7405X	96 reactions

NEBNext rRNA Depletion Kit (Bacteria)

#E7850S	6 reactions
#E7850L	24 reactions
#E7850X	96 reactions

NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads

#E7860S	6 reactions
#E7860L	24 reactions
#E7860X	96 reactions

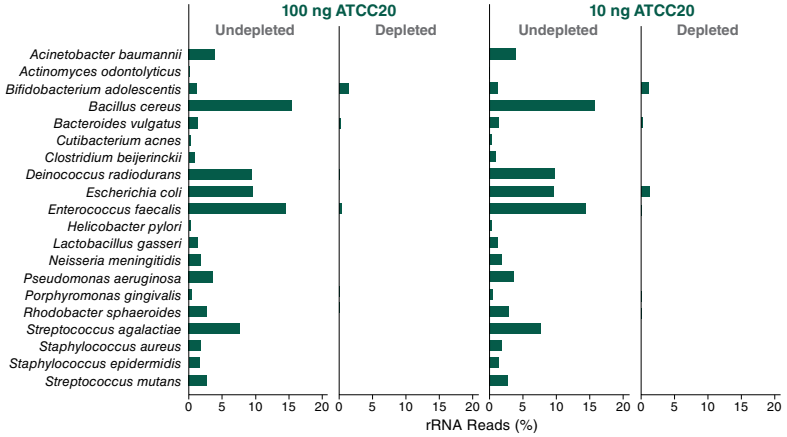
- *Suitable for low-quality (e.g., FFPE) and high-quality RNA*
- *Compatible with a broad range of input amounts: 10 ng–1 µg*
- *Superior depletion of abundant RNAs, with retention of RNAs of interest*
- *Fast workflow: 2 hours, with less than 10 minutes hands-on time*
- *Depleted RNA is suitable for RNA-seq, random-primed cDNA synthesis, or other downstream RNA analysis applications*
- *Available with optional Agencourt® RNAClean® XP Beads*

Description: The NEBNext rRNA Depletion Kit (Human/ Mouse/Rat) employs an RNase H-based method (1,2) to deplete cytoplasmic (5S, 5.8S, 18S, 28S, human ITS, ETS) and mitochondrial (12S and 16S) rRNA from human total RNA preparations.

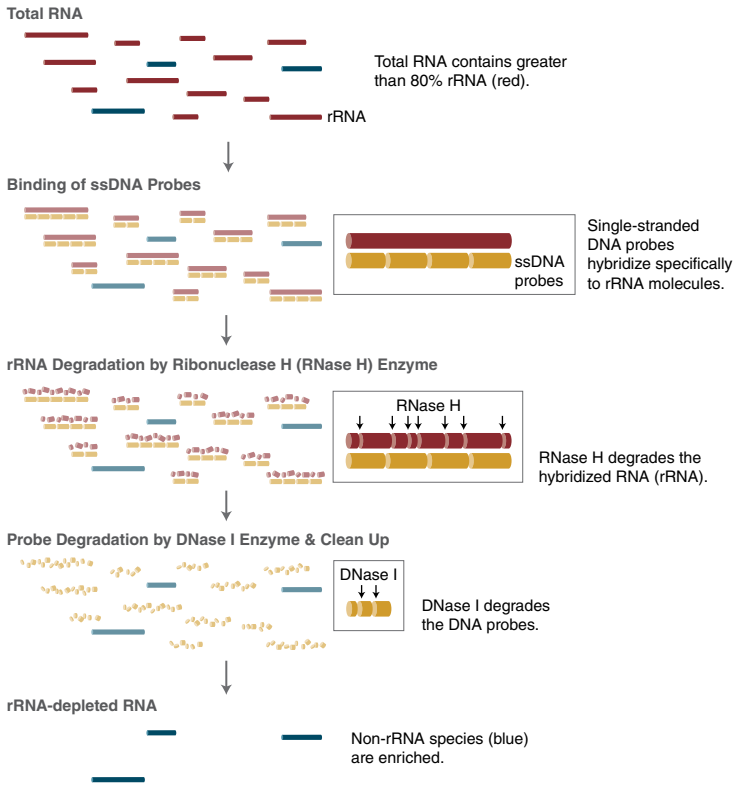
Specific enrichment of bacterial mRNAs is challenging due to their lack of poly(A) tails, precluding the use of oligo d(T)-based enrichment methods. For these samples, specific removal of bacterial rRNAs is an efficient way to enrich for RNAs of interest.

The NEBNext rRNA Depletion Kit (Bacteria) targets removal of rRNA (5S, 16S and 23S) from gram-positive and gram-negative organisms, from monocultures or samples with mixed bacterial species.

(1) Adiconis, X. et al (2013) Nature Methods, 10, 623–629.
(2) Morlon, J.D. et al (2012) PLoS One, 77 e42882.



Depletion of ribosomal RNA enriches for RNAs of interest, and maintains expression correlation, across a mock community of bacterial species and a range of input amounts. *Total RNA was extracted from a lyophilized pool of 20 different bacterial organisms (ATCC® #MSA-2002). Ribosomal RNA was depleted using the NEBNext rRNA Depletion Kit (Bacteria). RNA-seq libraries were prepared from untreated and depleted RNA using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina followed by paired-end sequencing (2 x 75 bp). 4 Million read pairs were sampled (seqtk) from each library, mapped to a composite genome (Bowtie 2.3.2) before counting reads on genes (htseq-count) and correlating their levels. Effective depletion of sequences overlapping with annotated rRNA regions was observed at 100 ng and 10 ng of input RNA for most of the organisms. Correlation analysis of the transcripts indicates consistent transcript expression regardless of treatment or input amount.*



NEBNext rRNA Depletion Kit Workflow.

NEBNext® Globin & rRNA Depletion Kits

NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)

#E7750S	6 reactions
#E7750L	24 reactions
#E7750X	96 reactions

NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads

#E7755S	6 reactions
#E7755L	24 reactions
#E7755X	96 reactions

- *Efficient, specific depletion of globin mRNA and rRNA*
- *Suitable for low- and high-quality RNA*
- *Broad range of input amounts: 10 ng–100 µg*
- *Superior deletion of abundant RNAs*
- *Fast workflow: 2 hours, with less than 10 minutes hands-on time*

GLOBIN-ZERO® is a registered trademark of Illumina, Inc.

Description: The great majority of RNA in blood samples is comprised of globin mRNA as well as cytoplasmic and mitochondrial ribosomal RNAs (rRNA). These highly abundant RNA species can conceal the biological significance of less abundant transcripts, and so their efficient and specific removal is desirable.

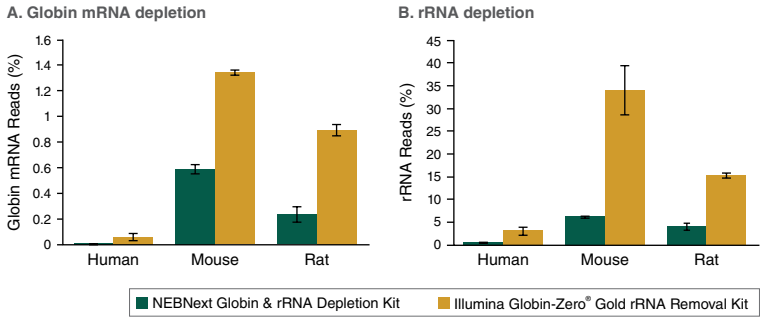
The NEBNext Globin & rRNA Depletion Kit (Human/ Mouse/Rat) employs the NEBNext RNase H-based RNA depletion workflow to deplete the following:

- Globin mRNA (HBA1/2, HBB, HBD, HBM, HBG1/2, HBE1, HBQ1 and HBZ)
- Cytoplasmic rRNA (5S, 5.8S, 18S, 28S, ITS and ETS)
- Mitochondrial rRNA (12S and 16S)

The kit is effective with human, mouse and rat total RNA preparations, both intact and degraded. The resulting depleted RNA is suitable for RNA-seq, random-primed cDNA synthesis, or other downstream RNA analysis.

This kit can also be used following poly(A) mRNA enrichment (e.g., using the NEBNext poly(A) mRNA Magnetic Isolation Module, NEB #E7490), so that the final depleted RNA contains only mRNA of interest and no non-coding RNA.

This kit is available with or without RNAClean beads.



NEBNext Globin & rRNA Depletion Kit efficiently removes Globin mRNA and rRNA. *Ribosomal RNA (rRNA) and globin mRNA were depleted from Human, Mouse, and Rat Whole Blood Total RNA (100 ng) using the NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) (NEB #E7750) or the Globin-Zero® Gold rRNA Removal Kit (Illumina #GZG1224). Libraries were prepared from the depleted RNA using the NEBNext Ultra II Directional RNA Library Prep Kit for Illumina and sequenced on an Illumina NextSeq instrument (2 x 75 bp). Reads were down sampled to 20 million reads per sample for analysis, and were identified as globin mRNA (A) or rRNA (B) using Mirabait (6 or more, 25-mers). The data represents an average of 3-4 replicates. Error bars indicate standard error.*

Customized Depletion of Unwanted RNA

NEBNext RNA Depletion Core Reagent Set

#E7865S	6 reactions	$259
#E7865L	24 reactions	$942
#E7865X	96 reactions	$3393

NEBNext RNA Depletion Core Reagent Set with RNA Sample Purification Beads

#E7870S	6 reactions	$267
#E7870L	24 reactions	$982
#E7870X	96 reactions	$3534

- *Compatible with a broad range of input amounts: 10 ng–1 µg*
- *Suitable for low-quality or high-quality RNA*
- *Fast workflow: 2 hours, with less than 10 minutes hands-on time*

NEBNext® Custom RNA Depletion Design Tool

Design oligos for depletion of unwanted RNA from any organism, when used in the NEBNext RNA depletion workflow.

https://depletion-design.neb.com/

Description: In RNA-seq, highly expressed transcripts with minimal biological interest, such as ribosomal RNA (rRNA) can dominate readouts and mask detection of more informative low-abundance transcripts. This challenge is amplified when working with sample types for which pre-designed RNA depletion kits are not available. The NEBNext RNA Depletion Core Reagent set provides a customized approach to deplete unwanted RNA from any organism, using probe sequences designed with the user-friendly NEBNext Custom RNA Depletion Design Tool.

The efficient RNase-H-based workflow, and close tiling of probes designed using the online tool, enables effective depletion from both low- and high-quality RNA, with a broad range of input amounts.

STEP 1: Use the online NEBNext Custom RNA Depletion Design Tool to obtain custom probe sequences, by entering the sequence of your target RNA.

STEP 2: Order ssDNA probe oligonucleotides from your trusted oligo provider.

STEP 3: Use the probes with the NEBNext Custom RNA Depletion Core Reagent Set or in combination with other NEBNext RNA Depletion Kits

NEW

NEBNext® High Input Poly(A) mRNA Isolation Module

#E3370S 24 reactions

Companion Products:

NEBNext Poly(A) mRNA Magnetic Isolation Module
#E7490S 24 reactions
#E7490L 96 reactions

NEBNext Ultra II Directional RNA Library Prep Kit for Illumina
#E7760S 24 reactions
#E7760L 96 reactions

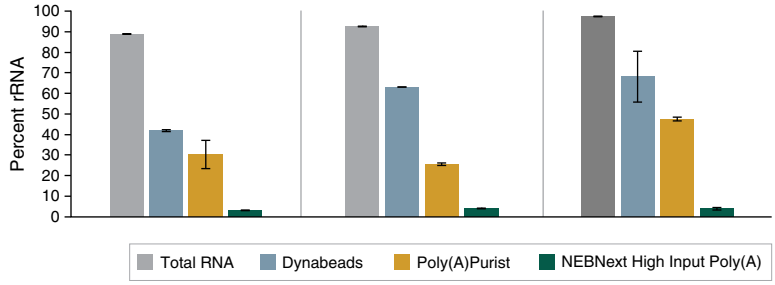
NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads
#E7765S 24 reactions
#E7765L 96 reactions

NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)
#E7400S 6 reactions
#E7400L 24 reactions
#E7400X 96 reactions

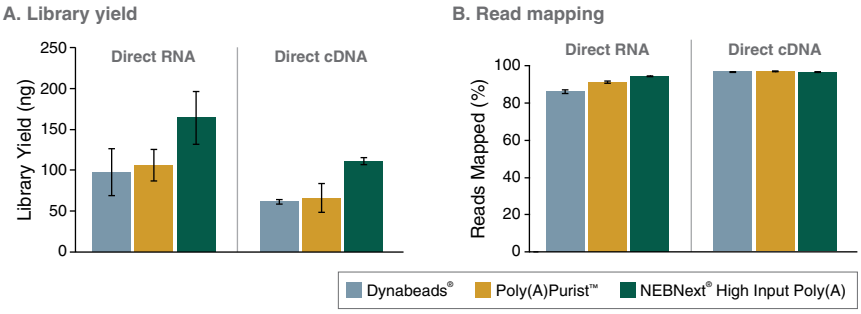
NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads
#E7405S 6 reactions
#E7405L 24 reactions
#E7405X 96 reactions

- *High inputs: 5 –50 µg total RNA per reaction*
- *Low elution volume*
- *Fast workflow*
- *Automation compatible*

The NEBNext High Input Poly(A) mRNA Isolation Module is designed to isolate intact poly(A)+ RNA from high inputs (5-50 µg per reaction) of previously isolated total RNA, using oligo $d(T)_{25}$-coupled paramagnetic beads. Intact mRNA can be obtained in approximately one hour, and eluted in small volumes. For inputs less than 5 µg, the NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB #E7490) is recommended.



The NEBNext® High Input Poly(A) mRNA Isolation Module produces low ribosomal RNA retention, across sample types. *Poly(A) RNA was enriched using Dynabeads® mRNA Purification Kit (Invitrogen®), Poly(A) Purist™ MAG (Invitrogen) or the NEBNext High Input Poly(A) mRNA Magnetic Isolation Module from 50 µg Universal Human Reference RNA (UHR, Agilent) or RNA extracted from mouse kidney tissue or S. cerevisiae (yeast) using the Monarch Total RNA Miniprep Kit. Percent ribosomal RNA (rRNA) of total or poly(A)-enriched RNA samples was determined from sequencing of triplicate (UHR and mouse poly(A) samples) or duplicate (total RNA and yeast poly(A) RNA samples) experiments, with standard deviation. Libraries were prepared from 40 ng poly(A)-enriched RNA using the NEBNext Ultra II Directional RNA Library Prep Kit and sequenced on an Illumina NextSeq 550 instrument. Six million reads were sampled from each library.*



RNA from the NEBNext® High Input Poly(A) mRNA Isolation Module produces higher library yields for nanopore sequencing, with good read mapping. *400 ng of poly(A)-enriched Universal Human Reference (UHR) RNA, enriched using the stated methods, was prepared for Direct RNA Sequencing (ONT #SQK-RNA002) on a GridION® sequencer (Oxford Nanopore Technologies®). 100 ng of poly(A)-enriched UHR RNA, enriched using the stated methods, was prepared for Direct cDNA Sequencing (ONT SQK-DCS109) on a GridION sequencer.*
A. *Library yields were assessed using Qubit® dsDNA High Sensitivity Assay Kit (Invitrogen); shown are the average of replicates with standard deviation.*
B. *Average mapping percentages of reads from replicate Direct RNA and Direct cDNA sequencing runs with standard deviation.*

NEBNext® Poly(A) mRNA Magnetic Isolation Module

#E7490S 24 reactions..... $72
#E7490L 96 reactions..... $259

Description: The NEBNext Poly(A) mRNA Magnetic Isolation Module is designed to isolate intact poly(A)+ RNA from previously-isolated total RNA. The technology is based on the coupling of Oligo $d(T)_{25}$ to 1 µm paramagnetic beads which are then used as the solid support for the direct binding of poly(A)+ RNA. Thus, the procedure permits the manual processing of multiple samples and can be adapted for automated high-throughput applications. Additionally, magnetic separation technology permits elution of intact mRNA in small volumes eliminating the need for precipitating the poly(A)+ transcripts in the eluent. Intact poly(A)+ RNA which is fully representative of the mRNA population of the original sample can be obtained in less than one hour.

NEBNext® Single Cell/Low Input RNA Library Prep

NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina
#E6420S 24 reactions
#E6420L 96 reactions

NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module
#E6421S 24 reactions
#E6421L 96 reactions

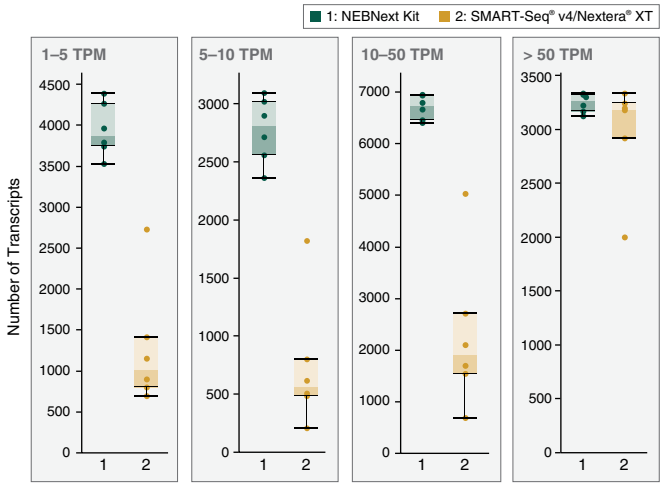
NEBNext Single Cell Lysis Module
#E5530S 96 reactions

- *Generate the highest yields of high-quality full-length transcript sequencing libraries from single cells, or as little as 2 pg–200 ng total RNA*
- *Experience unmatched detection of low abundance transcripts*
- *Rely on consistent transcript detection for a wide range of input amounts and sample types*
- *Obtain full-length, uniform transcript coverage, regardless of input amount or sample type*
- *Use with cultured or primary cells, or total RNA*
- *Save time with a fast, streamlined workflow, minimal handling steps and hands-on time*
- *Utilize a single-tube protocol from cell lysis to cDNA*
- *Enzymatic DNA fragmentation, end repair and dA-tailing reagents are in a single enzyme mix, utilizing a single protocol, regardless of GC content*
- *Available with or without library construction reagents*

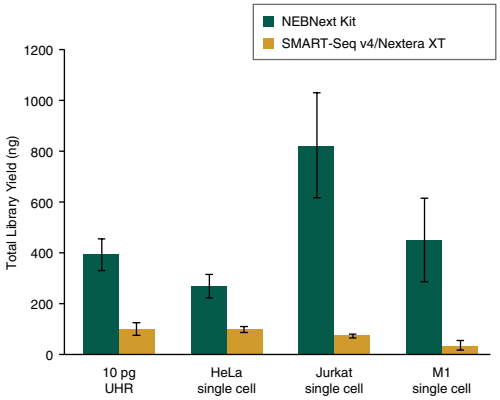
Description: The unique workflow of the NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina meets the demand for a highly sensitive, yet robust method that consistently generates high-quality, full-length transcript sequencing data from a single cell or ultra-low input RNA.

Optimized cDNA synthesis and amplification steps incorporate template switching, and utilize a unique protocol and suite of reagents.

cDNAs are generated directly from single cells or 2 pg–200 ng RNA, and even low-abundance transcripts are represented in the high yields of cDNA obtained. This is followed by library construction that incorporates the Ultra II FS enzymatic DNA fragmentation/end repair/dA-tailing mix in a simple and efficient workflow.



Increased transcript detection with the NEBNext Single Cell/Low Input RNA Library Prep Kit. *Sequencing libraries were generated from Jurkat single cells (6 replicates) using the NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Clontech # 634891) plus the Nextera® XT DNA Library Prep Kit (Illumina #FC-131-1096). Libraries were sequenced on an Illumina NextSeq 500 using paired-end mode (2 x 76 bp). TPM = Transcripts per Kilobase Million. Each dot represents the number of transcripts identified at the given TPM range, and each box represents the median, first and third quartiles per replicate and method. Salmon 0.6 was used for read mapping and quantification of all GENCODE v25 transcripts. Panels show the number of transcripts detected within the following TPM ranges: 1-5, 5-10, 10-50 and > 50 TPM. Increased identification of low abundance transcripts is observed with the NEBNext libraries.*



Higher library yields with the NEBNext Single Cell/Low Input RNA Library Prep Kit. *Sequencing libraries were generated from HeLa, Jurkat and M1 single cells or 10 pg of Universal Human Reference (UHR) RNA (Agilent #740000) with recommended amounts of ERCC RNA Spike-In Mix I (Thermo Fisher Scientific® #4456740). The NEBNext Single Cell/Low Input RNA Library Prep Kit, or the SMART-Seq v4 Ultra Low Input RNA Kit for Sequencing (Clontech #634891) plus the Nextera XT DNA Library Prep Kit (Illumina #FC-131-1096) were used. Error bars indicate standard deviation for 6-11 replicates. For the NEBNext workflow ~80% of the cDNA was used as input into sequencing library preparation, and libraries were amplified with 8 PCR cycles. For the SMART-Seq v4/Nextera XT workflow, as recommended, 125 pg of cDNA was used as input in sequencing library preparation and 12 PCR cycles were used for amplification. Error bars indicate standard deviation for 6-11 replicates.*

NEBNext® Small RNA Library Prep Kits

NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1)
#E7300S 24 reactions
#E7300L 96 reactions

NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2)
#E7580S 24 reactions
#E7580L 96 reactions

NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48)
#E7560S 96 reactions

NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible)
#E7330S 24 reactions
#E7330L 96 reactions

- *Minimal adaptor-dimer formation*
- *High yields*
- *Input RNA can be total RNA*
- *Suitable for methylated small RNAs (e.g, piRNAs) as well as unmethylated small RNAs*

The novel NEBNext Small RNA workflow has been optimized to minimize adaptor-dimers while producing high-yield, high-diversity libraries. Adaptors and primers are included in the Small RNA kits, and multiplexing options are available. The Multiplex kit contains index primers, and the Multiplex-Compatible kit enables use with your own barcode primers.

Input 100 ng – 1 µg

3´ Adaptor Ligation	Primer Hybridization	5´ Adaptor Ligation	First Strand Synthesis	PCR Enrichment	Size Selection	Total Workflow
NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1 NEB #E7300, Set 2 NEB #E7580)						
• 3´ Ligation Enzyme Mix • 3´ Ligation Reaction Buffer (2X) • 3´ SR Adaptor	• SR RT Primer	• 5´ Ligation Enzyme Mix • 5´ Ligation Reaction Buffer (10X) • 5´ SR Adaptor • Nuclease-Free Water	• RNase Inhibitor, Murine • M-MuLV Reverse Transcriptase (RNase H⁻) • First Strand Synthesis Reaction Buffer	• LongAmp® *Taq* 2X Master Mix • SR Primer • Index Primers 1–12 (Set 1) • Index Primers 13–24 (Set 2)	• Gel Loading Dye, Blue (6X) • Quick-Load® pBR322 DNA-MspI Digest • DNA Gel Elution Buffer (1X) • Linear Acrylamide • TE Buffer	**Hands-On Time** 30 min **Total Time** 6 hrs
NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48) (NEB #E7560)						
• 3´ Ligation Enzyme Mix • 3´ Ligation Reaction Buffer (2X) • 3´ SR Adaptor	• SR RT Primer	• 5´ Ligation Enzyme Mix • 5´ Ligation Reaction Buffer (10X) • 5´ SR Adaptor • Nuclease-Free Water	• RNase Inhibitor, Murine • M-MuLV Reverse Transcriptase (RNase H⁻) • First Strand Synthesis Reaction Buffer	• LongAmp *Taq* 2X Master Mix • SR Primer • NEBNext Index 1-48 Primers for Illumina	• Gel Loading Dye, Blue (6X) • Quick-Load pBR322 DNA-MspI Digest • DNA Gel Elution Buffer (1X) • Linear Acrylamide • TE Buffer	**Hands-On Time** 30 min **Total Time** 6 hrs
NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible) (NEB #E7330)						
• 3´ Ligation Enzyme Mix • 3´ Ligation Reaction Buffer (2X) • 3´ SR Adaptor	• SR RT Primer	• 5´ Ligation Enzyme Mix • 5´ Ligation Reaction Buffer (10X) • 5´ SR Adaptor • Nuclease-Free Water	• RNase Inhibitor, Murine • M-MuLV Reverse Transcriptase (RNase H⁻) • First Strand Synthesis Reaction Buffer	• LongAmp *Taq* 2X Master Mix • SR Primer • Index Primer 1	• Gel Loading Dye, Blue (6X) • Quick-Load pBR322 DNA-MspI Digest • DNA Gel Elution Buffer (1X) • Linear Acrylamide • TE Buffer	**Hands-On Time** 30 min **Total Time** 6 hrs

NEBNext® Magnetic Separation Rack

#S1515S 24 tubes

- *Fast separations in purification and size-selection steps in next generation sequencing workflows*
- *Small-scale separation of magnetic particles*
- *Anodized aluminum rack with Neodymium Iron Boron (NdFeB) rare earth magnets, the most powerful commercially available*
- *24 tube capacity: 8- and 12-strip 0.2 ml PCR tubes or individual 0.2 ml PCR tubes*

Description: Next generation sequencing library preparation workflows include magnetic bead-based purification and size-selection steps and it is important for library yield and quality that bead separation be highly efficient and fast.

The NEBNext Magnetic Separation Rack was designed for this application and contains rare earth Neodymium Iron Boron (NdFeB) magnets, the most powerful commercially available magnets, in an anodized aluminium rack. The rack holds 24 (0.2 ml) tubes, and is compatible with single tubes or strip tubes.

NEBNext® Adaptors & Primers for Illumina

NEBNext Multiplex Oligos for Illumina
(Unique Dual Index UMI Adaptors DNA Set 1)
#E7395S 96 reactions
#E7395L 384 reactions

NEW
NEBNext Multiplex Oligos for Illumina
(Unique Dual Index UMI Adaptors DNA Set 2)
#E7874S 96 reactions
#E7874L 384 reactions

NEW
NEBNext Multiplex Oligos for Illumina
(Unique Dual Index UMI Adaptors DNA Set 3)
#E7876S 96 reactions
#E7876L 384 reactions

NEW
NEBNext Multiplex Oligos for Illumina
(Unique Dual Index UMI Adaptors DNA Set 4)
#E7878S 96 reactions
#E7878L 384 reactions

NEBNext Multiplex Oligos for Illumina
(Unique Dual Index UMI Adaptors RNA Set 1)
#E7416S 96 reactions
#E7416L 384 reactions

NEBNext Multiplex Oligos for Illumina
(96 Unique Dual Index Primer Pairs)
#E6440S 96 reactions
#E6440L 384 reactions

NEBNext Multiplex Oligos for Illumina
(96 Unique Dual Index Primer Pairs Set 2)
#E6442S 96 reactions
#E6442L 384 reactions

NEBNext Multiplex Oligos for Illumina
(96 Unique Dual Index Primer Pairs Set 3)
#E6444S 96 reactions
#E6444L 384 reactions

NEBNext Multiplex Oligos for Illumina
(96 Unique Dual Index Primer Pairs Set 4)
#E6446S 96 reactions
#E6446L 384 reactions

NEW
NEBNext Multiplex Oligos for Illumina
(96 Unique Dual Index Primer Pairs Set 5)
#E6448S 96 reactions
#E6448L 384 reactions

NEBNext Multiplex Oligos for Enzymatic Methyl-seq
(Unique Dual Index Primer Pairs)
#E7140S 24 reactions
#E7140L 96 reactions

NEBNext Multiplex Oligos for Illumina
(Dual Index Primers Set 1)
#E7600S 96 reactions

NEBNext Multiplex Oligos for Illumina
(Dual Index Primers Set 2)
#E7780S 96 reactions

NEBNext Multiplex Oligos for Illumina
(Index Primers Set 1)
#E7335S 24 reactions
#E7335L 96 reactions

NEBNext Multiplex Oligos for Illumina
(Index Primers Set 2)
#E7500S 24 reactions
#E7500L 96 reactions

NEBNext Multiplex Oligos for Illumina
(Index Primers Set 3)
#E7710S 24 reactions
#E7710L 96 reactions

NEBNext Multiplex Oligos for Illumina
(Index Primers Set 4)
#E7730S 24 reactions
#E7730L 96 reactions

NEBNext Multiplex Oligos for Illumina
(96 Index Primers)
#E6609S 96 reactions.
#E6609L 384 reactions

NEBNext Adaptor Dilution Buffer
#B1430S 9.6 ml

Description: Designed for use in library prep for DNA, ChIP DNA and RNA (but not Small RNA), the NEBNext Multiplex Oligos for Illumina are an essential component of the NGS sample prep workflow. Offering a range of indexing strategies, from Unique Dual Index UMI Adaptors to the truncated, hairpin-loop NEBNext Adaptor, meant for use with UDI, dual, and single index primers, the NEBNext Multiplex Oligos can support NGS across a wide range of formats. Optimized for performance in recommended applications, there's an NEBNext indexing option tailored to you. NEBNext Oligos can be used with NEBNext products, and with other standard Illumina-compatible library preparation protocols.

Single or dual barcode primer options are available. Unique dual index primer pairs are available to address the "index hopping" seen with certain Illumina sequencing instruments.

Unique Dual Index UMI Adaptors (available for both DNA and RNA library prep) offer a ready-to-ligate adaptor for correction of PCR duplicates and errors, while improving the detection of single-nucleotide variants (SNVs). When read without the UMI sequence, the full-length adaptor enables PCR-free DNA library prep.

- *Index strategies are optimized by application*
- *Index Primers are available for NGS library prep workflows that include an amplification step*
- *Index Adaptors enable PCR-free workflows and incorporation of UMIs for error correction/ deduplication*
- *Extensively QC'd for purity and increased library yields*
- *Flexibility for use with NEBNext library preparation kits and other standard, Illumina-compatible library preparation methods*
- *Provided with index-pooling guidelines and sample sheets*

Multiplex Oligos Selection Chart

	 SINGLE INDEX	 DUAL INDEX	 UNIQUE DUAL INDEX	 UNIQUE DUAL INDEX UMIs
NEB PRODUCTS	NEB #E7335 NEB #E7500 NEB #E7710 NEB #E7730 NEB #E6609	NEB #E7600 NEB #E7780	NEB #E6440 NEB #E6442 NEB #E6444 NEB #E6446 NEB #E6448 NEB #E7140	NEB #E7395 NEB #E7874 NEB #E7876 NEB #E7878 NEB #E7416
Contains UMI	No	No	No	Yes
Addresses Index Hopping	No	No	Yes	Yes
Indexing Strategy	Index Primer	Index Primer	Index Primer	Index Adaptor
Adaptor Included	NEBNext Adaptor (Loop)	NEBNext Adaptor (Loop)	NEBNext Adaptor (Loop)	Unique Dual Index UMI Adaptor
Applications	DNA-seq, RNA-seq (except small RNA)	DNA-seq, RNA-seq (except small RNA)	DNA-seq, RNA-seq (except small RNA)	DNA-seq, RNA-seq (except small RNA)
Number of Indices for Multiplexing	up to 144	up to 384	up to 384	up to 96
Compatible with EM-seq™	Yes*	Yes*	Yes*	No
Compatible with EpiMark® Bisulfite Sequencing	Yes**	Yes**	Yes**	No
Number of Sets Available; Formats and Indices Available	Five; Sets 1-4 (12 indices/set): Individual vials 96 Index: premixed plate	Two; Individual vials containing 8 i5 primers and 12 i7 primers for combinatorial mixing	Five; 96 indices in premixed, foil-sealed 96-well plates, including a version for EM-seq (up to 120 indices, either 96-well plate or 24 vial format)	Four sets for DNA, One set for RNA; Adaptors with 96 indices in premixed, foil-sealed 96-well plate (DNA-seq OR RNA-seq) and primers

* Requires the use of the EM-seq Adaptor; Single, dual and unique dual index are all compatible; NEB recommends using the Unique Dual Index Primers found in the NEBNext Enzymatic Methyl-seq Kit (NEB #E7120) or the NEBNext Multiplex Oligos for EM-seq (NEB #E7140), both supplied with the NEBNext EM-seq Adaptor; For higher levels of multiplexing, Unique Dual Index Primers Sets 3 and 4 (NEB #E6444 and #E6446) are also validated for EM-seq.

** Requires use of NEBNext EM-seq adaptor from NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs, #E7140S/L.

NEBNext® ARTIC Products for SARS-CoV-2 Sequencing

NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina)
#E7658S 24 reactions
#E7658L 96 reactions

NEBNext ARTIC SARS-CoV-2 Library Prep Kit (Illumina)
#E7650S 24 reactions
#E7650L 96 reactions

NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies)
#E7660S 24 reactions
#E7660L 96 reactions

NEW
NEBNext ARTIC SARS-CoV-2 RT-PCR Module
#E7626S 24 reactions
#E7626L 96 reactions

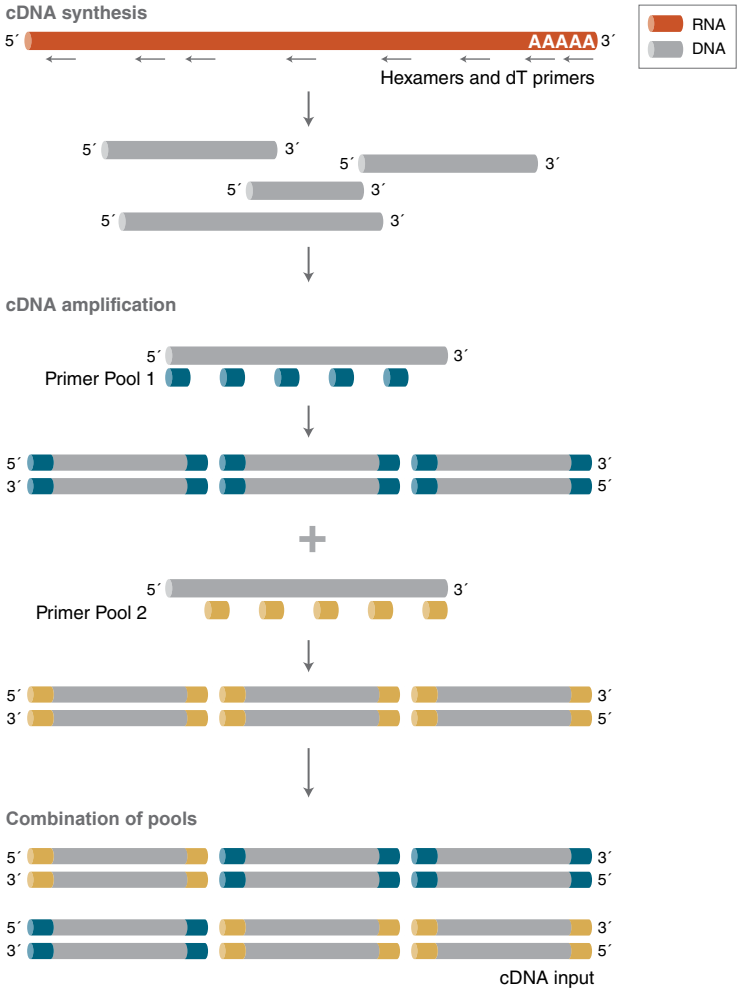
- *Streamlined, high-efficiency protocol*
- *Ample amplicon yields from a wide range of viral genome inputs*
- *Improved SARS-CoV-2 genome coverage depth with a more balanced primer pool*
- *Available for Illumina and Oxford Nanopore Technologies sequencing platforms*
- *No requirement for amplicon normalization prior to Illumina library preparation*

The NEBNext ARTIC kits were developed in response to the critical need for reliable and accurate methods for sequencing viral pathogens, specifically SARS-CoV-2. These kits, for long and short read sequencing, were based on the original work of the ARTIC Network (1). The ARTIC SARS-CoV-2 sequencing workflow is a multiplexed amplicon-based whole-viral-genome sequencing approach.

NEBNext ARTIC Companion kits include primers and reagents for RT-PCR from SARS-CoV-2 gRNA and downstream library preparation for Illumina and Oxford Nanopore Technologies sequencing.

The optimized primers and reagents for RT-PCR deliver uniform, ample amplicon yields from gRNA across a wide copy number range, and library prep and sequencing can be performed downstream of a single RT-PCR procedure.

For Illumina applications, a novel DNA polymerase formulation for the enrichment of next-generation sequencing libraries eliminates the need to normalize amplicon concentrations prior to library preparation. Two library prep options are available: The NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina) incorporates enzymatic cDNA fragmentation, and generates libraries with inserts in the 150 bp range. The NEBNext ARTIC SARS-CoV-2 Library Prep Kit (Illumina) does not include DNA fragmentation and library inserts are in the 400 bp range.



ARTIC workflow.

(1) Josh Quick 2020. nCoV-2019 sequencing protocol v2 (GunIt). protocols.io https://dx.doi.org/10.17504/protocols.io.bdp7i5rn

Reagents for Oxford Nanopore Technologies® Sequencing

NEBNext Companion Module for Oxford Nanopore Technologies Ligation Sequencing
#E7180S 24 reactions
#E7180L 96 reactions

NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies)
#E7660S 24 reactions
#E7660L 96 reactions

Companion Products:

Monarch HMW DNA Extraction Kit for Cells & Blood
#T3050S 5 preps
#T3050L 50 preps

Monarch HMW DNA Extraction Kit for Tissue
#T3060S 5 preps
#T3060L 50 preps

Monarch Genomic DNA Purification Kit
#T3010S 50 preps
#T3010L 150 preps

- *Component volumes tailored for use with many SQK-LSK109 and SQK-LSK110 workflows*
- *Simplified ordering and inventory management*
- *Compatible with all devices: MinION®, GridION®, PromethION™, Flongle®*
- *No unnecessary buffers or excess reagents*

Many NEBNext and NEB products are recommended for use in multiple sample prep workflows for Oxford Nanopore Technologies sequencing, for a range of sample types and applications.

The NEBNext Companion Module for Oxford Nanopore Technologies Ligation Sequencing includes the NEBNext DNA repair, end repair and ligation reagents recommended in Oxford Nanopore Ligation library preparation. These are provided at volumes designed for use in several protocols alongside Oxford Nanopore Technologies SQK-LSK109 and SQK-LSK110.

The NEBNext Companion Module for Oxford Nanopore Technologies Ligation Sequencing Includes:

- NEBNext FFPE DNA Repair Mix (0.048 ml)
- NEBNext FFPE DNA Repair Buffer (0.084 ml)
- NEBNext Ultra II End Prep Enzyme Mix (0.072 ml)
- NEBNext Ultra II End Prep Reaction Buffer (0.084 ml)
- Quick T4 DNA Ligase (0.240 ml)

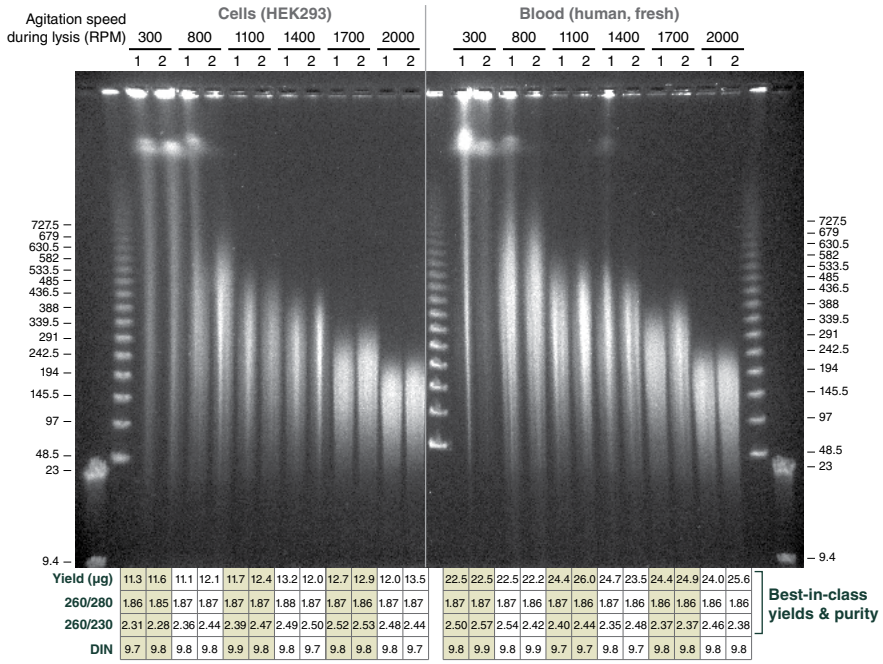
The NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies) is designed for sequencing of SARS-CoV-2 using the ARTIC protocol and the Oxford Nanopore Technologies platform. Optimized primers and reagents for RT-PCR deliver uniform, ample amplicon yields from gRNA across a wide copy number range.

- Streamlined, high-efficiency protocol
- Ample amplicon yields from a wide range of viral genome inputs
- Improved SARS-CoV-2 genome coverage depth with a more balanced primer pool

Also Available:

Monarch DNA Extraction for Oxford Nanopore Sequencing

Long read sequencing technologies, including Oxford Nanopore sequencing, require high quality extracted DNA. For the longest reads, the Monarch® HMW DNA Extraction kits enable isolation of DNA in the Mb range. The HMW DNA Extraction Kit for Tissue (NEB #T3060) is effective with a variety of tissues, bacteria and other samples (yeast, insect, amphibian), and the HMW DNA Extraction Kit for Cells & Blood (NEB #T3050) isolates HMW DNA from cultured cells and whole blood. When reads < 80 kb are required, the Monarch Genomic DNA Purification Kit (NEB #T3010) produces genomic DNA with a typical peak size of > 50 kb.



DNA fragment size is tunable based on agitation speed during lysis. *Preps were performed on duplicate aliquots of 1 x 10⁶ HEK 293 cells and 500 µl fresh human blood. Samples were agitated at the indicated speed during the lysis step to control the fragmentation of the DNA. Equal amounts of DNA from the replicates (cells: 500 ng; blood: 650 ng) were resolved by PFGE (1% agarose gel, 6 V/cm, 13°C for 20 hours, switch times ramped from 0.5–94 seconds on a BioRad CHEF-DR III System). Yield and purity ratios of the individual preps are shown in the accompanying tables. Lambda PFG Ladder and Lambda DNA-Hind III Digest (NEB #N0341 and #N3012) were used as molecular weight standards. Yield, purity ratios and DINs of the individual preps are shown in the accompanying tables.*

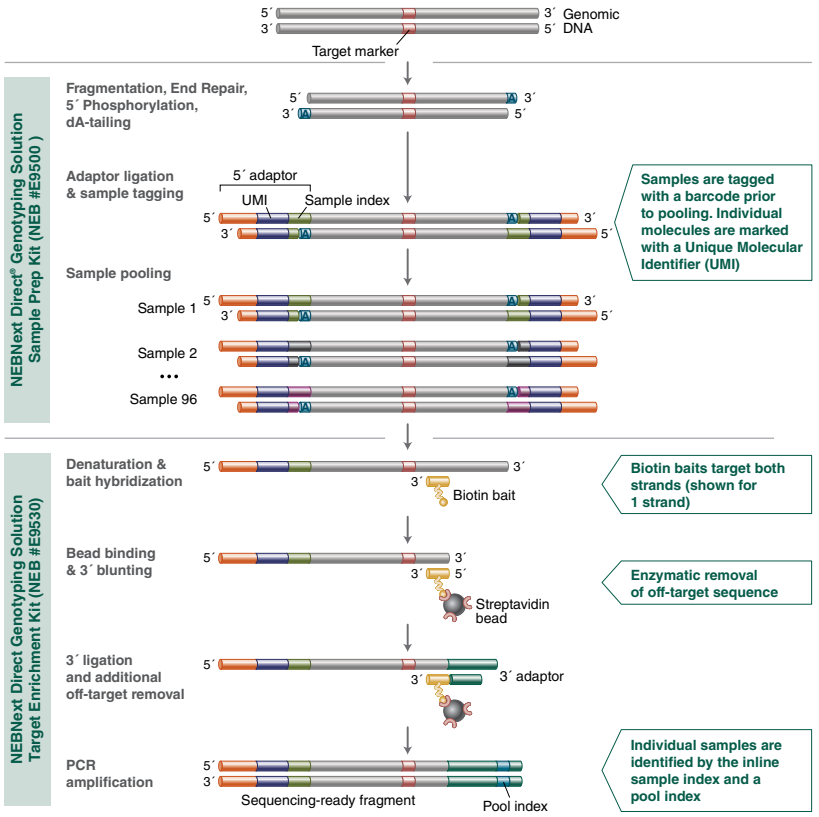
NEBNext Direct® Genotyping Solution

#E9530B-S 8 reactions
#E9500B-S 96 reactions

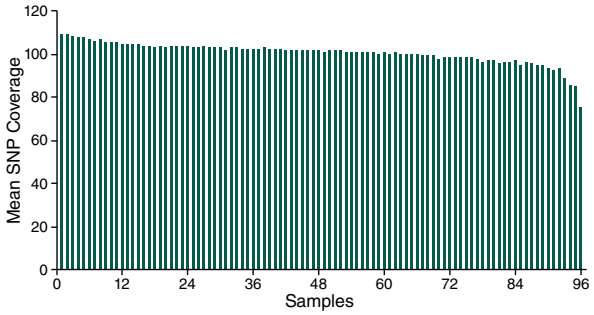
- *Ideal solution for genotyping hundreds to thousands of markers*
- *Reduce costs and streamline workflow through pre-capture pooling of up to 96 samples*
- *Maximize sequencer efficiency through dual barcode sample indexing plus Unique Molecular Identifier*
- *Unparalleled target coverage uniformity through unique capture-based enrichment*
- *Eliminate marker dropouts with finely tuned bait design*
- *Increase sample throughput using the 1-day, automatable workflow*

Visit **www.neb.com/E9500** to learn more.

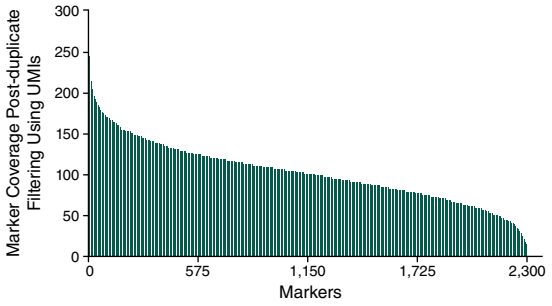
Description: The NEBNext Direct Genotyping Solution combines highly multiplexed, capture-based enrichment with maximum efficiency next-generation sequencing to deliver cost-effective, high-throughput, genotyping for a wide variety of applications. Applicable for marker ranges spanning 100-5,000 markers, pre-capture multiplexing of up to 96 samples combined with dual indexed sequencing allowing extremely high levels of sample multiplexing in a single Illumina sequencing run.



NEBNext Direct Genotyping Solution workflow.



NEBNext Direct Genotyping Solution demonstrates similar coverage across 96 pooled samples. *Mean SNP coverage of 2,309 SolCAP markers across 96 samples. 25 ng of purified tomato DNA was used for each sample. Samples were index-tagged and pooled prior to hybridization and Libraries were sequenced on an Illumina MiSeq with 20 cycles of Read 1 to sequence the 12 base UMI and 8 base sample index, and 75 cycles of Read 2 to sequence the targets.*



Mean Coverage across 2309 markers within a single sample. *Histogram of coverage across each of the 2,309 SolCAP markers demonstrates evenness of enrichment across targets and coverage levels sufficient for genotyping calls. These data represent enrichment of a single tomato sample pooled with 95 others prior to hybridization. 25 ng of purified tomato DNA was used for each sample. Samples were index-tagged and pooled prior to hybridization and libraries were sequenced on an Illumina MiSeq with 20 cycles of Read 1 to sequence the 12 base UMI and 8 base sample index, and 75 cycles of Read 2 to sequence the targets.*

NEBNext® Immune Sequencing Kits (Human & Mouse)

NEBNext Immune Sequencing Kit (Human)

#E6320S	24 reactions
#E6320L	96 reactions

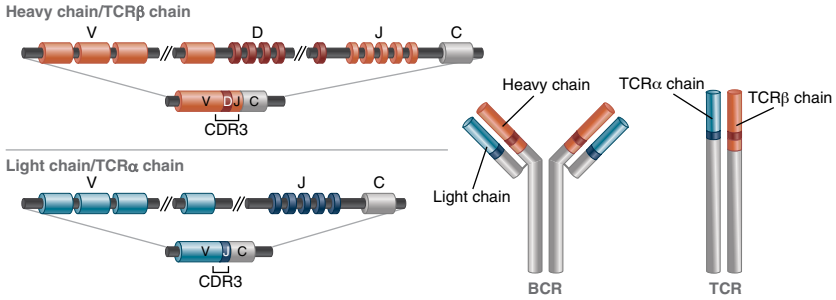
NEBNext Immune Sequencing Kit (Mouse)

#E6330S	24 reactions
#E6330L	96 reactions

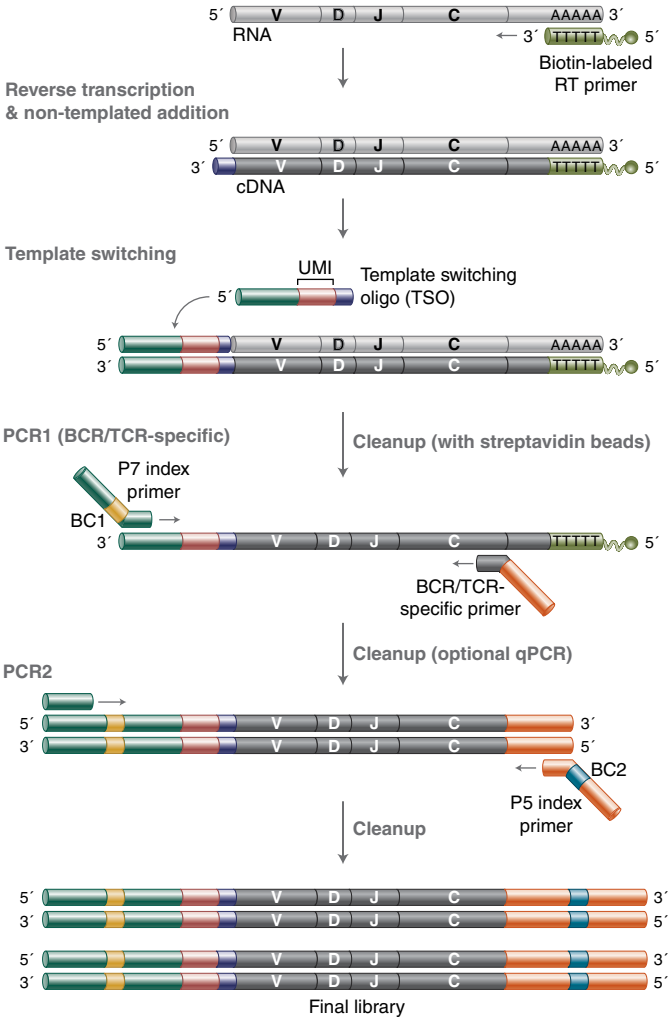
Description: The NEBNext Immune Sequencing Kits (Human & Mouse) enable exhaustive profiling of somatic mutations in the full-length immune gene repertoires of B cells and T cells, via the expression of complete antibody chains. A unique, UMI-based mRNA barcoding process allows PCR copies derived from an individual molecule to be converted to a consensus sequence. This improves sequence accuracy and eliminates PCR bias.

Immune repertoire sequencing is frequently used to analyze immune responses, both current and distant. Areas of particular interest include characterization of autoimmune diseases, oncology, discovery of neutralizing antibodies against infectious disease, tumor-infiltrating lymphocytes and use as a tool to study residual disease. Recent improvements in read lengths and throughputs of next-generation sequencing (NGS) platforms have resulted in a rise in the popularity of immune repertoire sequencing.

- *Generation of full-length variable sequences (including isotype information), allowing downstream antibody synthesis and functional characterization not possible with approaches sequencing only the CDR3 region*
- *Eliminated use of variable region primers, reducing primer pool complexity and realizing unbiased and simultaneous recovery of B-cell and T-cell receptor transcripts*
- *Minimized PCR bias and improved sequencing accuracy by allowing a consensus to be generated from duplicate sequencing reads originating from the same transcript; UMIs enable accurate quantitation of each clone present in the sample*
- *Optimized high target-capture efficiency for immune repertoire sequencing and analysis from sub-microgram quantities of total RNA*



Simplified representation of the structure of an antibody or TCR. *Simplified representation of the structure of an antibody or TCR showing the outcome of V(D)J recombination in mature lymphocytes.*



NEBNext Immune Sequencing Kit Workflow.

NEBNext® Microbiome DNA Enrichment Kit

#E2612S	6 reactions
#E2612L	24 reactions

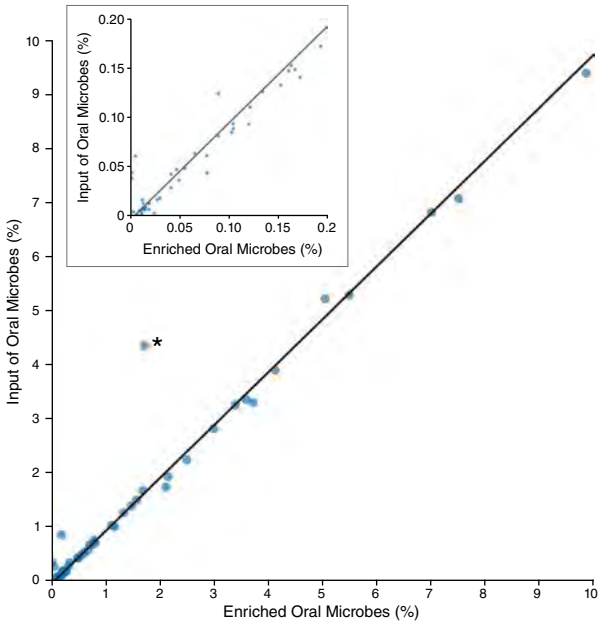
Description: The NEBNext Microbiome DNA Enrichment Kit facilitates separation of microbial DNA from methylated host DNA (including human) by selective binding and removal of the CpG methylated host DNA (1).

Functional Validation: Each set of reagents are functionally validated by enriching *E. coli* DNA from a mixture of *E. coli* and human DNA. Enrichment is evaluated through library construction and sequencing of the enriched sample on an Illumina sequencer.

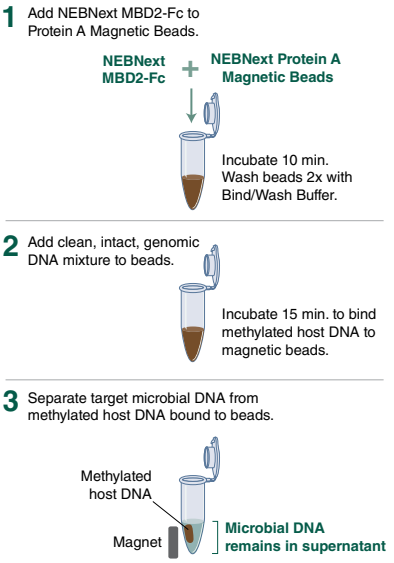
(1) Feehery, G.R., et al. (2013) *PLoS One*, 8: e76096
(2) Chen, T., et al. (2010) *Database*, Vol. 2010, Article ID baq013, doi: 10.1093/database/baq013
(3) Langmead, B., et al. (2009) *Genome Biol.* 10:R25 doi: 10.1186/gb-2009-10-3-r25

Kit Includes:

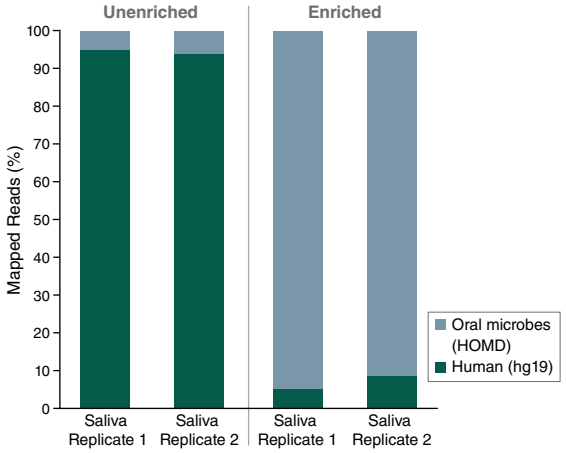
- NEBNext MBD2-Fc Protein
- NEBNext Bind/wash Buffer
- 16s rRNA Universal Gene Bacteria Control Primers
- RPL30 Human DNA Control Primers
- NEBNext Protein A Magnetic Beads



Microbiome Diversity is Retained after Enrichment with the NEBNext Microbiome DNA Enrichment Kit. *DNA was purified from pooled human saliva DNA (Innovative Research) and enriched using the NEBNext Microbiome DNA Enrichment Kit. Libraries were prepared from unenriched and enriched samples, followed by sequencing on the SOLiD 4 platform. The graph shows a comparison between relative abundance of each bacterial species listed in HOMD[2] before and after enrichment with the NEBNext Microbiome DNA Enrichment Kit. Abundance is inferred from the number of reads mapping to each species as a percentage of all reads mapping to HOMD. High concordance continues even to very low abundance species (inset). We compared 501M 50 bp SOliD 4 reads in the enriched dataset to 537M 50 bp SOLiD 4 reads in the unenriched dataset. Reads were mapped using Bowtie 0.12.7[3] with typical settings (2 mismatches in a 28 bp seed region, etc). * Niesseria flavescens – This organism may have unusual methylation density, allowing it to bind the enriching beads at a low level. Other Niesseria species (N. mucosa, N. sicca and N. elognata) are represented, but do not exhibit this anomalous enrichment.*



NEBNext Microbiome DNA Enrichment Kit workflow.



Salivary Microbiome DNA Enrichment. *DNA was purified from pooled human saliva DNA (Innovative Research) and enriched using the NEBNext Microbiome DNA Enrichment Kit. Libraries were prepared from unenriched and enriched samples and sequenced on the SOLiD 4 platform. The graph shows percentages of 500 M–537 M SOLiD™4 50 bp reads that mapped to either the Human reference sequence (hg19) or to a microbe listed in Human Oral Microbiome Database (HOMD) [2]. (Because the HOMD collection is not comprehensive, ~80% of reads in the enriched samples do not map to either database.) Reads were mapped using Bowtie 0.12.7[3] with typical settings (2 mismatches in a 28 bp seed region, etc.).*

NEBNext® Ultra™ II Q5® Master Mix

#M0544S	50 reactions
#M0544L	250 reactions
#M0544X	500 reactions

Companion Products:

NEBNext Q5 Hot Start HiFi PCR Master Mix

#M0543S	50 reactions
#M0543L	250 reactions

NEBNext High-Fidelity 2X PCR Master Mix

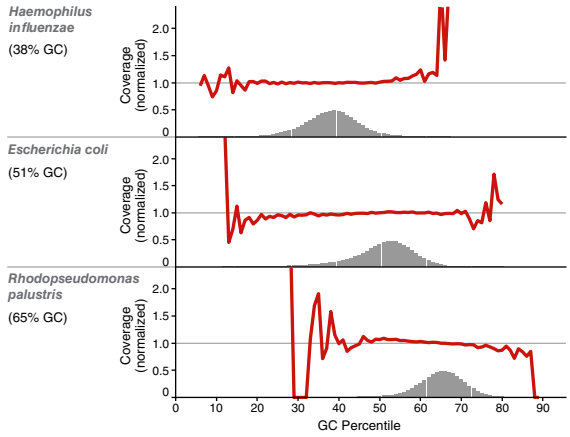
#M0541S	50 reactions
#M0541L	250 reactions

- *Next generation sequencing library preparation*
- *High-fidelity amplification*
- *Uniform GC coverage*
- *Improves sequencing library coverage of known difficult regions of the human genome*

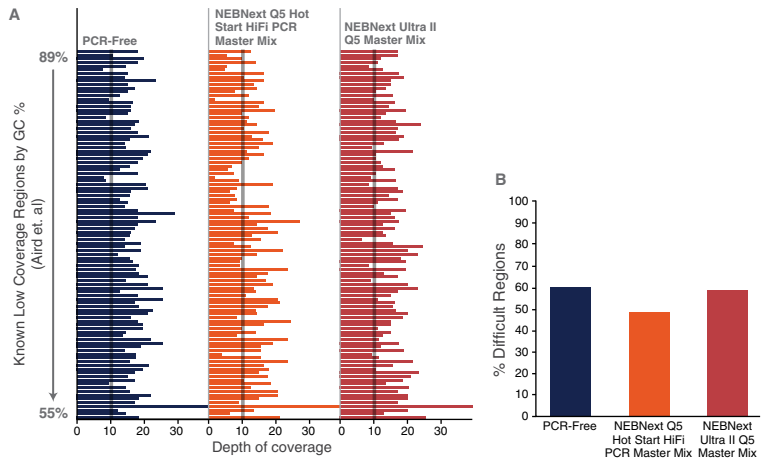
Description: The NEBNext Ultra II Q5 Master Mix is a new formulation of Q5 DNA Polymerase that has been optimized for robust, high-fidelity amplification of next-generation sequencing (NGS) libraries. This formulation further improves the uniformity of amplification of libraries, including superior performance with GC-rich regions.

The polymerase component of the master mix, Q5 High-Fidelity DNA Polymerase, is a novel thermostable DNA polymerase that possesses 3´→5´ exonuclease activity, and is fused to a processivity-enhancing Sso7d domain. Q5 also has the highest fidelity available (> 100-fold higher than that of *Taq* DNA Polymerase and ~12-fold higher than that of *Pyrococcus furiosus* (Pfu) DNA Polymerase), resulting in ultra-low error rates.

The NEBNext Ultra II Q5 Master Mix is an aptamer-based hot start formulation that allows convenient room temperature reaction set up. The convenient 2X master mix format contains dNTPs, Mg^{++} and a proprietary buffer, and requires only the addition of primers and DNA template for robust amplification. NEBNext Ultra II Q5 Master Mix is also included in the NEBNext Ultra II DNA Library Prep Kit for Illumina.



NEBNext Ultra II Q5 Master Mix provides uniform GC coverage for microbial genomic DNA with a broad range of GC composition. *Libraries were made using 100 ng of the genomic DNAs shown and the NEBNext Ultra II DNA Library Prep Kit. Libraries were amplified using the NEBNext Ultra II Q5 Master Mix, and sequenced on an Illumina MiSeq. GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each GC% is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library. NEBNext Ultra II Q5 Master Mix provides uniform GC coverage regardless of the GC content of the DNA.*



NEBNext Ultra II Q5 Master Mix provides improved coverage of known low-coverage regions of the human genome. *Libraries were prepared from Human NA19240 genomic DNA. One library was not amplified. The other two libraries were amplified using 5 cycles of PCR with NEBNext Q5 Hot Start HiFi PCR Master Mix (NEB #M0543) or with NEBNext Ultra II Q5 Master Mix (NEB #M0544). Libraries were sequenced on an Illumina NextSeq 500. 420 million 75 bp reads were randomly extracted from each dataset, representing an average coverage of 10X. Reads were mapped to the GRCh37 reference genome using Bowtie 2.2.4. Reads on each region were counted using bedtools v2.19.1. A: The number of reads overlapping distinct low coverage regions of the human genome (1) are shown for each library. B: From the 420 million 75 bp reads randomly extracted from each dataset, 10X coverage was expected. The % of difficult regions covered at > 10X are shown for each library. The NEBNext Ultra II Q5 Master Mix provides improved coverage of these known low coverage regions, without drop-outs, and shows similar coverage to the unamplified sample. (1) Popatov, V. and Ong, J.L. (2017). Examining Sources of Error in PCR by Single-Molecule Sequencing. PLoS ONE. 12(1):e0169774.*

NEBNext® Library Quant Kit for Illumina®

#E7630S 100 reactions
#E7630L 500 reactions

Companion Product:

NEBNext Library Dilution Buffer
#B6118S 15 ml

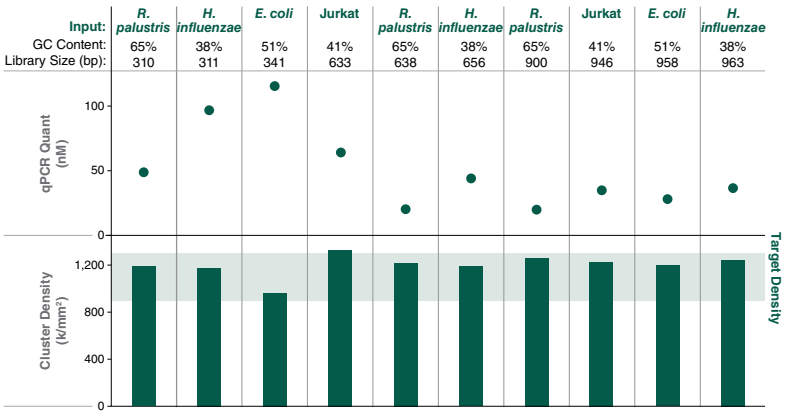
- *Provides more accurate and reproducible quant values than alternative methods and kits*
- *Compatible with libraries with a broad range of insert sizes and GC content, made by a variety of methods*
- *Includes 6 standards, but only requires 4, allowing more libraries to be quantitated per kit*
- *Supplied with a convenient Library Dilution Buffer*
- *The NEBNext Library Quant Master Mix requires only the addition of primers*
- *Utilizes a single extension time for all libraries, regardless of insert size*
- *Library quant values can be easily calculated using NEB's online tool, at NEBioCalculator.neb.com*
- *ROX is included in the kit, for use with qPCR instruments that require a reference dye for normalization*

Description: Accurate quantitation of next-generation sequencing libraries is essential for maximizing data output and quality from each sequencing run. For Illumina sequencing specifically, accurate quantitation of libraries is critical to achieve optimal cluster densities, a requirement for optimal sequence performance. qPCR is considered to be the most accurate and effective method of library quantitation, providing considerably higher consistency and reproducibility than electrophoresis or spectrophotometry, which measure total nucleic acid concentration. Amplification-based methods quantitate only those molecules that contain both adaptor sequences, thereby providing a more accurate estimate of the concentration of the library molecules that can be sequenced.

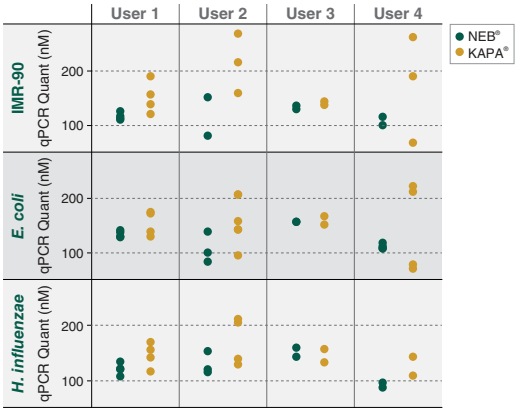
The NEBNext Library Quant Kit delivers significant improvements to qPCR-based library quantitation for next gen sequencing. The NEBNext Library Quant Kit for Illumina contains components that are optimized for qPCR-based quantitation of libraries prepared for Illumina next-generation sequencing platforms. The NEBNext Library Quant Kit contains primers which target the P5 and P7 Illumina adaptor sequences and a set of 6 high-quality, pre-diluted DNA standards to enable reliable quantitation of diluted DNA libraries between 150–1000 bp.

Kit Includes:

- NEBNext Library Quant Master Mix
- NEBNext Library Quant Primer Mix
- NEBNext Library Dilution Buffer
- NEBNext Library Quant DNA Standard 1
- NEBNext Library Quant DNA Standard 2
- NEBNext Library Quant DNA Standard 3
- NEBNext Library Quant DNA Standard 4
- NEBNext Library Quant DNA Standard 5
- NEBNext Library Quant DNA Standard 6
- ROX (High)
- ROX (Low)



With NEBNext, optimal cluster density is achieved from quantitated libraries with a broad range of library size and GC content. *Libraries of 310–963 bp from the indicated sources were quantitated using the NEBNext Library Quant Kit, then diluted to 8 pM and loaded onto a MiSeq (v2 chemistry; MCS v2.4.1.3). Library concentrations ranged from 7–120 nM, and resulting raw cluster density for all libraries was 965–1300 k/mm2 (ave. =1199). Optimal cluster density was achieved using concentrations determined by the NEBNext Library Quant Kit for all library sizes.*



Three 340–400 bp libraries were quantitated by 4 different users 2–4 times using either the NEBNext or Kapa Library Quantification Kit (Universal). *A notable improvement in quantitation consistency was observed for concentrations determined by the NEBNext Kit (orange) versus those from the Kapa kit (gray).*

	Reagent Preparation	Library Dilution	Set Up	qPCR	Data Analysis	Total Workflow
Hands-On	5 min.	10 min.	25 min.	1 min.	10 min.	**51 min.**
Total	5 min.	10 min.	25 min.	60 min.	10 min.	**1 hr. 50 min.**

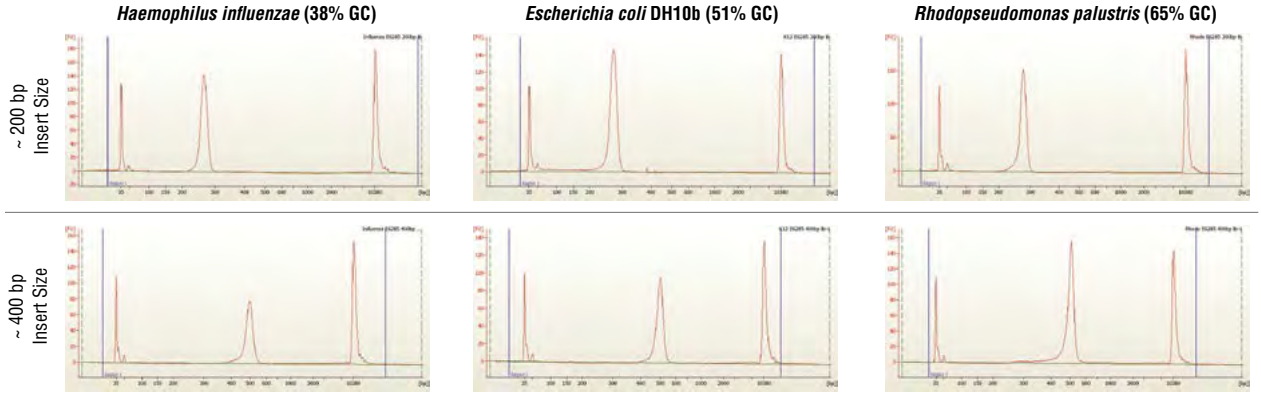
NEBNext Reagents for Ion Torrent™: DNA Library Preparation

NEBNext kits are available for DNA library preparation for Ion Torrent, with or without enzymatic DNA fragmentation. In addition to stringent QCs on individual components, the NEBNext DNA kits are functionally validated by library preparation of a genomic DNA library, followed by Ion Torrent sequencing. Reagent lots are reserved specifically for inclusion in NEBNext kits. Most of these reagents are provided in master mix format, reducing the number of vials provided in the kits, and reducing pipetting steps. Adaptors and primers for singleplex libraries are supplied in the kits. For multiplexed libraries, the Ion XPress™ Barcode Adaptors from Thermo Fisher Scientific can be used.

Input 10 ng – 1 µg*

Fragmentation	End Repair	Adaptor Ligation/Fill-In	PCR Enrichment	Total Workflow
NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent (NEB #E6285)				
• DNA Fragmentation Master Mix • DNA Fragmentation Reaction Buffer	• End Repair Enzyme Mix • End Repair Reaction Buffer (10X)	• Adaptors for Ion Torrent • T4 DNA Ligase • T4 DNA Ligase Buffer for Ion Torrent (10X) • *Bst* 2.0 WarmStart® DNA Polymerase	• Primers for Ion Torrent • NEBNext Q5 Hot Start HiFi PCR Master Mix	**Hands-On Time** 12 min. **Total Time** 110 min. – 133 min.
NEBNext Fast DNA Library Prep Set for Ion Torrent (NEB #E6270)				
	• End Repair Enzyme Mix • End Repair Reaction Buffer (10X)	• Adaptors for Ion Torrent • T4 DNA Ligase • T4 DNA Ligase Buffer for Ion Torrent (10X) • *Bst* 2.0 WarmStart DNA Polymerase	• Primers for Ion Torrent • NEBNext Q5 Hot Start HiFi PCR Master Mix	**Hands-On Time** 12 min. **Total Time** 110 min. – 133 min.

**Note that a minimum of 100 ng is recommended when used in conjunction with Ion Express Barcode Adaptors.*



Varying GC Content Libraries. *0.5 µg of DNA from 3 different genomes with varying GC content were used to construct 200 bp and 400 bp libraries using the NEBNext Fast DNA Fragmentation and Library Prep Set for Ion Torrent, analyzed by the Agilent Bioanalyzer.*

NEBNext Reagents for DNA Library Preparation – Ordering Information

Kits for Illumina DNA Library Preparation		NEB #	Size
DNA & ChIP	NEBNext UltraExpress DNA Library Prep Kit	E3325S/L	24/96 rxns
	NEBNext Ultra II DNA Library Prep Kit for Illumina	E7645S/L	24/96 rxns
	NEBNext Ultra II DNA Library Prep with Sample Purification Beads	E7103S/L	24/96 rxns
	NEBNext Ultra II FS DNA Library Prep Kit for Illumina	E7805S/L	24/96 rxns
	NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads	E6177S/L	24/96 rxns
	NEBNext Ultra II DNA PCR-free Library Prep Kit for Illumina	E7410S/L	24/96 rxns
	NEBNext Ultra II DNA PCR-free Library Prep with Sample Purification Beads	E7415S/L	24/96 rxns
	NEBNext Ultra II FS DNA PCR-free Library Prep Kit for Illumina	E7430S/L	24/96 rxns
	NEBNext Ultra II FS DNA PCR-free Library Prep with Sample Purification Beads	E7435S/L	24/96 rxns
	NEBNext Enzymatic Methyl-seq Kit	E7120S/L	24/96 rxns
	NEBNext Enzymatic 5hmC-seq Kit	E3350S/L	24/96 rxns
	NEBNext FFPE DNA Library Prep Kit	E6650S/L	24/96 rxns
	NEBNext UltraShear FFPE DNA Library Prep Kit	E6655S/L	24/96 rxns
Modules & Enzymes		**NEB #**	**Size**
DNA & ChIP	NEBNext Enzymatic Methyl-seq Conversion Module	E7125S/L	24/96 rxns
	NEBNext Enzymatic 5hmC-seq Conversion Module	E3365S/L	24/96 rxns
	NEBNext FFPE DNA Repair v2 Module	E7360S/L	24/96 rxns
	NEBNext Microbiome DNA Enrichment Kit	E2612S/L	6/24 rxns
	NEBNext UltraShear	M7634S/L	24/96 rxns
	NEBNext Ultra II FS DNA Module	E7810S/L	24/96 rxns
	NEBNext Ultra II End Repair/dA-Tailing Module	E7546S/L	24/96 rxns
	NEBNext Ultra II Ligation Module	E7595S/L	24/96 rxns
	NEBNext Ultra II Q5 Master Mix	M0544S/L/X	50/250/500 rxns
	NEBNext dsDNA Fragmentase	M0348S/L	50/250 rxns
	NEBNext End Repair Module	E6050S/L	20/100 rxns
	NEBNext dA-Tailing Module	E6053S/L	20/100 rxns
	NEBNext Quick Ligation Module	E6056S/L	20/100 rxns
	NEBNext Q5 Hot Start HiFi PCR Master Mix	M0543S/L	50/250 rxns
	NEBNext High-Fidelity 2X PCR Master Mix	M0541S/L	50/250 rxns
	NEBNext FFPE DNA Repair Mix	M6630S/L	24/96 rxns
	NEBNext Q5U Master Mix	M0597S/L	50/250 rxns
	NEBNext dsDNA Fragmentase Reaction Buffer v2	B0349S	6 ml
Adaptors & Primers		**NEB #**	**Size**
	NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 1)	E7395S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 2)	E7874S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 3)	E7876S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors DNA Set 4)	E7878S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	E6440S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 2)	E6442S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 3)	E6444S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 4)	E6446S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 5)	E6448S/L	96/384 rxns
	NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)	E7140S/L	24/96 rxns
	NEBNext Multiplex Oligos for Enzymatic 5hmC-seq (Unique Dual Index Primer Pairs)	E3360S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 rxns
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2)	E7780S	96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	E7335S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 2	E7500S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)	E7710S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)	E7730S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 rxns
	NEBNext Adaptor Dilution Buffer	#B1430S	1 x 9.6 ml
Target Enrichment		**NEB #**	**Size**
	NEBNext Direct Genotyping Solution	E9500B-S	96 rxns
	NEBNext Direct Genotyping Solution	E9530B-S	8 rxns
	NEBNext Immune Sequencing Kit (Human)	E6320S/L	24/96 rxns
	NEBNext Immune Sequencing Kit (Mouse)	E6330S/L	24/96 rxns
Library Quantitation		**NEB #**	**Size**
	NEBNext Library Quant Kit for Illumina	E7630S/L	100/500 rxns
	NEBNext Library Dilution Buffer	B6118S	15 ml
	NEBNext Library Quant DNA Standards	E7642S	500 rxns

Products for Oxford Nanopore DNA Library Preparation		NEB #	Size
DNA	NEBNext Companion Module for Oxford Nanopore Technologies Ligation Sequencing	E7180S/L	24/96 rxns

Products for Ion Torrent DNA Library Preparation		NEB #	Size
DNA	NEBNext Fast DNA Library Prep Set for Ion Torrent	E6270L	50 rxns
	NEBNext Fast DNA Fragmentation & Library Prep Set for Ion Torrent	E6285L	50 rxns

DNA Enrichment		NEB #	Size
DNA	NEBNext Microbiome DNA Enrichment Kit	E2612S/L	6/24 rxns
DNA Repair		**NEB #**	**Size**
DNA	NEBNext FFPE DNA Repair v2 Module	E7360S/L	24/96 rxns
	NEBNext FFPE DNA Repair Mix	M6630S/L	24/96 rxns
Modules & Enzymes		**NEB #**	**Size**
DNA	NEBNext UltraShear	M7634S/L	24/96 rxns
	NEBNext Ultra II Q5 Master Mix	M0544S/L/X	50/250/500 rxns
	NEBNext Q5 Hot Start HiFi PCR Master Mix	M0543S/L	50/250 rxns
	NEBNext High-Fidelity 2X PCR Master Mix	M0541S/L	50/250 rxns
	NEBNext dsDNA Fragmentase	M0348S/L	50/250 rxns
	NEBNext dsDNA Fragmentase Reaction Buffer v2	B0349S	6 ml
Magnetic Separation		**NEB #**	**Size**
	NEBNext Magnetic Separation Rack	S1515S	24 tubes

Kit is the Group Leader for Next Generation Sequencing Product Development and has been with NEB for 8 years. Learn more about Kit in her video reel.

#NEBiographies

NEBNext Reagents for RNA Library Preparation – Ordering Information

Kits for Illumina RNA Library Preparation		NEB #	Size
Directional RNA	NEBNext UltraExpress RNA Library Prep Kit	E3330S/L	24/96 rxns
	NEBNext Ultra II Directional RNA Library Prep Kit for Illumina	E7760S/L	24/96 rxns
	NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads	E7765S/L	24/96 rxns
Non-directional RNA	NEBNext Ultra II RNA Library Prep Kit for Illumina	E7770S/L	24/96 rxns
	NEBNext Ultra II RNA Library Prep with Sample Purification Beads	E7775S/L	24/96 rxns
Small RNA	NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 1)	E7300S/L	24/96 rxns
	NEBNext Multiplex Small RNA Library Prep Set for Illumina (Set 2)	E7580S/L	24/96 rxns
	NEBNext Multiplex Small RNA Library Prep Kit for Illumina (Index Primers 1-48)	#E7560S	96 rxns
	NEBNext Small RNA Library Prep Set for Illumina (Multiplex Compatible)	E7330S/L	24/96 rxns
Single Cell	NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina	E6420S/L	24/96 rxns
SARS-CoV-2	NEBNext ARTIC SARS-CoV-2 Library Prep Kit (Illumina)	E7650S/L	24/96 rxns
	NEBNext ARTIC SARS-CoV-2 FS Library Prep Kit (Illumina)	E7658S/L	24/96 rxns
Modules & Enzymes		**NEB #**	**Size**
RNA	NEBNext RNA Depletion Core Reagent Set	E7865S/L/X	6/24/96 rxns
	NEBNext RNA Depletion Core Reagent Set with RNA Sample Purification Beads	E7870S/L/X	6/24/96 rxns
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)	E7750S/L/X	6/24/96 rxns
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E7755S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat)	E7400S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit v2 (Human/Mouse/Rat) with RNA Sample Purification Beads	E7405S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Bacteria)	E7850S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Bacteria) with RNA Sample Purification Beads	E7860S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	E6310S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E6350S/L/X	6/24/96 rxns
	NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 rxns
	NEBNext High Input Poly(A) mRNA Isolation Module	E3370S	24 rxns
	NEBNext Magnesium RNA Fragmentation Module	E6150S	200 rxns
	NEBNext Ultra II RNA First Strand Synthesis Module	E7771S/L	24/96 rxns
	NEBNext Ultra II Directional RNA Second Strand Synthesis Module	E7550S/L	24/96 rxns
	NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module	E6111S/L	20/100 rxns
	NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module	E6421S/L	24/96 rxns
	NEBNext Single Cell Lysis Module	E5530S	96 rxns
DNA	NEBNext Ultra II End Repair/dA-Tailing Module	E7546S/L	24/96 rxns
	NEBNext Ultra II Ligation Module	E7595S/L	24/96 rxns
	NEBNext Ultra Ligation Module	E7445L	96 rxns
	NEBNext End Repair Module	E6050S/L	20/100 rxns
	NEBNext dA-Tailing Module	E6053S/L	20/100 rxns
	NEBNext Quick Ligation Module	E6056S/L	20/100 rxns
	NEBNext Ultra II Q5 Master Mix	M0544S/L/X	50/250/500 rxns
	NEBNext Q5 Hot Start HiFi PCR Master Mix	M0543S/L	50/250 rxns
	NEBNext High-Fidelity 2X PCR Master Mix	M0541S/L	50/250 rxns
Adaptors & Primers		**NEB #**	**Size**
	NEBNext Multiplex Oligos for Illumina (Unique Dual Index UMI Adaptors RNA Set 1)	E7416S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs)	E6440S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 2)	E6442S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 3)	E6444S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 4)	E6446S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (96 Unique Dual Index Primer Pairs Set 5)	E6448S/L	96/384 rxns
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1)	E7600S	96 rxns
	NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 2)	E7780S	96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 1)	E7335S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 2	E7500S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 3)	E7710S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (Index Primers Set 4)	E7730S/L	24/96 rxns
	NEBNext Multiplex Oligos for Illumina (96 Index Primers)	E6609S/L	96/384 rxns
	NEBNext Adaptor Dilution Buffer	B1430S	1 x 9.6 ml

Library Quantitation for Illumina		NEB #	Size
	NEBNext Library Quant Kit for Illumina	E7630S/L	100/500 rxns
	NEBNext Library Dilution Buffer	B6118S	15 ml

Products for Oxford Nanopore DNA Library Preparation		NEB #	Size
RNA	NEBNext ARTIC SARS-CoV-2 Companion Kit (Oxford Nanopore Technologies)	E7660S/L	24/96rxns

Modules & Enzymes		NEB #	Size
RNA	NEBNext rRNA Depletion Kit (Human/Mouse/Rat)	E6310S/L/X	6/24/96 rxns
	NEBNext rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E6350S/L/X	6/24/96 rxns
	NEBNext Poly(A) mRNA Magnetic Isolation Module	E7490S/L	24/96 rxns
	NEBNext High Input Poly(A) mRNA Isolation Module	E3370S	24 rxns
	NEBNext Magnesium RNA Fragmentation Module	E6150S	200 rxns
	NEBNext Ultra II RNA First Strand Synthesis Module	E7771S/L	24/96 rxns
	NEBNext Ultra II Non-Directional RNA Second Strand Synthesis Module	E6111S/L	20/100 rxns
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat)	E7750S/L/X	6/24/96 rxns
	NEBNext Globin & rRNA Depletion Kit (Human/Mouse/Rat) with RNA Sample Purification Beads	E7755S/L/X	6/24/96 rxns
Magnetic Separation		**NEB #**	**Size**
	NEBNext Magnetic Separation Rack	S1515S	24 tubes

Featured Online Tools

NEBNext® Selector v1.0

Use this tool to guide you through selection of NEBNext reagents for next generation sequencing sample preparation. Try it out at **NEBNextSelector.neb.com**

NEBNext® Custom RNA Depletion Design Tool

This tool designs probes to be used with the NEBNext RNA Depletion Core Reagent Set or to supplement an existing NEBNext depletion kit for the depletion of unwanted RNA species. Try it out at **https://depletion-design.neb.com/**.

Meet three members of the Sales and Marketing Team from our subsidiary office in the UK (pictured left to right). Dawn joined the team in 2003 and works in Sales and Marketing Support. Amanda has been with NEB since 1998 and is the UK Marketing Communications Manager. Shuoya joined in 2020 and is a Marketing Specialist.

Will a warming climate cause more vector-borne diseases?

Warming climates create favorable conditions for disease-carrying vectors like mosquitoes and ticks that typically burden tropical and sub-tropical regions. It expands their geographical range, extends their disease transmission season, and even changes their biting behavior. While modeling various climate change scenarios is complex, the consensus among scientists is that the risk of contracting a vector-borne disease in a warming world will increase; by 2050, disease-carrying mosquitoes could reach approximately 500 million more people than they do currently.

However, in some cases, there is interplay between factors and predictions is less clear – an increase in temperature may not necessarily lead to an increase in disease. Many factors must be considered, including interactions between the vector and the disease, altitude, changes in humidity and rainfall, and the vector life cycle. Disease spread may increase in some areas and decrease in others.

This is illustrated by the spread of the dengue virus in a warming climate. Dengue is the fastest-spreading mosquito-borne illness in the world; 40% of the world's population is at risk for dengue infection. It is transmitted by the *Aedes aegypti* mosquito in busy urban areas and causes severe flu-like symptoms with fever, headaches, and muscle and joint pain. More severe cases include blood vessel leakage, constant vomiting, and even death. It is thought that climate change will increase the risk of dengue infections with longer seasons and broader geographic distribution in the areas it currently burdens – Asia, Europe, Central and South America and sub-Saharan Africa.

Success in controlling the spread of Dengue has come about in a unique and innovative way using a bacterial endosymbiont, *Wolbachia*, which exists in about half of all the insects in the world. *Aedes aegypti* does not typically carry *Wolbachia*; it shortens its lifespan if infected. Cytoplasmic incompatibility results in the inability of the dengue virus and *Wolbachia* to coexist, prevening replication and spread. The World Mosquito Program has drastically decreased dengue outbreaks by releasing *Aedes aegypti* infected with *Wolbachia*.

Researchers have recently observed that climate change may adversely affect the *Wolbachia* biocontrol program. Studies have shown that fluctuating temperatures cause *Wolbachia* to struggle to reproduce and be passed to the next generation of mosquitoes. During heat waves, the temperature increase has only temporary effects (1); however, *Wolbachia*-infected mosquitoes released into populations in hot climates could die out over time.

Temperature sensitivity of the *Aedes aegypti* mosquito is increased both by dengue and *Wolbachia* infection (2). Both have the potential to decrease the lifespan of the mosquito. On the other hand, an increase in temperature leads to a rise in dengue viral replication, making the overall outcome difficult to predict.

The range of potential scenarios illustrates the need for more research to formulate mitigation strategies. Disease surveillance is essential to detect outbreaks as early as possible, especially in heavily burdened, low-resource regions. Accelerated vaccine development and distribution are paramount and require global investment. Vector breeding sites, such as wetlands, should be closely monitored. There is a growing awareness that careful observation and preparedness with predictive models and adaptive responses are necessary to prevent vulnerable populations from becoming burdened with a new health crisis.

(1) Ross, P.A., et al. (2020) *PLoS Negl. Trop. Dis.* PMID: 31971938
(2) Ware-Gilmore, F., et al. (2021) *PLoS Negl. Trop. Dis.* PMID: 34292940

Meadow at sunset. A flock of mosquitoes.
Credit: vovik_mar, Adobe Stock

Listen to an NEB research scientist talk about the expanding range of lyme disease.

Markers & Ladders (DNA, RNA & Protein)

A wide range of ladders and markers to meet your macromolecule quantification needs.

New England Biolabs provides a wide range of ladders and markers, featuring exceptional quality, uniform band intensities, convenient band spacing and easy-to-identify reference bands.

Double-stranded DNA markers are available for conventional electrophoresis. Conventional electrophoresis markers (size range: ~10 to 2.3 x 10^4 bp) include: HindIII and BstEII digests of Lambda DNA, BstNI and MspI digests of pBR322 DNA, and a HaeIII digest of ΦX174 DNA.

We also supply a series of DNA ladders ranging from 10 bp to 48.5 kb. Our 100 bp DNA ladder, 1 kb DNA Ladder and 1 kb Plus DNA Ladder are available in several formats: Conventional, Quick-Load® using either non-fluorescing purple dye or bromophenol blue as a tracking dye, and TriDye™ containing three dyes to track gel migration. Our Quick-Load Purple DNA ladders utilize our purple loading dye that improves band visibility by casting no UV shadow and are supplied with a vial of purple dye.

Our RNA ladders and markers have a size range of 17 to 9,000 bases. Both ssRNA ladders are supplied with 2X sample buffer and feature a higher intensity fragment to serve as a reference band. The dsRNA ladders are suitable for use as a size standard in dsRNA and RNAi analysis on both denaturing polyacrylamide and agarose gels.

For your protein analysis needs, NEB offers a selection of highly pure protein standards. Sizes range from 10–250 kDa, which is ideal for accurate molecular weight determination for a wide range of expressed proteins.

Featured Products

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184 1 kb Plus DNA Ladder for Safe Stains

184 TriDye Ultra Low Range DNA Ladder

187 Color Prestained Protein Standard, Broad Range (10–250 kDa)

Featured Tools & Resources

Visit **www.neb.com/DNAladders** to find selection charts for NEB's DNA markers and ladders.

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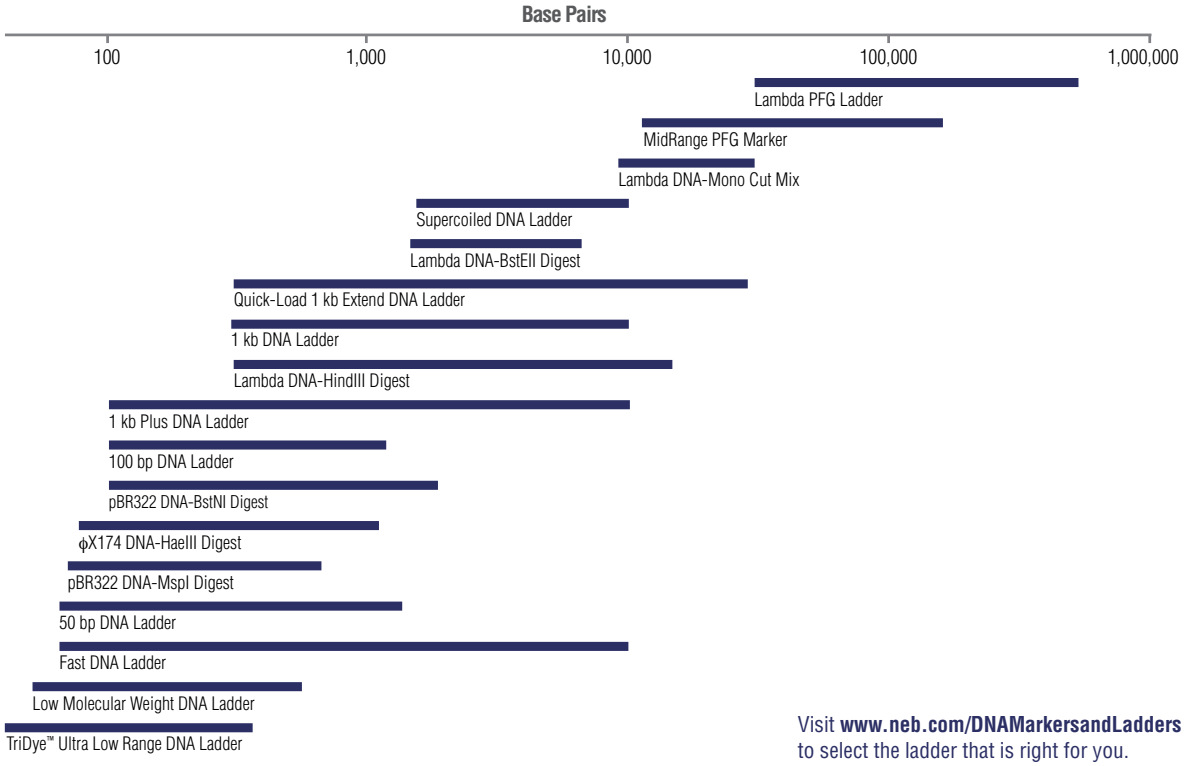
Size Ranges of DNA Ladders



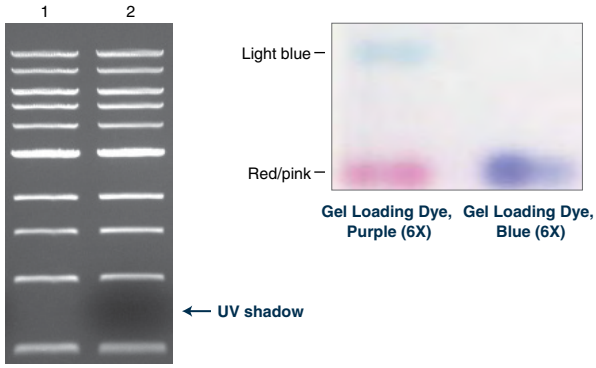
Visit **www.neb.com/DNAMarkersandLadders** to select the ladder that is right for you.

Purple Loading Dye

Gel Loading Dye, Purple (6X)
#B7024S 4 ml

Gel Loading Dye, Purple (6X), no SDS
#B7025S 4 ml

Our Gel Loading Dye, Purple (6X) (with and without SDS) is supplied with all unstained DNA Ladders, sharpens bands and eliminates the UV shadow seen with other dyes. These pre-mixed loading buffers contain a combination of two dyes, Dye 1 (pink/red) and Dye 2 (blue). The red dye serves as the tracking dye for both agarose and non-denaturing polyacrylamide gel electrophoresis. The two dyes separate upon electrophoresis; the red band is the major indicator and migrates similarly to Bromophenol Blue on agarose gels. Specifically chosen, this dye does not leave a shadow under UV light. EDTA is also included to chelate magnesium (up to 10 mM) in enzymatic reactions, thereby stopping the reaction. The dyes also contain Ficoll, which creates brighter and tighter bands when compared to glycerol loading dyes. Gel Loading Dye, Purple (6X) contains SDS, which often results in sharper bands, as some restriction enzymes are known to remain bound to DNA following cleavage.



The Gel Loading Dye, Purple (6X) (Lane 1) included in the Quick-Load Purple 1 kb DNA Ladder does not cast a UV shadow over the underlying bands, unlike the Gel Loading Dye, Blue (6X) (Lane 2).

DNA Ladders

1 kb DNA Ladder
#N3232S 200 gel lanes
#N3232L 1,000 gel lanes

100 bp DNA Ladder
#N3231S 100 gel lanes
#N3231L 500 gel lanes

1 kb Plus DNA Ladder
#N3200S 200 gel lanes
#N3200L 1,000 gel lanes

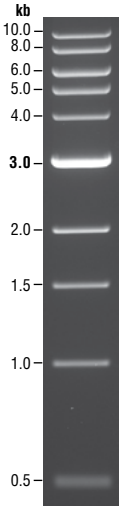
50 bp DNA Ladder
#N3236S 200 gel lanes
#N3236L 1,000 gel lanes

Low Molecular Weight DNA Ladder
#N3233S 100 gel lanes
#N3233L 500 gel lanes

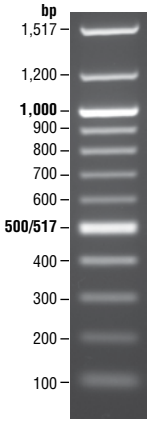
PCR Marker
#N3234S 100 gel lanes
#N3234L 500 gel lanes

NEB offers a variety of DNA Ladders with sizes ranging from 10 bp to 48.5 kb for use in agarose gel electrophoresis.

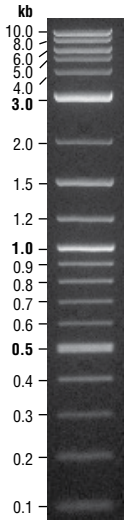
- Stable at room temperature
- Sharp, uniform bands
- Easy-to-identify reference bands
- Supplied with 1 vial of Gel Loading Dye, Purple (6X), no SDS
- Can be used for sample quantification (see www.neb.com for mass values)



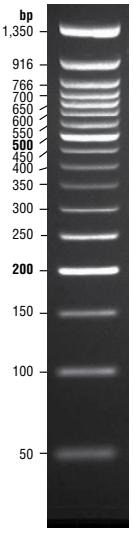
1 kb DNA Ladder
1 kb DNA Ladder visualized by ethidium bromide staining on a 0.8% TAE agarose gel.



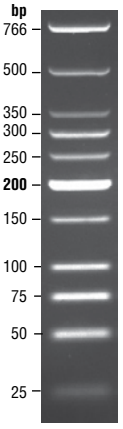
100 bp DNA Ladder
100 bp DNA Ladder visualized by ethidium bromide staining on a 1.3% TAE agarose gel.



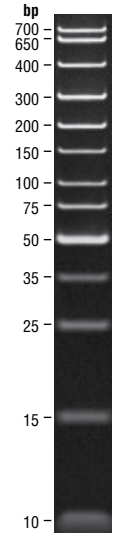
1 kb Plus DNA Ladder
1 kb Plus DNA Ladder visualized by ethidium bromide staining on a 1.0% TBE agarose gel.



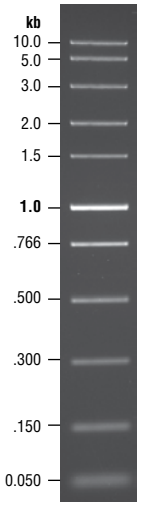
50 bp DNA Ladder
1.0 µg of 50 bp DNA Ladder visualized by ethidium bromide staining on a 3% TBE agarose gel.



Low Molecular Weight DNA Ladder
0.5 µg of LMW DNA Ladder visualized by ethidium bromide staining on a 3% TBE agarose gel.

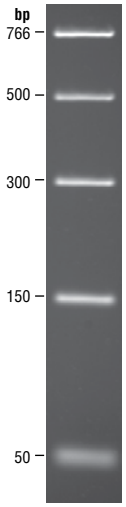


TriDye Ultra Low Range DNA Ladder
0.5 µg of Ultra Low Range DNA Ladder visualized by ethidium bromide staining on a 20% polyacrylamide gel.

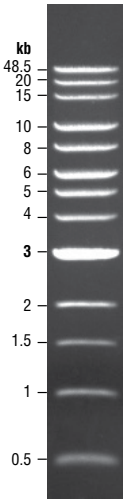


Fast DNA Ladder*
1.2% TBE agarose gel.

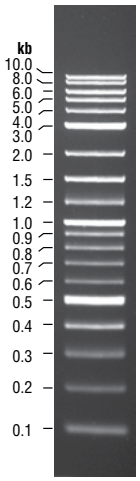
** Fast DNA Ladder can be used for fast electrophoresis systems as well as standard electrophoresis. It is in a ready-to-load format with a xylene cyanol dye.*



PCR Marker
0.3 µg of PCR Marker visualized by ethidium bromide staining on a 1.8% TBE agarose gel.



Quick-Load 1 kb Extend DNA Ladder
0.5 µg of Quick-Load 1 kb Extend DNA Ladder visualized by ethidium bromide staining on a 0.5% TBE agarose gel.



1 kb Plus DNA Ladder for Safe Stains**
1.0% TBE agarose gel.

*** 1 kb Plus DNA Ladder for Safe Stains, optimized for use with GelRed®, GelGreen® and SYBR® precast gels.*

Usage Notes: Dilution of these markers is recommended in TE or other buffer of minimal ionic strength. DNA may denature if diluted in dH_2O.

Individual DNA fragments, as seen in the 1 kb DNA Ladder, are available on request. Contact **info@neb.com** for more information.

DNA Ladders in Convenient Pre-mixed Formats

Quick-Load Purple 1 kb Plus DNA Ladder
#N0550S 250 gel lanes
#N0550L 750 gel lanes

Quick-Load Purple 1 kb DNA Ladder
#N0552S 125 gel lanes
#N0552L 375 gel lanes

Quick-Load Purple 100 bp DNA Ladder
#N0551S 125 gel lanes
#N0551L 375 gel lanes

Quick-Load Purple 50 bp DNA Ladder
#N0556S 250 gel lanes

Quick-Load Purple Low Molecular Weight DNA Ladder
#N0557S 125 gel lanes

1 kb Plus DNA Ladder for Safe Stains
#N0559S 1.25 ml

Fast DNA Ladder
#N3238S 1 ml

TriDye 1 kb Plus DNA Ladder
#N3270S 250 gel lanes

TriDye 1 kb DNA Ladder
#N3272S 125 gel lanes

TriDye 100 bp DNA Ladder
#N3271S 125 gel lanes

TriDye Ultra Low Range DNA Ladder
#N0558S 1.25 ml

Quick-Load 1 kb Plus DNA Ladder
#N0469S 250 gel lanes

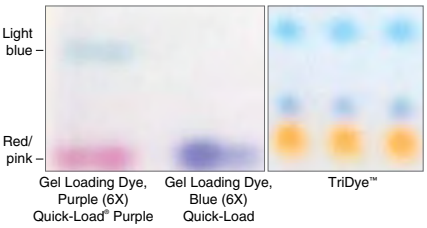
Quick-Load 1 kb DNA Ladder
#N0468S 125 gel lanes
#N0468L 375 gel lanes

Quick-Load 1 kb Extend DNA Ladder
#N3239S 125 gel lanes

Quick-Load 100 bp DNA Ladder
#N0467S 125 gel lanes
#N0467L 375 gel lanes

- Ready-to-load
- Uniform band intensities
- Easy-to-identify reference bands
- Defined mass profile for sample quantification.

Our 1 kb Plus, 1 kb and 100 bp DNA Ladders are offered in four formats. Conventional ladders are supplied with 1 vial of Gel Loading Dye, Purple (6X), no SDS. Quick-Load ladders use either non-fluorescing, purple dye or bromophenol blue as a tracking dye. TriDye ladders contain three dyes to facilitate monitoring of gel migration. Note that the TriDye Ultra Low Range DNA Ladder is suitable for both native polyacrylamide and agarose gels.



PFG Ladders

Lambda PFG Ladder
#N0341S 50 gel lanes

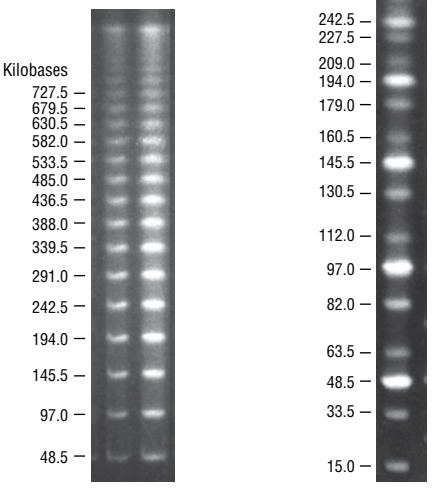
MidRange PFG Marker
#N0342S 50 gel lanes

λ DNA-Mono Cut Mix
#N3019S 100 gel lanes

The Lambda PFG Ladder consists of one GelSyringe dispenser, sufficient for 50 gel lanes. Successively larger concatemers of lambda DNA (*cI*857 *ind*1 *Sam*7) are embedded in 1% LMP agarose. Size range: 48.5–1,018 kb.

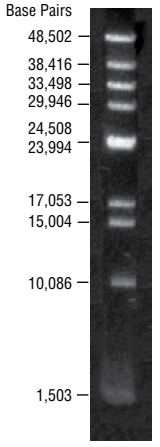
MidRange PFG Marker consists of concatemers of λ DNA isolated from the bacteriophage λ (*cI*857 *ind*1 *Sam*7) mixed with XhoI digested λ DNA embedded in 1% LMP agarose and supplied in a GelSyringe dispenser. XhoI produces fragments of 15.0 and 33.5 kb. These fragments anneal to and form concatemers with intact λ DNA. Size range: 15–291 kb.

The Lambda DNA-Mono Cut Mix is best separated by pulsed field gel electrophoresis, but can be alternatively used with standard electrophoresis systems. It is supplied in a liquid format. Size range: 1.5–48.5 kb.



Lambda PFG Ladder
1% agarose gel, 4.5 V/cm, 15°C for 48 hours. Switch times ramped from 5–120 seconds.

MidRange PFG Marker
1% agarose gel, 6 V/cm, 15°C for 24 hours. Switch times ramped from 1–25 seconds.



Lambda DNA-Mono Cut Mix
PFGE separation of 0.5 µg of Lambda DNA-Mono Cut Mix. 1% agarose gel, 0.5X TBE, 6 V/cm, 15°C for 20 hours. Switch times ramped from 0.5–1.5 seconds.

Conventional DNA Markers

λ DNA-HindIII Digest
#N3012S 150 gel lanes
#N3012L 750 gel lanes

λ DNA-BstEII Digest
#N3014S 150 gel lanes

ΦX174 DNA-HaeIII Digest
#N3026S 50 gel lanes
#N3026L 250 gel lanes

pBR322 DNA-BstNI Digest
#N3031L 250 gel lanes

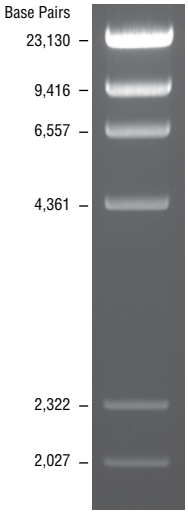
pBR322 DNA-MspI Digest
#N3032S 50 gel lanes

NEB offers a wide range of double-stranded DNA molecular weight markers for conventional agarose gel electrophoresis. These standards have a size range of approximately 10–23,000 base pairs.

The typical pattern generated by each of the conventional markers is shown below. The number of fragments generated by each marker, as well as the specific fragment sizes for each of the conventional markers can be found online.

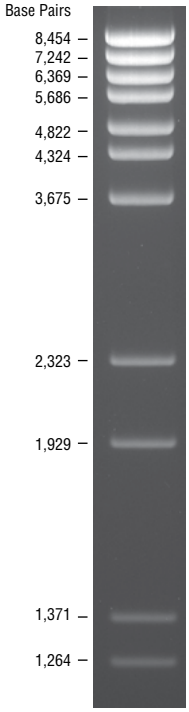
Usage Recommendation: Dilution of these markers is recommended in TE or other buffer of minimal ionic strength. DNA may denature if diluted in dH_2O.

The cohesive ends of fragments 1 and 4 of the Lambda DNA-HindIII and Lambda DNA-BstEII Digests can be separated by heating to 60°C for 3 minutes.



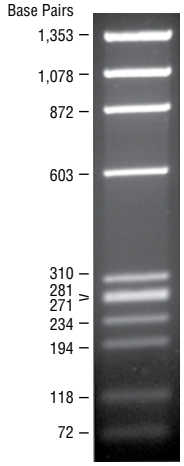
Lambda DNA-HindIII Digest

0.5 µg of Lambda DNA-HindIII Digest visualized by ethidium bromide staining. 1.0% agarose gel.



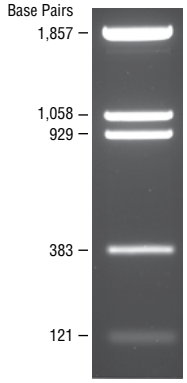
Lambda DNA-BstEII Digest

0.5 µg of Lambda DNA-BstEII Digest visualized by ethidium bromide staining. 1.0% agarose gel.



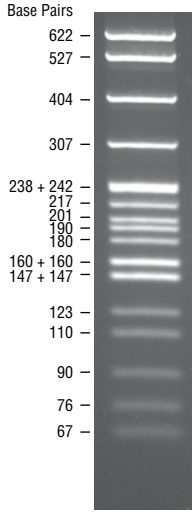
ϕX174 DNA-HaeIII Digest

0.5 µg of ϕX174 DNA-HaeIII Digest visualized by ethidium bromide staining. 1.7% agarose gel.



pBR322 DNA-BstNI Digest

0.5 µg of pBR322 DNA-BstNI Digest visualized by ethidium bromide staining. 1.4% agarose gel.



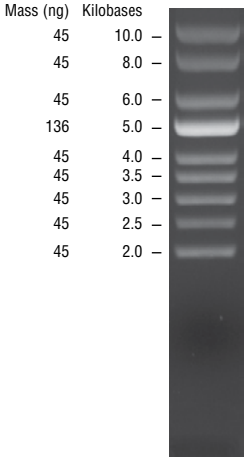
pBR322 DNA-MspI Digest

1.0 µg of pBR322 DNA-MspI Digest visualized by ethidium bromide staining. 3% agarose gel.

Hunter has worked at NEB for 32 years, spending 2 years in Shipping before moving to the Information Technology Department. Hunter loves celebrating holidays and organizes an NEB employee pumpkin carving contest and gingerbread decorating contest each year to bring the staff together.

Supercoiled DNA Ladder

#N0472S 100 gel lanes



Supercoiled DNA Ladder
0.5 µg/lane.
0.8% TAE agarose gel.

The Supercoiled DNA ladder contains 9 proprietary supercoiled plasmids, ranging in size from 2 to 10 kb, that are suitable for use as supercoiled molecular weight standards for agarose electrophoresis. The 5 kb plasmid has an increased intensity to serve as a reference band.

Concentration: 500 µg/ml

Note: This ladder may contain some traces of nicked DNA and dimers above the 10 kb plasmid. To minimize nicking of the supercoiled DNA, always use sterile pipette tips and avoid multiple freeze-thaw cycles. The migration of supercoiled plasmids in agarose gels can change depending on agarose concentration, buffer and electrophoresis conditions. Dilute in TE or other buffer of minimal ionic strength. DNA may denature if diluted in dH_2O. Centrifuge briefly and mix gently before use. We recommend loading 0.5 µg (1 µl) of the Supercoiled DNA Ladder diluted in sample buffer. This ladder was not designed for precise quantification of DNA mass, but can be used for approximating the mass of DNA in comparably intense samples of similar size.

The approximate mass of DNA in each of the bands in our Supercoiled DNA ladder is as follows (assuming a 0.5 µg loading):

Band	Base Pairs	DNA Mass
1	10,000	45 ng
2	8,000	45 ng
3	6,000	45 ng
4	**5,000**	**136 ng**
5	4,000	45 ng
6	3,500	45 ng
7	3,000	45 ng
8	2,500	45 ng
9	2,017	45 ng

RNA Markers & Ladders

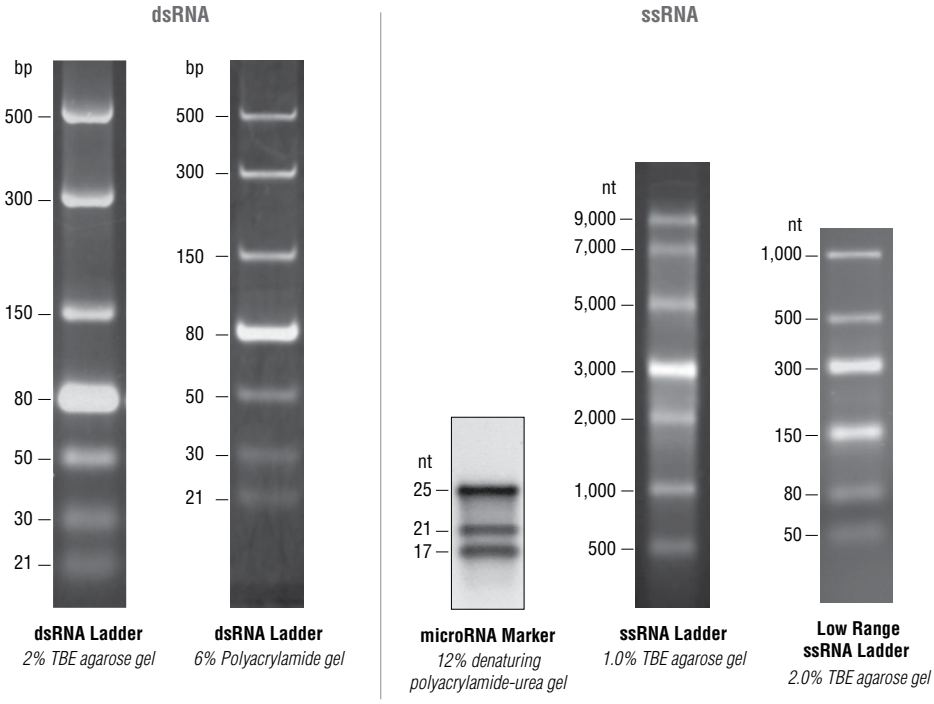
dsRNA Ladder
#N0363S 25 gel lanes

microRNA Marker
#N2102S 100 gel lanes

ssRNA Ladder
#N0362S 25 gel lanes

Low Range ssRNA Ladder
#N0364S 100 gel lanes

NEB offers several RNA Markers and Ladders with a size range from 17 to 9,000 bases. The Low Range ssRNA Ladder and the ssRNA Ladder are suitable for use as RNA size standards on denaturing or native gels. Both are supplied with RNA Loading Dye (2X) (NEB #B0363) and feature a higher intensity fragment to serve as a reference band. The microRNA Marker, provided in a ready-to-load denaturing solution, is ideally used as a size marker on denaturing polyacrylamide gels or northern blots and is best visualized stained with SYBR®-Gold. It is supplied with a 3´-biotinylated 21-mer oligonucleotide probe that can be labeled with g32-P-ATP and T4 PNK (NEB #M0201). The dsRNA Ladder is suitable for use as a size standard in dsRNA and RNAi analysis on both polyacrylamide and agarose gels.



dsRNA Ladder
2% TBE agarose gel

dsRNA Ladder
6% Polyacrylamide gel

microRNA Marker
12% denaturing polyacrylamide-urea gel

ssRNA Ladder
1.0% TBE agarose gel

Low Range ssRNA Ladder
2.0% TBE agarose gel

Protein Standards

Unstained Protein Standard, Broad Range (10-200 kDa)
#P7717S 150 gel lanes
#P7717L 750 gel lanes

Color Prestained Protein Standard, Broad Range (10-250 kDa)
#P7719S 150 gel lanes
#P7719L 750 gel lanes

Blue Prestained Protein Standard, Broad Range (11-250 kDa)
#P7718S 150 gel lanes
#P7718L 750 gel lanes

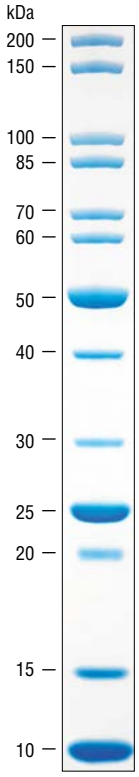
Companion Product:

Blue Protein Loading Dye
#B7703S 8 ml

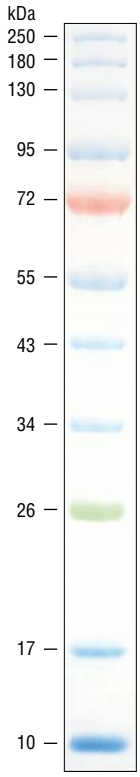
NEB offers a selection of highly pure protein standards available as unstained, blue prestained or color prestained (containing two colored reference bands for easy identification). Sizes range from 10 to 250 kDa which is ideal for calculating molecular weight determination for a wide range of expressed proteins. NEB protein standards provide uniform band intensities, convenient band spacing and easy-to-identify reference bands.

Recommended Load Volume: 3 µl

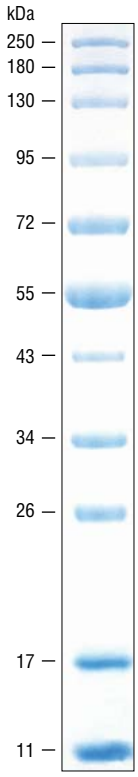
Note: For calculating molecular weight determinations, use NEB's Unstained Protein Standard, Broad Range.



Unstained Protein Standard, Broad Range (10–200 kDa)
10–20% Tris-glycine SDS-PAGE Gel



Color Prestained Protein Standard, Broad Range (10–250 kDa)
10–20% Tris-glycine SDS-PAGE Gel



Blue Prestained Protein Standard, Broad Range (11–250 kDa)
10–20% Tris-glycine SDS-PAGE Gel

Meet three members of the Sales and Marketing Team from our subsidiary office in France (pictured left to right). Pierre-Grégoire is a Business Analyst, Muriel is the Operational Marketing Manager and Patrick is the National Sales and Marketing Manager.

Restoring the soil – is the answer right under our feet?

Soils are living dynamic ecosystems deep-rooted in climate change via the soil-plant-atmospheric interface. Minerals, water, gases, and organic matter coalesce into a medium containing vast communities of organisms, particularly microbes. Soil degradation from short-sighted farming practices impacts long term soil productivity and climate in a negative feedback loop. Regenerative agriculture can restore soil to improve human health and the environment.

Essential life-supporting soil functions are driven by microbial biomass and biodiversity. Plant, fungi and animal life directly depend on the constitution of soil microbiomes. Scientists have only scratched the surface of the global soil microbiome, but it is the largest biome, holding at least a quarter of the planet's biodiversity. Soil microbiomes include protozoa, fungi, bacteria, archaea, viruses and fungi. By nature, soil fertility is driven by the surface area of mineral particles and bioavailability of carbon sources for breakdown. Plants sequester carbon by drawing down carbon dioxide from the atmosphere. Carbon is then leached out to microbes in the rhizosphere region near roots. Bacterial and fungal communities synergistically regulate the recycling of carbon, nitrogen, phosphorus, sulfur and other elements. Microbes decompose necromass into flows of organic matter which promote plant growth. Sometimes they can also produce antibiotics that protect plants from pathogens. Fungal and bacterial polysaccharide or glycoprotein biofilms also improve soil structure. Microbe activities vary based on climate conditions like water and oxygen availability, temperature, and pH. The key is that microbes and plants cooperate to cycle carbon and nitrogen in concerted, self-regulated systems.

Agricultural impacts to climate change are complex. Principally, farming influences the soil-plant-atmospheric interface, which feeds into planet-wide levels of free or dissolved energy absorbing gases. These gases affect climate. Intense land tillage, emissions from high energy use, and the broad effects of synthetic fertilizers and pesticides lead to soil nutrient loss, erosion, compaction, contamination, emissions, and loss of biodiversity and organic carbon. Broad acting synthetic fertilizers and pesticides upset soil ecosystems. Excess nitrogen from chemical fertilizers converts to the potent greenhouse gas nitrous oxide. Intense tillage releases this gas while eroding and compacting topsoil. Associated ammonia runs off into waterways to form anoxic dead zones. Nitrogen-fixing soil bacteria are suppressed by synthetic fertilizers. In turn, food crops sequester less carbon dioxide gas from the air. Synthetic fertilizer fed crops grow to be less nutritious and less able to uptake carbon dioxide from the atmosphere. These common agricultural practices ultimately weaken ecosystems on a global scale.

Soil represents massive sustainability opportunities to raise crop yields with lower emissions. Half of all habitable land on Earth is dedicated to agriculture, but only fifteen percent undergoes regenerative practices. Monetary carbon credit offsets do not fully compensate for these losses. To address global food shortages, custom solutions must fit harmoniously with stakeholders to drive uptake of sustainable soil management. Perennial crops with deep roots can restore soil carbon. Year-round nitrogen sequestering cover crop rotations like clover, beans and peas are an age-old answer. Bioreactors, bacteria bio-inoculants, and biological fungicides and pesticides offer modern solutions. Adoption is crucial, and farmers cannot carry the full burden for the common good. The shared goal is to increase soil capacity to grow nutritious, carbon sequestering crops – to work with the earth, instead of against it.

Hear about agroforestry, a promising soil stewardship solution.

Panoramic shot of soil erosion caused by water, aerial view.
Credit: yelantsevv, Adobe Stock

Genome Editing

Programmable nucleases for your applications.

Easily changing the sequence specificity of a DNA binding protein enables many new possibilities for the detection and manipulation of DNA, including for genome editing – creating targeted changes in the DNA of living cells. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) -associated (Cas) nucleases have been adapted from bacterial immune systems into useful tools for biotechnology. Cas nucleases including Cas9 and Cas12a, are attractive for genome editing because they are easily programmable with a single guide RNA or crRNA/tracrRNA to introduce a double-stranded break at a specific target. The resultant breaks are repaired by cellular machinery, in some applications using an exogenous repair template.

The ease of programming Cas nucleases, and their conversion into nicking endonucleases, or DNA binding proteins without nuclease activity has expanded their use to include delivering a specific cargo to a locus for applications including visualization, activation, repression and base editing. Furthermore, Cas nuclease and their variants are useful tools for the detection and manipulation of DNA *in vitro*.

Featured Products

192 **EnGen® Spy Cas9 HF1**

192 **EnGen Lba Cas12a (Cpf1)**

192 **EnGen Sau Cas9**

194 **EnGen Mutation Detection Kit**

195 **EnGen sgRNA Synthesis Kit, *S. pyogenes***

Featured Tools & Resources

Visit **www.neb.com/GenomeEditing** for more information, including our feature article and latest brochure.

RI	EnGen Spy Cas9 HF1	192, 193
RI	EnGen Spy Cas9 NLS	192, 193
	EnGen Mutation Detection Kit	192, 194
	EnGen sgRNA Synthesis Kit, *S. pyogenes*	192, 195
RI	EnGen Spy Cas9 Nickase	192, 193
RI	EnGen Spy dCas9 (SNAP-tag)	192, 193
RI	EnGen Lba Cas12a (Cpf1)	192, 193
RI	EnGen Sau Cas9	192, 193
RI	Cas9 Nuclease, *S. pyogenes*	192, 193
	Monarch Total RNA Miniprep Kit	192
	Monarch RNA Cleanup Kit (50 µg)	192
	Q5 Site-Directed Mutagenesis Kit (with or without Competent Cells)	192
	Q5 High-Fidelity DNA Polymerases	192
	NEBuilder HiFi DNA Assembly Master Mix	192
	NEBuilder HiFi DNA Assembly Cloning Kit	192
	HiScribe T7 mRNA Kit with CleanCap Reagent AG	192
	HiScribe T7 ARCA mRNA Kit (with or without tailing)	192
	HiScribe T7 High Yield RNA Synthesis Kit	192
	HiScribe T7 Quick High Yield RNA Synthesis Kit	192
RI	T7 Endonuclease I	192
	Tth Argonaute (TtAgo)	193

RI Recombinant Enzyme

Featured NEB Products Supporting CRISPR Workflows

New England Biolabs provides reagents to support a broad variety of CRISPR/Cas genome editing approaches. From introduction of Cas and single guide RNA (sgRNA) on plasmids, to direct introduction of Cas ribonucleoprotein (RNP) and detection of edits using next generation sequencing or enzymatic mutation detection, NEB provides reagents that simplify and shorten genome editing workflows.

Product	CRISPR/Cas9 Application	NEB #	Size
NEW **EnGen Spy Cas9 HF1**	• High-fidelity *in vitro* cleavage of dsDNA. • Genome engineering by direct introduction of active ribonucleoproteins. • Recognizes 5´-NGG-3´ PAM.	#M0667T #M0667M	500 pmol 2,500 pmol
EnGen Spy Cas9 NLS	• *in vitro* cleavage of dsDNA. • Genome engineering by direct introduction of active ribonucleoproteins. • Recognizes 5´-NGG-3´ PAM.	#M0646T #M0646M	500 pmol 2,500 pmol
EnGen Mutation Detection Kit	• Determination of the targeting efficiency of genome editing protocols	#E3321S	25 reactions
EnGen sgRNA Synthesis Kit, *S. pyogenes*	• Generation of microgram quantities of custom sgRNA. • Recognizes 5´-NGG-3´ PAM.	#E3322V #E3322S	10 reactions 20 reactions
EnGen Spy Cas9 Nickase	• *in vitro* nicking of dsDNA. • Genome engineering by direct introduction of active nickase complexes. • Recognizes 5´-NGG-3´ PAM.	#M0650S #M0650T	90 pmol 500 pmol
EnGen Spy dCas9 (SNAP-tag	• Programmable binding of DNA. • Compatible with SNAP-tag substrates for visualization and enrichment. • Recognizes 5´-NGG-3´ PAM.	#M0652S #M0652T	90 pmol 500 pmol
EnGen Lba Cas12a (Cpf1)	• *in vitro* cleavage of dsDNA. • Genome engineering by direct introduction of active nuclease complexes. • Signal generation for sequence detection assays. • Recognizes 5´-TTTN PAM.	#M0653S #M0653T	70 pmol 2,000 pmol
EnGen Sau Cas9	• *in vitro* cleavage of dsDNA. • Genome engineering by direct introduction of active nuclease complexes. • Recognizes 5´-NNGRRT-3´ PAM.	#M0654S #M0654T	90 pmol 500 pmol
Cas9 Nuclease, *S. pyogenes*	• *in vitro* cleavage of dsDNA. • Genome engineering by direct introduction of active ribonucleoproteins.	#M0386S #M0386T #M0386M	90 pmol 500 pmol 2,500 pmol
Monarch® Total RNA Miniprep Kit	• Purification of total RNA, with a binding capacity of up to 100 µg	#T2010S	50 preps
Monarch RNA Cleanup Kit (50 µg)	• Purification of sgRNA, with a capacity of up to 50 µg	#T2040S #T2040L	10 preps 100 preps
Q5® Site-Directed Mutagenesis Kit (with or without Competent Cells)	• Insertion of target sequence into a Cas9-sgRNA construct and modification of HDR templates	#E0554S #E0552S	10 reactions 10 reactions
Q5 High-Fidelity DNA Polymerases	• High-fidelity construct generation for use with CRISPR workflows and for sequencing	Multiple	Multiple
NEBuilder® HiFi DNA Assembly Master Mix	• Single-tube, isothermal generation of the Cas9-sgRNA construct and HDR templates	#E2621S #E2621L #E2621X	10 reactions 50 reactions 250 reactions
NEBuilder HiFi DNA Assembly Cloning Kit	• Single-tube, isothermal generation of the Cas9-sgRNA construct and HDR templates	#E5520S	10 reactions
HiScribe® T7 mRNA Kit with CleanCap® Reagent AG	• Generation of Cas9 mRNA wtih CleanCap Reagent AG cap	#E2080S	20 reactions
HiScribe T7 ARCA mRNA Kit (with or without tailing)	• Generation of Cas9 mRNA with ARCA cap	#E2060S #E2065S	20 reactions 20 reactions
HiScribe T7 High Yield RNA Synthesis Kit	• Generation of sgRNA and Cas9 mRNA	#E2040S	50 reactions
HiScribe T7 Quick High Yield RNA Synthesis Kit	• Generation of sgRNA and Cas9 mRNA	#E2050S	50 reactions
T7 Endonuclease I	• Determination of the editing efficiency of genome editing experiments	#M0302S #M0302L	250 units 1,250 units

Programmable Nucleases

The highest efficiency strategy for genome editing with CRISPR/Cas nucleases is direct introduction of Cas/guide RNA complexes. This method further simplifies CRISPR/Cas workflows and has been reported to increase on-target editing activity and reduce off-target events. NEB provides purified Cas9 nucleases from *S. pyogenes* and *S. aureus*, and Cas12a (Cpf1) nuclease from *Lachnospiraceae* bacterium ND2006. In addition, NEB provides variants of Cas9 from *S. pyogenes*, including nicking endonuclease and endonuclease deficient versions. NEB also provides *Thermus thermophilus argonaute* (TtAgo), a programmable DNA endonuclease which requires a short 5´-phosphorylated single-stranded DNA guide to target its activity to a specific corresponding sequence on a substrate.

Product	NEB #	Features	Size
NEW **EnGen Spy Cas9 HF1**	#M0667T #M0667M	• Reduced off-target cleavage • Ideal for direct introduction of Cas9/sgRNA complexes • Dual NLS for improved transport to the nucleus • Compatible with EnGen sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322) and the EnGen Mutation Detection Kit (NEB #E3321)	500 pmol 2,500 pmol
EnGen Spy Cas9 NLS	#M0646T #M0646M	• Ideal for direct introduction of Cas9/sgRNA complexes • Dual NLS for improved transport to the nucleus • Compatible with EnGen sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322) and the EnGen Mutation Detection Kit (NEB #E3321)	500 pmol 2,500 pmol
Cas9 Nuclease, *S. pyogenes*	#M0386S #M0386T #M0386M	• Ideal for *in vitro* digestion of dsDNA • Compatible with EnGen sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322) and the EnGen Mutation Detection Kit (NEB #E3321) • For help with oligo design, try EnGen sgRNA Template Oligo Designer	90 pmol 500 pmol 2,500 pmol
EnGen Spy Cas9 Nickase	#M0650S #M0650T	• Variant of Cas9 nuclease differing by a point mutation (D10A) in the RuvC nuclease domain • Capable of generating nicks, but not cleaving DNA • DNA double strand breaks can be generated, with reduced off-target cleavage, by targeting two sites with EnGen Cas9 Nickase in close proximity • Compatible with the EnGen sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322)	90 pmol 500 pmol
EnGen Spy dCas9 (SNAP-tag	#M0652S #M0652T	• An inactive mutant of Cas9 nuclease that retains programmable DNA binding activity • The N-terminal SNAP-tag allows for covalent attachment of fluorophores, biotin, and a number of other conjugates useful for visualization and target enrichment • Compatible with the EnGen sgRNA Synthesis Kit, S. pyogenes (NEB #E3322)	90 pmol 500 pmol
EnGen Lba Cas12a (Cpf1)	#M0653S #M0653T	• 5´-TTTN-3´ PAM sequence opens up additional genomic regions for targeting • Shorter, 40-44 base guide RNA • Two nuclear localization signals for improved transport to the nucleus • 5´ overhanging termini on cleavage products • Active from 16 to 48°C • Lachnospiraceae bacterium ND2006 (Lba) Cas12a maintains activity at lower temperatures than the Acidaminococcus orthologs, permitting editing in ectothermic organisms such as zebra fish and xenopus • High concentration enzyme can be used for microinjection, electroporation and lipofection	70 pmol 2,000 pmol
EnGen Sau Cas9	#M0654S #M0654T	• 5´-NNGRRT-3´ PAM • Dual NLS for improved transport to nucleus • High concentration enzyme can be used for microinjection electroporation and lipofection • Cleaves 3 bases upstream of PAM, blunt-ended cleavage	90 pmol 500 pmol
***Tth* Argonaute (TtAgo)**	#M0665S	• Short 16-18 oligonucleotide 5´ phosphorylated ssDNA guides are cost effective and can be phosphorylated with T4 Polynucleotide Kinase • Guide target RNA sequence selection is not limited by the requirement of adjacent sequence motif • HIghly active on ssDNA and most dsDNA substrates (generates a nick in dsDNA substrates), with mild activity on ssRNA substrates • Recommended for in vitro applications	50 pmol

Learn more about genome editing.

EnGen® Mutation Detection Kit

#E3321S 25 reactions

Companion Products:

Q5 Hot Start High-Fidelity 2X Master Mix
#M0494S 100 reactions
#M0494L 500 reactions
#M0494X 500 reactions

Quick-Load Purple 1 kb Plus DNA Ladder
#N0550S 250 gel lanes
#N0550L 750 gel lanes

Monarch PCR & DNA Cleanup Kit (5 µg)
#T1030S 50 preps
#T1030L 250 preps

EnGen Spy Cas9 NLS
#M0646T 500 pmol
#M0646M 2,500 pmol

Cas9 Nuclease, *S. pyogenes*
for high (20X) concentration
#M0386T 500 pmol
#M0386M 2,500 pmol

#M0386S 90 pmol

EnGen Sau Cas9
#M0654S 90 pmol

for high (20X) concentration
#M0654T 500 pmol

T7 Endonuclease I
#M0302S 250 units
#M0302L 1,250 units

- *T7 Endonuclease-based detection of genome editing events*

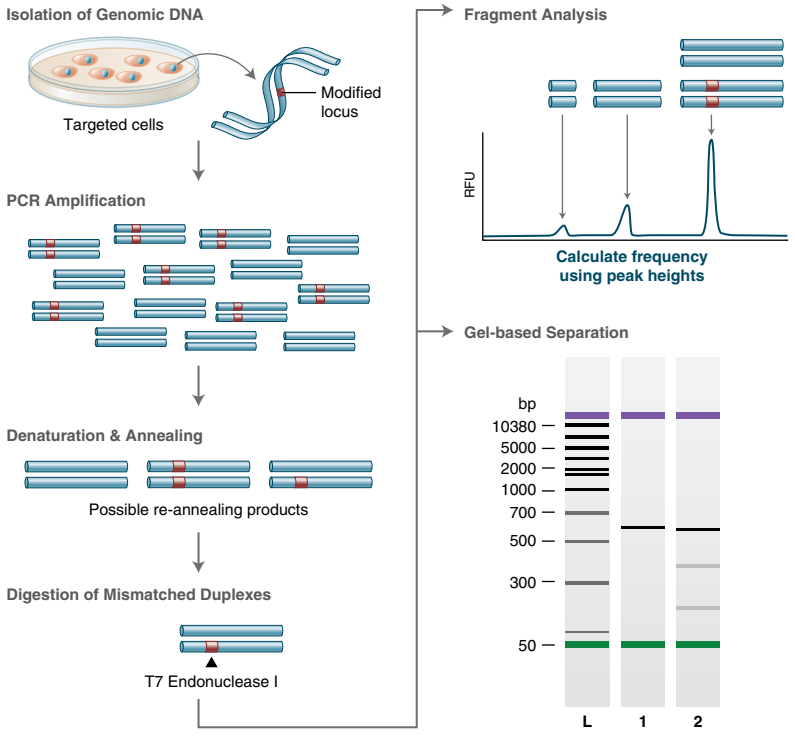
Description: The EnGen Mutation Detection Kit provides reagents for detection of on-target genome editing events. In the first step, regions targeted for genome editing (i.e. CRISPR/Cas9, TALENs, Zinc-finger Nucleases) are amplified using Q5 Hot Start High-Fidelity 2X Master Mix. Upon denaturation and re-annealing, heteroduplexes are formed when mutations from insertions and deletions (indels) are present in the amplicon pool. In the second step, annealed PCR products are digested with EnGen T7 Endonuclease I, a structure-specific enzyme that will recognize mismatches larger than 1 base. Both strands of the DNA are cut when a mismatch is present, which results in the formation of smaller fragments. Analysis of the resulting fragments provides an estimate of the efficiency of the genome editing experiments.

The EnGen Mutation Detection Kit includes a Control Template and Primer Mix that can be used as a control for the PCR reaction and T7 Endonuclease I digestion. The Control Template and Primer Mix provided contains two plasmids and primers that when amplified, denatured and re-annealed will form heteroduplexes that contain a 10-base insertion. This structure is a substrate for T7 Endonuclease I. The digestion of the 600 bp heteroduplex containing amplicon yields products of 200 bp and 400 bp. 600 bp parental homoduplexes are uncleaved, and are easily distinguished from cleaved heteroduplexes when separated and visualized by agarose gel electrophoresis or fragment analysis instrument.

The protocol has been optimized so that PCR products generated by the Q5 Hot Start High-Fidelity 2X Master Mix can be introduced directly into the T7 Endonuclease I digestion without the need for purification. Digestion of the heteroduplex is complete in only 15 minutes, and Proteinase K is included to stop the reaction efficiently. Additional Q5 Hot Start High-Fidelity 2X Master Mix is also included to allow for optimization of target site amplification before digestion.

Kit Includes:

- Q5 Hot Start High-Fidelity 2X Master Mix
- NEBuffer 2
- Control Template and Primer Mix
- Proteinase K, Molecular Biology Grade
- Quick-Load Purple 1 kb Plus DNA Ladder
- Gel Loading Dye, Purple (6X), no SDS
- EnGen T7 Endonuclease I



Genomic DNA is amplified with primers bracketing the modified locus. *PCR products are then denatured and re-annealed yielding three classes of possible structures. Duplexes containing a mismatch greater than one base are digested by T7 Endonuclease I. The DNA is then electrophoretically separated and fragment analysis is used to estimate targeting efficiency.*

EnGen® sgRNA Synthesis Kit, *S. pyogenes*

#E3322V	10 reactions
#E3322S	20 reactions

Companion Products:

EnGen Spy Cas9 NLS
#M0646T 500 pmol
#M0646M 2,500 pmol

EnGen Spy Cas9 HF1
#M0667T 500 pmol
#M0667M 2,500 pmol

Monarch RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

EnGen Spy Cas9 Nickase
#M0650S 90 pmol

for high (20X) concentration
#M0650T 500 pmol

EnGen Spy dCas9 (SNAP-tag)
#M0652S 90 pmol

for high (20X) concentration
#M0652T 500 pmol

EnGen Mutation Detection Kit
#E3321S 25 reactions

DNase I (RNase-free)
#M0303S 1,000 units
#M0303L 5,000 units

- *Rapid generation of microgram quantities of sgRNAs in less than one hour*

EnGen® sgRNA Template
Oligo Designer

Configure target-specific DNA oligos design for use with the EnGen sgRNA Synthesis Kit, *S. pyogenes* with our oligo design tool accessible at **sgrna.neb.com**

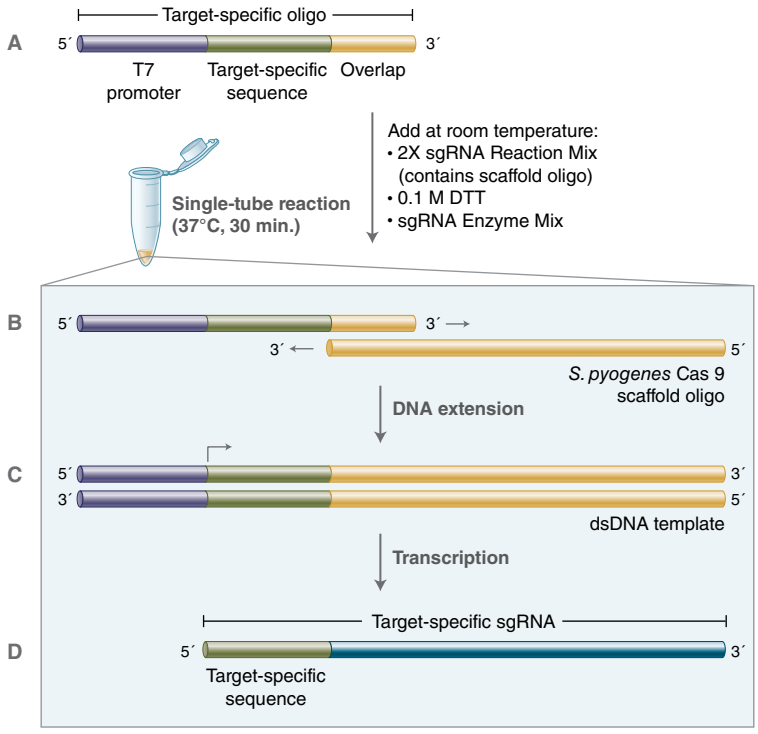
The EnGen sgRNA Synthesis Kit, *S. pyogenes* provides a simple and quick method for transcribing high yields of sgRNA in a single 30 minute reaction, using the supplied reagents and target-specific DNA oligos designed by the user.

In nature, S. pyogenes Cas9 is programmed with two separate RNAs, the crRNA and tracrRNA. The crRNA, or CRISPR RNA sequence contains approximately 20 nucleotides of homology complementary to the strand of DNA opposite and upstream of a PAM (Protospacer Adjacent Motif) (NGG) sequence. The tracrRNA, or transactivating crRNA, contains partial complementary sequence to the crRNA as well as the sequence and secondary structure that is recognized by Cas9. These sequences have been adapted for use in the lab by combining the tracrRNA and crRNA into one long single guide RNA (sgRNA) species capable of complexing with Cas9 to recognize and cleave the target DNA.

The EnGen sgRNA Synthesis Kit, S. pyogenes combines an S. pyogenes Cas9-specific Scaffold Oligo (included in the EnGen 2X sgRNA Reaction Mix) that is partially complementary to the target-specific oligos designed by the user. The two oligos anneal at the overlapping region and are filled in by the DNA polymerase, creating a double-stranded DNA (dsDNA) template for transcription. Synthesis of the dsDNA template and transcription of RNA occur in a single reaction, resulting in the generation of a functional sgRNA.

Kit Includes:

- EnGen sgRNA Enzyme Mix
- EnGen 2X sgRNA Reaction Mix, *S. pyogenes*
- DNase I (RNase-free)
- EnGen sgRNA Control Oligo, *S. pyogenes*
- Dithiothreitol (DTT)



A. *The target-specific oligo contains the T7 promoter sequence, ~20 nucleotides of target-specific sequence and a 14 nucleotide overlap sequence complementary to the S. pyogenes Cas9 Scaffold Oligo supplied in the reaction mix. Target-specific oligos (or EnGen sgRNA Control Oligo, S. pyogenes) are mixed with the EnGen 2X sgRNA Reaction Mix (NTPs, dNTPs, S. pyogenes Cas9 Scaffold Oligo), 0.1 M DTT and the EnGen sgRNA Enzyme Mix (DNA and RNA polymerases) at room temperature. B. At 37°C the two oligos anneal at the 14 nucleotide overlap region of complementarity. C. The DNA polymerase extends both oligos from their 3´ ends creating a double-stranded DNA template. D. The RNA polymerase recognizes the double-stranded DNA of the T7 promoter and initiates transcription. The resulting sgRNA contains the target-specific/crRNA sequence as well as the tracrRNA. All steps occur in a single reaction during a 30 minute incubation at 37°C.*

Direct air capture – a promising technology for augmenting strategies to reduce CO_2 emissions

To reach the 350 parts per million (ppm) CO_2 emission goal set by the international scientific community, it is necessary to introduce technology that removes previously emitted CO_2 directly from the air. This is because, in addition to the CO_2 we are adding to the atmosphere, CO_2 has a long half-life and remains in the atmosphere for hundreds of years.

Technology is currently available that sequesters carbon and can capture approximately 90% of carbon emissions at the source. For example, industrial plants can effectively capture emissions spewed from smokestacks, as CO_2 is emitted at a high concentration. The challenge with direct air capture (DAC) is that it requires the capture and isolation of CO_2 that is dispersed at a much lower concentration in the atmosphere.

DAC is not a new technology; it has been used since the 1940s in submarines and spacecraft to clear the internal air of CO_2. However, in these examples, captured carbon was mainly released back into the atmosphere. Now, development is focused not only on the removal of CO_2 from the atmosphere, but the fate of the CO_2 that has been removed.

One method of DAC draws air into a system of corrugated sheets wetted with CO_2-absorbent liquid, where it is converted to carbonate. Turbulence within the system ensures maximum contact between the CO_2 and the solution. Additional research is focused on a membrane-based system that can separate the CO_2 from the solution. The captured CO_2 can be stored underground or under a seabed or used to make gasoline or jet fuel. DAC is currently an expensive process, and companies have no economic reason to participate. Tax incentives, credits, or a market to trade CO_2 at competitive rates will more likely garner the interest of the big industrial polluters to become involved in technologies that repurpose CO_2. When stored underground, there is also the possibility of leakage back into the atmosphere or even a build-up of pressure that creates seismic activity. Alternatively, captured CO_2 can be combined with hydrogen to produce hydrocarbons to make gasoline or jet fuel at a very economical rate. Even when this is burned as fuel and released back into the atmosphere, it is still considered a sustainable solution. Additional uses for captured CO_2 are in fertilizer, carbonated drinks, and building materials such as concrete.

The expansion of DAC cannot be viewed as an excuse to become complacent when it comes to replacing fossil fuel burning with sustainable sources of low-carbon energy. Currently, much of the funding for DAC comes from oil companies, which store the CO_2 underground in old oil fields. This builds up pressure and dislodges the remaining hydrocarbon from the oil, making it easier to extract (Enhanced Oil Recovery). At present, most CO_2 sequestered using DAC technology is used to extract more oil. This is a massive paradox – DAC is essentially just cleaning up some of the CO_2 waste that the oil companies are emitting, which is waste that DAC is contributing to!

The main strategy should always be to lower CO_2 emissions before they pollute the atmosphere. If this technology gains a stronger foothold, the urgency for action to reduce emissions should still be maintained if we are to avoid the more severe predicted effects of climate change.

Learn more about direct air capture.

CO_2 carbon capture plant – climate change solutions technology, Iceland.
Credit: Adam Sébire/Stocksy, Adobe Stock

RNA Reagents

A broad portfolio of reagents to support RNA research.

RNA molecules play a multitude of cellular roles in all kingdoms of life, perhaps reflecting the hypothetical, prehistoric RNA world. RNA is an essential carrier of genetic information, can scaffold molecular interactions, catalyze chemical reactions, and influences gene expression.

In the last several years, our understanding of RNAs as regulatory molecules in the cell has dramatically changed. Many new classes of small and large non-coding RNAs, with largely unexplored functions, have been reported, ushering in a renaissance of RNA-focused research in biology.

Small RNAs play a major role in the post-transcriptional regulation of gene expression in eukaryotic and prokaryotic organisms. Small RNAs play central roles in CRISPR pathways of adaptive immunity. Researchers have capitalized on these pathways to enable analyses of gene function not previously possible.

Introducing RNA, and in particular mRNA, into cells is an exciting new platform for inducing phenotypic changes in cells with implications for therapy, vaccines, and research applications.

New England Biolabs continues its strong tradition of providing high quality reagents to support RNA research. Our expanding range of products includes tools for the synthesis, processing, cleanup, isolation, analysis, amplification, copying and cloning of RNA molecules. Further, all NEB products pass stringent quality control assays to ensure the highest level of functionality and purity.

Featured Products

201 **HiScribe® T7 mRNA Kit with CleanCap® Reagent AG**

201 **HiScribe T7 High Yield RNA Synthesis Kits**

203 **HiScribe T7 ARCA mRNA Kit (with tailing)**

207 **Faustovirus Capping Enzyme**

211 **Template Switching RT Enzyme Mix**

Featured Tools & Resources

200 **Avoiding Ribonuclease Contamination**

213 **RNA Ligase Activity Chart**

View our **video tutorial** describing **high yield *in vitro* synthesis** of both **capped and uncapped mRNA**.

View our **video for avoiding ribonuclease contamination**.

Learn more about RNA modifications.

Avoiding Ribonuclease Contamination 200

RNA Synthesis

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RI Recombinant Enzyme

Avoiding Ribonuclease Contamination

Maintaining the integrity of RNA is a critical aspect of nearly all applications that use it. RNA is more susceptible to degradation than DNA, due to the ability of the 2´ hydroxyl groups to act as nucleophiles. Many ribonucleases (RNases) bypass the need for metal ions by taking advantage of the 2´ hydroxyl group as a reactive species. These RNases are resistant to metal chelating agents and, some of them, like RNase A family enzymes, can survive prolonged boiling or autoclaving. RNase A-type enzymes rely on active site histidine residues for catalytic activity (1) and can be inactivated by the histidine-specific alkylating agent diethyl pyrocarbonate (DEPC).

Sources of RNase Contamination:

RNases are found in all cell types and organisms from prokaryotes to eukaryotes. RNases generally have very high specific activity, meaning miniscule amounts of contamination in an RNA sample is sufficient to destroy the RNA. The major sources of RNase contamination include:

- Aqueous solutions, reagents used in experiments
- Exposure to RNase from environmental sources (lab surfaces, aerosols from pipetting, ungloved hands, etc.)

Laboratory Precautions (2,3):

NEB's enzymes have been purified free of ribonucleases. However, it is possible to reintroduce RNases during the course of experimentation from various sources. RNase contamination can be prevented with a few common sense laboratory procedures:

- Always wear gloves during an experiment and change them often, especially after contact with skin, hair or other potentially RNase-contaminated surfaces, such as doorknobs, keyboards and animals.
- Use RNase-free solutions. Use RNase-free certified, disposable plasticware and filter tips whenever possible.
- Maintain a separate area for RNA work. Carefully clean the surfaces.
- Decontaminate glassware by baking at 180°C or higher for several hours or by soaking in freshly prepared 0.1% (v/v) DEPC in water or ethanol for 1 hour, followed by draining and autoclaving. Autoclaving will destroy any unreacted DEPC which can otherwise react with other proteins and RNA.
- Decontaminate polycarbonate or polystyrene materials (e.g., electrophoresis tanks) by soaking in 3% hydrogen peroxide for 10 minutes. Remove peroxide by extensively rinsing with RNase-free water prior to use.

Preparation of Solutions (2,3):

Preparation of solutions using the following suggestions can help prevent RNase contamination:

- As an alternative to the historic use of DEPC, which can inhibit enzymatic reactions if not completely removed, we have found that Milli-Q® (Millipore) purified water is sufficiently free of RNases for most RNA work. NEB also offers Nuclease-free Water (NEB #B1500).
- DEPC treatment of solutions is accomplished by adding 1 ml DEPC (Sigma) per liter of solution, stirring for 1 hour, and autoclaving for one hour to remove any remaining DEPC. [Note: Compounds with primary amine groups (e.g., Tris) which will react with DEPC, cannot be DEPC-treated. Other compounds, which are not stable during autoclaving, cannot be DEPC-treated].
- Solutions and buffers (e.g. DTT, nucleotides, manganese salts) should be prepared by dissolving the solid (highest available purity) in autoclaved DEPC-treated or Milli-Q water and passing the solution through a 0.22 µm filter to sterilize.

Inhibitors of Ribonucleases:

RNA can also be protected from RNase activity by using one of the following RNase inhibitors:

- RNase Inhibitor, Murine, (NEB #M0314) is the murine version of RNase inhibitor. It has the same inhibition profile as human or porcine inhibitors, but is more stable due to improved resistance to oxidation (4). The inhibitor requires low concentrations of DTT (< 1 mM) to maintain activity, making it ideal for reactions where low DTT concentration is required (e.g., real-time RT-PCR).
- RNase Inhibitor, Human Placenta, (NEB #M0307), a recombinant protein of human placenta, is a specific inhibitor for RNases A, B and C. Similar to the Murine RNase Inhibitor, it is compatible with many enzymatic reactions involving RNA (e.g., *in vitro* transcription, RT-PCR, ligation, etc.).
- Ribonucleoside Vanadyl Complex (NEB #S1402) is a transition-state analog inhibitor of RNase A-type enzymes with $K_i = 1 \times 10^{-5}$ M. This complex is compatible with many RNA isolation procedures, but it should not be used in the presence of EDTA. The complex also inhibits many other enzymes used in RNA work (5).

Find tips for avoiding RNA contamination.

References:

(1) Fersht, A.R. (1977) *Enzyme Structure and Mechanism* Freeman, Reading, PA, 325–329.
(2) Blumberg, D.D. (1987) *Methods Enzymol.*, 152, 20–24.
(3) Sambrook, J., et al. (1989). *Molecular Cloning: A Laboratory Manual*, (2nd ed.). Cold Spring Harbor: Cold Spring Harbor Laboratory Press., 7.3–7.5.
(4) Kim, B.M. et al. (1999) *Protein Science*, 8, 430–434.
(5) Berger, S.L. (1987) *Methods Enzymol.*, 152, 227–234.

NEW

HiScribe® T7 mRNA Kit with CleanCap® Reagent AG

#E2080S 20 reactions

Companion Products:

Q5 Site-Directed Mutagenesis Kit
#E0554S 10 reactions

Monarch RNA Cleanup Kit (500 µg)
#T2050S 10 preps
#T2050L 100 preps

Monarch RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

- *Streamlined workflow with single-step co-transcriptional capping*
- *CleanCap Reagent AG trinucleotide cap technology results in a natural Cap-1 structure, maximizing translatability and minimizing immune response from synthetic mRNA*
- *High capping efficiency*
- *Optimized for high yields*
- *Suitable for full- or partial- modified nucleotide substitution*

The HiScribe T7 mRNA Kit with CleanCap Reagent AG utilizes an optimized RNA synthesis formulation and trinucleotide cap analog technology for co-transcriptionally capping mRNAs that contain a natural Cap-1 structure, in a single simplified reaction without compromising RNA yield. The kit contains individual vials of NTPs and CleanCap Reagent AG to allow for partial of complete substitution of modified NTPs, with a total kit yield of 1.8 mg of mRNA. Cap-1 mRNA synthesized can be used in many downstream applications, including transfections, microinjections, *in vitro* translation, preclinicial mRNA therapeutic mRNA studies, as well as RNA structure and function analysis.

Reagents Supplied:

- T7 RNA Polymerase Mix
- LiCl Solution
- DNase I (RNase-free)
- CLuc AG Control Template
- 10X T7 CleanCap Reagent AG Reaction Buffer
- CleanCap Reagent AG
- ATP
- GTP
- CTP
- UTP

HiScribe® T7 High Yield RNA Synthesis Kits

HiScribe T7 High Yield RNA Synthesis Kit
#E2040S 50 reactions

HiScribe T7 Quick High Yield RNA Synthesis Kit
#E2050S 50 reactions

- *Synthesis of long and short RNA transcripts*
- *Incorporation of modified nucleotides*
- *Incorporation of labeled nucleotides*
- *Generation of capped RNA using cap analogs*
- *Synthesis of radioactively labeled probes with high or low specific activity*

Description: NEB's HiScribe T7 High Yield RNA Synthesis Kits offer robust *in vitro* RNA transcription of many kinds of RNA, including internally labeled and co-transcriptionally capped transcripts. Utilizing T7 RNA Polymerase, these kits achieve efficient transcription with small amounts of template, and can generate up to 180 µg per reaction, or up to 30–40 µg of capped RNA using cap analog. RNA generated can be used in a variety of applications, including RNA structure/function studies, ribozyme biochemistry, probes for RNase protection assays and hybridization-based blots, anti-sense RNA and RNAi experiments, microarray analysis, microinjection, and *in vitro* translation and RNA vaccines.

The HiScribe T7 High Yield RNA Synthesis Kit is an extremely flexible system, with separate NTPs included for flexible reaction setup. The HiScribe T7 Quick High Yield RNA Synthesis Kit is provided in master mix format for fast reaction setup. Each kit can yield up to 9 mg of RNA.

The HiScribe T7 High Yield RNA Synthesis Kit Includes:

- T7 RNA Polymerase Mix
- 10X T7 Reaction Buffer
- ATP, GTP, UTP, CTP (100 mM)
- FLuc Control Template

The HiScribe T7 Quick High Yield RNA Synthesis Kit Includes:

- T7 RNA Polymerase Mix
- LiCl Solution
- NTP Buffer Mix
- FLuc Control Template
- DNase I (RNase-free)

Watch our webinar on tools for RNA synthesis.

HiScribe® SP6 RNA Synthesis Kit

#E2070S 50 reactions

Companion Products:

Monarch RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

DNase I-XT
#M0570S 1,000 units
#M0570L 5,000 units

DNase I (RNase-free)
#M0303S 1,000 units
#M0303L 5,000 units

RNA Loading Dye, (2X)
#B0363S 4 ml

Faustovirus Capping Enzyme
#M2081S 500 units
#M2081L 2,500 units

Vaccinia Capping System
#M2080S 400 units

Monarch RNA Cleanup Kit (10 µg)
#T2030S 10 preps
#T2030L 100 preps

Monarch RNA Cleanup Kit (500 µg)
#T2050S 10 preps
#T2050L 100 preps

E. coli Poly(A) Polymerase
#M0276S 100 units
#M0276L 500 units

mRNA Cap 2'-O-Methyltransferase
#M0366S 2,000 units

3´-O-Me-m^{7}G(5')ppp(5')G RNA Cap Structure Analog
#S1411S 1 µmol
#S1411L 5 µmol

G(5')ppp(5')A RNA Cap Structure Analog
#S1406S 1 µmol
#S1406L 5 µmol

G(5')ppp(5')G RNA Cap Structure Analog
#S1407S 1 µmol
#S1407L 5 µmol

m^{7}G(5')ppp(5')G RNA Cap Structure Analog
#S1404S 1 µmol
#S1404L 5 µmol

m^{7}G(5')ppp(5')A RNA Cap Structure Analog
#S1405S 1 µmol
#S1405L 5 µmol

Use Monarch RNA Cleanup Kits to purify your synthesized RNA.

Description: The HiScribe SP6 RNA Synthesis Kit is designed for the *in vitro* transcription of RNA using SP6 RNA Polymerase. This kit is suitable for synthesis of high yield RNA transcripts and allows for incorporation of cap analogs (not included) or modified nucleotides (not included) to obtain capped, biotin-labeled or dye-labeled RNA. The kit is also capable of synthesizing high specific activity radiolabeled RNA for use as probes or targets.

RNA synthesized from this kit is suitable for many applications including RNA structure and function studies, ribozyme biochemistry, probes for RNase protection or gel shift assays, hybridization-based blots, anti-sense RNA or RNAi experiments, microarray analysis, microinjection and *in vitro* translation studies.

This kit contains sufficient reagents for 50 reactions of 25 µl each. Each standard reaction yields ≥ 80 µg of RNA from 1 µg SP6 Control Template DNA. Each kit can yield ≥ 4 mg of RNA.

Kit Includes:

- SP6 Reaction Buffer
- SP6 RNA Polymerase Mix
- SP6 Control Template
- ATP (Tris)
- GTP (Tris)
- UTP (Tris)
- CTP (Tris)
- DNase I (RNase-free)
- LiCl Solution

Andrew joined NEB in 2016 and is an Associate Director of Business Development. Learn more about Andrew's role at NEB in his video reel.

#NEBiographies

RNA REAGENTS

Cloned at NEB · Recombinant Enzyme · PCR Enzyme · Hot Start/WarmStart · Requires SAM · 37° Incubation Temperature · 65° Heat Inactivation

HiScribe® T7 ARCA Kits

HiScribe T7 ARCA mRNA Kit
#E2065S 20 reactions

HiScribe T7 ARCA mRNA Kit (with tailing)
#E2060S 20 reactions

Companion Products:

DNase I (RNase-free)
#M0303S 1,000 units
#M0303L 5,000 units

RNA Loading Dye, (2X)
#B0363S 4 ml

- *Synthesis of capped and tailed mRNA*
- *Incorporation of modified nucleotides*
- *Template removal and mRNA purification reagents included*

Description: Most eukaryotic mRNAs require a 7-methyl guanosine (m7G) cap structure at the 5´ end and a Poly(A) tail at the 3´ end to be efficiently translated. By using a DNA template encoding a poly(A) tail, the HiScribe T7 ARCA mRNA Kit can be used to synthesize capped and tailed mRNAs. The cap structure is added to the mRNA by co-transcriptional incorporation of Anti-Reverse Cap Analog (ARCA, NEB #S1411) using T7 RNA Polymerase. The transcription reaction can be set up easily by combining the ARCA/NTP Mix, T7 RNA Polymerase Mix and a suitable DNA template. The kit also allows for partial incorporation of 5mCTP, PseudoUTP and other modified nucleotides into mRNA. mRNAs synthesized with the kit can be used for cell transfection, microinjection, *in vitro* translation and RNA vaccines. Poly(A) tail is incorporated during the transcription reaction. The kit also includes DNase I and LiCl for DNA template removal and quick mRNA purification.

The HiScribe T7 ARCA mRNA Kit (with tailing) is designed for quick production of ARCA capped and poly(A) tailed mRNA *in vitro* from templates without encoded poly(A) tails.

The HiScribe T7 ARCA mRNA Kit Includes:

- T7 RNA Polymerase Mix
- ARCA/NTP Mix
- DNase I (RNase-free)
- LiCl Solution
- CLuc Control Template

The HiScribe T7 ARCA mRNA Kit (with tailing) Includes:

- T7 RNA Polymerase Mix
- ARCA/NTP Mix
- DNase I (RNase-free)
- *E. coli* Poly(A) Polymerase
- Poly(A) Polymerase Reaction Buffer
- LiCl Solution
- CLuc Control Template

Advantages:

- Quick reaction setup and streamlined protocol
- Enables partial incorporation of 5mCTP, Pseudo-UTP and other modified CTP and UTP
- High quality components ensure mRNA integrity

EnGen® sgRNA Synthesis Kit, *S. pyogenes*

#E3322V 10 reactions
#E3322S 20 reactions

Companion Products:

EnGen Spy Cas9 NLS
#M0646T 500 pmol
#M0646M 2,500 pmol

EnGen Spy Cas9 HF1
#M0667T 500 pmol
#M0667M 2,500 pmol

Monarch RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

EnGen Spy Cas9 Nickase
#M0650S 90 pmol

for high (20X) concentration
#M0650T 500 pmol

EnGen Spy dCas9 (SNAP-tag)
#M0652S 90 pmol

for high (20X) concentration
#M0652T 500 pmol

EnGen Mutation Detection Kit
#E3321S 25 reactions

DNase I (RNase-free)
#M0303S 1,000 units
#M0303L 5,000 units

- *Rapid generation of microgram quantities of sgRNAs in less than one hour*

The EnGen sgRNA Synthesis Kit, *S. pyogenes* provides a simple and quick method for transcribing high yields of sgRNA in a single 30 minute reaction, using the supplied reagents and target-specific DNA oligos designed by the user.

In nature, *S. pyogenes* Cas9 is programmed with two separate RNAs, the crRNA and tracrRNA. The crRNA, or CRISPR RNA sequence contains approximately 20 nucleotides of homology complementary to the strand of DNA opposite and upstream of a PAM (Protospacer Adjacent Motif) (NGG) sequence. The tracrRNA, or transactivating crRNA, contains partial complementary sequence to the crRNA as well as the sequence and secondary structure that is recognized by Cas9. These sequences have been adapted for use in the lab by combining the tracrRNA and crRNA into one long single guide RNA (sgRNA) species capable of complexing with Cas9 to recognize and cleave the target DNA.

The EnGen sgRNA Synthesis Kit, S. pyogenes combines an S. pyogenes Cas9-specific Scaffold Oligo (included in the EnGen 2X sgRNA Reaction Mix) that is partially complementary to the target-specific oligos designed by the user. The two oligos anneal at the overlapping region and are filled in by the DNA polymerase, creating a double-stranded DNA (dsDNA) template for transcription. Synthesis of the dsDNA template and transcription of RNA occur in a single reaction, resulting in the generation of a functional sgRNA.

Kit Includes:

- EnGen sgRNA Enzyme Mix
- EnGen 2X sgRNA Reaction Mix, *S. pyogenes*
- DNase I (RNase-free)
- EnGen sgRNA Control Oligo, *S. pyogenes*
- Dithiothreitol (DTT)

Recommended HiScribe RNA Synthesis Kits by Application

Application		T7 Kits					SP6 Kits
		HiScribe T7 High Yield RNA Synthesis Kit NEB #E2040S	HiScribe T7 Quick High Yield RNA Synthesis Kit NEB #E2050S	HiScribe T7 ARCA mRNA Kit NEB #E2065S	HiScribe T7 ARCA mRNA Kit (with tailing) NEB #E2060S	HiScribe T7 mRNA Kit with CleanCap Reagent AG NEB #E2080S	HiScribe SP6 RNA Synthesis Kit NEB #E2070S
Probe labeling	Fluorescent labeling: FAM, Cyanine (Cy) dyes, etc. • Fluorescent *in situ* hybridization (FISH)	●					●
	Non-fluorescent labeling: Biotin, Digoxigenin • *In situ* hybridization • Blot hybridization with secondary detection • Microarray		●				●
	High specific activity radiolabeling • Blot hybridization • RNase protection	●					●
mRNA & RNA for transfection	Streamlined high yield CleanCap Reagent AG capped RNA synthesis • Template encoded poly(A) tails • Non polyadenylated transcripts • Transfection • Microinjection • *In vitro* translation					●	
	Streamlined mRNA synthesis with ARCA co-transcriptional capping and enzymatic poly(A) tailing • Transfection • Microinjection • *In vitro* translation				●		
	Streamlined ARCA capped RNA synthesis • Template encoded poly(A) tails • Non polyadenylated transcripts • Transfection • Microinjection • *In vitro* translation			●			
	Co-transcriptional capping with alternate cap analogs • Transfection • Microinjection • *In vitro* translation	●	●				●
	Post-transcriptional capping with Vaccinia Capping System • Transfection • Microinjection • *In vitro* translation	●	●				●
	Complete substitution of NTPs: 5-mC, pseudouridine, etc.	●				●	●
	Partial substitution of NTPs: 5-mC, pseudouridine, etc.		●	●	●	●	●
	Unmodified RNA		●			●	●
	Hairpins, short RNA, dsRNA • Gene knockdown		●				●
Structure, function, & binding studies	Complete substitution of NTPs • Aptamer selection • Isotopic labeling	●					●
	Partial substitution of one or more NTPs • Aptamer selection • Structure determination		●				●
	Unmodified RNA • SELEX • Structure determination		●				●

Cloned at NEB · RR Recombinant Enzyme · PCR PCR Enzyme · Hot Start/WarmStart · SAM Requires SAM · 37° Incubation Temperature · 65° Heat Inactivation

RNA Polymerases

RR

T3 RNA Polymerase
#M0378S 5,000 units

T7 RNA Polymerase
#M0251S 5,000 units
#M0251L 4 25,000 units

T7 RNA Polymerase (High Concentration)
#M0460T 50,000 units

SP6 RNA Polymerase
#M0207S 2,000 units

Hi-T7 RNA Polymerase
#M0658S 5,000 units

Hi-T7 RNA Polymerase (High Concentration)
#M0470T 50,000 units

- *Radiolabeled RNA probe preparation*
- *RNA generation for in vitro translation*
- *RNA generation for studies of RNA structure, processing and catalysis*

Description: Initiation of transcription with T3, T7 and SP6 RNA Polymerases is highly specific for the T3, T7 and SP6 phage promoters, respectively. Cloning vectors have been developed which direct transcription from the T3, T7 or SP6 promoter through polylinker cloning sites. These vectors allow *in vitro* synthesis of defined RNA transcripts from a cloned DNA sequence. T7 RNA Polymerase (High Concentration) is offered at a 20-fold higher concentration than our standard T7 RNA Polymerase and is ideal for experienced users interested in building and optimizing their own *in vitro* transcription reactions.

Hi-T7 RNA Polymerase is an engineered, thermoactive T7 RNA Polymerase. Hi-T7 uses T7 RNA Polymerase Promoters. It can increase capping efficiency and eliminate dsRNA by-product formation during synthesis. Hi-T7 RNA Polymerase (High Concentration) is offered at a 20-fold higher concentration than our standard Hi-T7 RNA Polymerase and is ideal for experienced users interested in building and optimizing their own *in vitro* transcription reactions.

Reaction Conditions: 1X RNAPol Reaction Buffer. Supplement with 0.5 mM each ATP, UTP, GTP, CTP (not included) and DNA template containing the appropriate promoter. Incubate at 37°C (T3, T7 and SP6) or 50°C (Hi-T7). Protocols involving high concentration T7 and Hi-T7 RNA Polymerases are to be designed and optimized by the user.

Unit Definition: One unit is defined as the amount of enzyme required to incorporate 1 nmol ATP into an acidinsoluble material in 1 hour at 37°C or 50°C for Hi-T7. Unit assay conditions can be found at www.neb.com.

Concentration: T3 RNA Polymerase: 50,000 units/ml. T7 RNA Polymerase: 50,000 units/ml. T7 RNA Polymerase (High Concentration): 1,000,000 units/ml. SP6 RNA Polymerase: 20,000 units/ml. Hi-T7 RNA Polymerase: 50,000 units/ml. Hi-T7 RNA Polymerase (High Concentration): 1,000,000 units/ml.

E. coli Poly(A) Polymerase

NEBU RR 37°

#M0276S 100 units
#M0276L 500 units

Companion Products:

Adenosine 5'-Triphosphate (ATP)
#P0756S 1 ml
#P0756L 5 ml

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

- *Labeling of RNA with ATP or cordycepin 5´-triphosphate*
- *Poly(A) tailing of RNA for cloning or affinity purification*
- *Enhances translation of RNA transferred into eukaryotic cells*

Description: *E. coli* Poly(A) Polymerase catalyzes the template independent addition of AMP from ATP to the 3´ end of RNA.

Source: An *E. coli* strain that carries the cloned Poly(A) Polymerase gene from *E. coli* (1).

Reaction Conditions: Poly(A) Polymerase Reaction Buffer, 37°C. Supplement with 1 mM ATP.

Reagents Supplied:

- Poly(A) Polymerase Reaction Buffer
- Adenosine-5'-Triphosphate (ATP)

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 1 nmol of AMP into RNA in a 20 µl volume in 10 minutes at 37°C.

Concentration: 5,000 units/ml

Poly(U) Polymerase

NEB2 RR 37° 65°

#M0337S 60 units

Companion Products:

Ribonucleotide Solution Set
#N0450S 10 µmol
#N0450L 50 µmol

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

- *Labeling of RNA with UTP*
- *Poly(U) tailing of RNA for cloning*

Description: Poly(U) Polymerase catalyzes the template-independent addition of UMP from UTP or AMP from ATP to the 3´ end of RNA.

Source: An *E. coli* strain that carries the cloned poly(U) polymerase gene of *Schizosaccharomyces pombe* Cid1.

Reaction Conditions: NEBuffer 2, 37°C. Supplement with 0.5 mM UTP. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that incorporates 1 nmol of UMP into RNA in a 50 µl volume in 10 minutes at 37°C.

Concentration: 2,000 units/ml

Note: Poly(U) Polymerase in NEBuffer 2 will incorporate rNMP from rNTP into RNA. Tailing length of poly(U) varies with UTP. Poly(U) Polymerase is highly processive under low primer concentrations (<100 pmol).

E. coli RNA Polymerase, Core Enzyme & Holoenzyme

E. coli RNA Polymerase, Core Enzyme
#M0550S 100 units

E. coli RNA Polymerase, Holoenzyme
#M0551S 50 units

- *RNA synthesis from E. coli promoter*
- *Transcription initiation studies*
- *In vitro translation with PURExpress*

Description: *E. coli* RNA Polymerase Core Enzyme consists of 5 subunits designated α, α, β´, β, and ω. The enzyme is free of sigma factor and does not initiate specific transcription from bacterial and phage DNA promoters. The enzyme retains the ability to transcribe RNA from nonspecific initiation sequences. Addition of sigma factors will allow the enzyme to initiate RNA synthesis from specific bacterial and phage promoters. The core enzyme has a molecular weight of ~ 400 kDa.

E. coli RNA Polymerase Holoenzyme is the core enzyme saturated with sigma factor 70. The Holoenzyme initiates RNA synthesis from sigma 70 specific bacterial and phage promoters.

Reaction Conditions: 1X *E. coli* RNA Polymerase Reaction Buffer, 0.5 mM of each rNTP and DNA template. Incubate at 37°C.

Unit Definition: One unit is the amount of enzyme required to incorporate 1 nmol of NTP into RNA in 10 minutes at 37°C. Unit assay conditions can be found at www.neb.com.

Concentration: 1,000 units/ml

Pyrophosphatases

Pyrophosphatase, Inorganic (*E. coli*)
#M0361S 10 units
#M0361L 50 units

Pyrophosphatase, Inorganic (yeast)
#M2403S 10 units
#M2403L 50 units

Thermostable Inorganic Pyrophosphatase
#M0296S 250 units
#M0296L 1,250 units

NudC Pyrophosphatase
#M0607S 250 pmol

- *Increasing RNA yield in transcription reactions*
- *Enhancing DNA replication*

Description: Inorganic pyrophosphatase (PPase) catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate.

$P207^{-4} + H_20 \rightarrow 2HP04^{-2}$

Source: Pyrophosphatase, Inorganic (*E. coli*) is prepared from a clone of the *E. coli* inorganic pyrophosphatase gene.

Pyrophosphatase, Inorganic (yeast) is an *E. coli* strain containing a genetic fusion of the *Saccharomyces cerevisiae ppa* gene and the gene coding for *Mycobacterium xenopi* GyrA intein. Developed by BioHelix Corporation, now a wholly owned subsidiary of Quidel Corporation, and produced at New England Biolabs.

Thermostable Inorganic Pyrophosphatase is an *E. coli* strain carrying a plasmid encoding a pyrophosphatase from the extreme thermophile *Thermococcus litoralis*.

NudC is a NUDIX pyrophosphatase that efficiently hydrolyzes NAD^{+-} and NADH-capped RNA, generating a ligatible 5´ monophosphate on the RNA (NAD^+ decapping or deNADding). Deletion of the nudC gene has been shown to increase the fraction of NAD^{+-} capped RNA in *E. coli*.

Unit Definition: One unit is defined as the amount of enzyme that will generate 1 µmol of phosphate per minute from inorganic pyrophosphate under standard reaction conditions.

1 µM of NudC hydrolyzes 200 µM or more NAD^+ into NMN^+ and AMP in 1X NEBuffer r3.1 and 5 mM DTT at 37°C for 30 min.

Concentration: Pyrophosphatase, Inorganic (*E. coli*) and Pyrophosphatase, Inorganic (yeast): 100 units/ml. Thermostable Inorganic Pyrophosphatase: 2,000 units/ml. NudC Pyrophosphatase: 10 µM

Ribonucleotides

Ribonucleotide Solution Set
#N0450S 10 µmol
#N0450L 50 µmol

Ribonucleotide Solution Mix
#N0466S 10 µmol
#N0466L 50 µmol

Description: Ribonucleotide Solution Set consists of four separate solutions of ATP, GTP, CTP and UTP, pH 7.5, as sodium salts. Each nucleotide is supplied at 100 mM.

Ribonucleotide Solution Mix consists of a buffered equimolar solution of ribonucleotide triphosphates (rATP, rCTP, rGTP and rUTP), pH 7.5, as sodium salts. Each nucleotide is supplied at a concentration of 25 mM (total rNTP concentration equals 100 mM).

Note: To ensure maximum activity upon long-term storage, aliquot and store at –80°C

Sce PUS1

#M0526S 5,000 pmol

- *Sequence-specific pseudouridine modification is an alternative to randomly incorporated modified nucleosides by RNA polymerases*

Description: Sce Pseudouridine Synthase I (Sce PUS1) converts Uridine to Pseudouridine in single-stranded RNA, with a preference for Uridines in single-stranded RNA regions over Uridines in double-stranded RNA. The optimal substrate is an unstructured RNA that is 15 nt long or longer.

Reaction Conditions: NEBuffer r1.1, 30°C. Heat inactivation: 55°C.

Concentration: 100 pmol/µl

NudC and Sce PUS1 are **Enzymes for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

NEW

Faustovirus Capping Enzyme

NEBU 37° 70° SAM

#M2081S 500 units
#M2081L 2,500 units

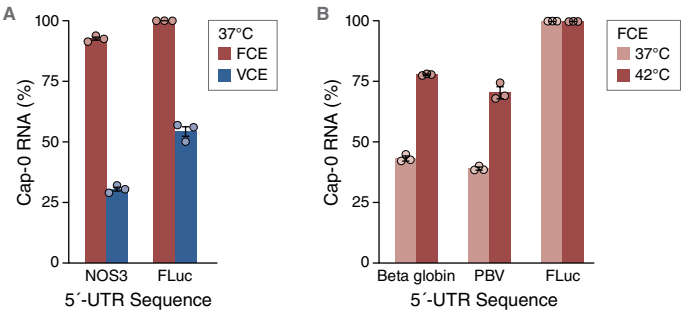
- *Experience improved capping efficiency, even on difficult substrates*
- *Achieve robust capping with less enzyme*
- *Set up reactions under a broad temperature range, for added flexibility*
- *Choose as an alternative to Vaccinia Capping System, with minimal optimization required*
- *Enable one-pot Cap-1 synthesis, as FCE is compatible with mRNA Cap 2´-O-Methyltransferase*
- *Benefit from no licensing fees from NEB for the use of FCE*

Faustovirus Capping Enzyme (FCE) catalyzes the addition of N7-methylguanosine cap (m7G) to the 5´ end of the triphosphorylated and diphosphorylated transcripts, producing Cap-0 RNA. FCE is a single-subunit enzyme that combines the three activities necessary to produce the Cap-0 structure – triphosphatase, guanylyltransferase, and (guanine-N7)-methyltransferase. FCE retains significant capping activity at low temperatures and tolerates reaction temperatures up to 55°C. In many cases, 1 µl of FCE (25 units) can cap over 100 µg of RNA in 1 hour at 37°C.

Reaction Conditions: FCE Capping Buffer, 37°C. Supplement with 0.5 mM GTP and 0.1 mM S-adenosylmethionine (SAM). Heat inactivation: 70°C for 10 minutes. Addition of EDTA to 5 mM is recommended to avoid RNA hydrolysis.

Unit Definition: One unit of Faustovirus Capping Enzyme is defined as the amount of enzyme required to convert 75 pmol of a 20-mer transcript to Cap-0 RNA in 30 minutes at 37°C.

Concentration: 25,000 units/ml



Faustovirus Capping Enzyme (FCE) offers increased capping efficiency and workflow optimization.

200 µg (~350 picomoles) of a 1.77 kb transcript having 5´-UTR sequences as indicated were treated with A) a limiting amount of FCE (25 units, 1 picomole, 20 nM in 50 µl) or VCE (10 units, 1 picomole, 20 nM in 50 µl) for 1 hour at 37°C or B) or for FCE only for 1 hour at 37°C or 42°C. Note that this is less than our recommended amount of enzyme highlighting the increased capping efficiency of FCE vs VCE. 50 µl reactions contained 0.1 mM SAM, and 0.5 mM GTP, 1X FCE Capping Buffer for FCE reactions or 1X Capping Buffer for VCE reactions. mRNA capping was measured using targeted RNase H cleavage and LC-MS.

Vaccinia Capping System

NEBU RR 37° SAM

#M2080S 400 units

Companion Product:

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

- *Capping mRNA prior to in vivo or in vitro translation*
- *Labeling 5´ end of mRNA*

Description: Based on the Vaccinia Virus Capping Enzyme, the Vaccinia Capping System provides the necessary components to add 7-methylguanylate cap structures (Cap-0) to the 5´ end of RNA. In eukaryotes, these terminal cap structures are involved in stabilization, transport and translation of mRNAs. Enzymatic production of capped RNA is an easy way to improve the stability and translational competence of RNA used for *in vitro* translation, transfection and microinjection. Alternatively, use of labeled GTP in a reaction provides a convenient way to label any RNA containing a 5´ terminal triphosphate.

This single enzyme is composed of two subunits (D1 and D12) and has three enzymatic activities (RNA triphosphatase and guanylyltransferase by the D1 subunit and guanine methyltransferase by the D12 subunit), all necessary for addition of a complete Cap-0 structure, m^7Gppp(5´)N. All capped structures are added in the proper orientation, unlike co-transcriptional addition of some cap analogs.

Source: An *E. coli* strain that carries the genes for the Vaccinia (WR) capping enzyme.

Reaction Conditions: Capping Buffer, 37°C. Supplement with 0.5 mM GTP and 0.1 mM S-adenosylmethionine (SAM).

Reagents Supplied:

- Capping Buffer
- S-adenosylmethionine (SAM)
- GTP

Unit Definition: One unit of Vaccinia Capping Enzyme is defined as the amount of enzyme required to incorporate 10 pmol of (α^{32}P) GTP into an 80 nt transcript in 1 hour at 37°C.

Concentration: 10,000 units/ml

mRNA Cap 2'-O-Methyltransferase

NEBU RR 37° SAM

#M0366S 2,000 units

- *Enhances translation of RNA*
- *Improving mRNA expression during microinjection and transfection*

Description: mRNA Cap 2´-O-Methyltransferase adds a methyl group at the 2´-O position of the first nucleotide adjacent to the cap structure at the 5´ end of the RNA. The enzyme utilizes S-adenosylmethionine (SAM) as a methyl donor to methylate capped RNA (Cap-0) resulting in a Cap-1 structure.

Source: An *E. coli* strain that carries the gene for the Vaccinia mRNA Cap 2´-O-Methyltransferase.

Reaction Conditions: Capping Buffer, 37°C. Supplement with 0.2 mM S-adenosylmethionine (SAM).

Reagents Supplied:

- Capping Buffer
- S-adenosylmethionine (SAM)

Unit Definition: One unit is defined as the amount of enzyme required to methylate 10 pmoles of 80 nt long capped RNA transcript in 1 hour at 37°C.

Concentration: 50,000 units/ml

RNA Cap Analog Selection Chart

The 5´ terminal m7 G cap present on most eukaryotic mRNAs is required for translation, *in vitro*, at the initiation level. For most RNAs, the cap structure increases stability, decreases susceptibility to exonuclease degradation, and promotes the formation of mRNA initiation complexes. Certain prokaryotic mRNAs with 5´ terminal cap structures are translated as efficiently as eukaryotic mRNA in a eukaryotic cell-free protein synthesizing system. Splicing of certain eukaryotic substrate RNAs has also been observed to require a cap structure.

Product	NEB #	Application	Size	Price
(ARCA) Anti-Reverse Cap Analog 3´-O-Me-m^{7}G(5')ppp(5')G RNA Cap Structure Analog	S1411S S1411L	• Ensures incorporation in correct orientation • Co-transcriptional capping with T7 (NEB #M0251), SP6 (NEB #M0207) and T3 RNA polymerases • Synthesis of m7G capped RNA for *in vitro* splicing assays • Synthesis of m7G capped RNA for transfection or microinjection	1 µmol 5 µmol	
Standard Cap Analog m^{7}G(5')ppp(5')G RNA Cap Structure Analog	S1404S S1404L	• Co-transcriptional capping with T7, SP6 and T3 RNA polymerases • Synthesis of m7G capped RNA for *in vitro* splicing assays • Synthesis of m7G capped RNA for transfection or microinjection	1 µmol 5 µmol	
Unmethylated Cap Analog G(5')ppp(5')G RNA Cap Structure Analog	S1407S S1407L	• Co-transcriptional capping with T7, SP6 and T3 RNA polymerases • Synthesis of unmethylated G capped RNA	1 µmol 5 µmol	
Methylated Cap Analog for A +1 sites m^{7}G(5')ppp(5')A RNA Cap Structure Analog	S1405S S1405L	• Co-transcriptional capping with T7 RNA polymerase from the phi2.5 promoter that contains an A at the transcription initiation site • Synthesis of m7G capped RNA for *in vitro* splicing assays • Synthesis of m7G capped RNA for transfection or microinjection	1 µmol 5 µmol	
Unmethylated Cap Analog for A +1 sites G(5')ppp(5')A RNA Cap Structure Analog	S1406S S1406L	• Co-transcriptional capping with T7 RNA polymerase from the phi2.5 promoter that contains an A at the transcription initiation site • Synthesis of unmethylated G capped RNA • Synthesis of A capped RNA	1 µmol 5 µmol	

3´-Desthiobiotin-GTP & 3´-Biotin-GTP

3´-Desthiobiotin-GTP
#N0761S 0.5 µmol

3´-Biotin-GTP
#N0760S 0.5 µmol

Description: 3´-Desthiobiotin-GTP or 3´-Biotin GTP are guanosine triphosphate (GTP) analogs which are modified at their 3´ position with desthiobiotin or biotin, respectively. When used with the Vaccinia Capping System, (NEB #M2080) these reagents enable affinity tagging of RNA triphosphate ends. Tagged RNAs are enriched by binding to Hydrophilic Streptavidin Magnetic Beads (NEB #S1421). Desthiobiotin-tagged RNAs can be eluted with free biotin. This approach is used in Cappable-seq, a method developed at NEB for directly enriching the 5´-ends of primary transcripts (1).

Reference:
(1) Ettwiller, L. et al. (2016) *BMC Genomics*, 17,199.

yDcpS

NEBU RR 37° 70°

#M0463S 4,000 units

- *mRNA decapping, enabling recapping with tagged-GTP analogs*
- *Biotinylation of 5´ ends of primary transcripts*
- *Recappable-seq*

Description: yDcpS decapping enzyme from *S. cerevisiae* hydrolyzes the phosphodiester bond between the gamma and beta phosphates of m^{7}G capped mRNA, leaving behind a diphosphorylated 5´ end and m^{7}GMP. yDcpS is capable of decapping full length mRNAs and the diphosphorylated 5´ end it leaves behind is suitable for recapping using Vaccinia Capping Enzyme.

Source: An *E. coli* strain carrying the *S. cerevisiae* gene *DCS1* (encoding yDcpS) on a plasmid.

Reaction Conditions: yDcpS Reaction Buffer, 37°C. Heat inactivation: 70°C.

Reagents Supplied:

• yDcpS Reaction Buffer

Unit Definition: One unit is defined as the amount of yDcpS required to convert 50% of a 500 nM solution of the following 25-mer m7G-capped RNA to a 5'-diphosphorylated form in a total reaction volume of 20 µl in 1 hour at 37°C: 5'-[m7Gppp]rGrUrArGrArArCrU rUrCrGrUrCrGrArGrUrArCrGrCrUrCrArA[3-6FAM]-3'

Concentration: 200,000 units/ml

mRNA Decapping Enzyme

NEBU 37° No

#M0608S 2,000 units

- *Efficient replacement for Tobacco Acid Pyrophosphatase*
- *Cap-0 and Cap-1 are removed with equal efficiency*
- *Suitable for 5´ RLM-RACE and RNA-seq*

Description: mRNA Decapping Enzyme catalyzes the removal of 7-methylguanosine cap (m^{7}G) from the 5´ end of mRNA, producing 5´ monophosphate and releasing m^{7}GDP. mRNA Decapping Enzyme is capable of decapping mRNAs of various lengths and removes both Cap-0 and Cap-1 structures with similar efficiency. mRNA Decapping Enzyme also converts 5´ triphosphate ends to 5´ monophosphate, albeit with reduced efficiency.

Source: An *E. coli* strain that carries a plasmid encoding the mRNA Decapping Enzyme.

Reaction Conditions: mRNA Decapping Enzyme Reaction Buffer, 37°C.

Reagents Supplied:

• mRNA Decapping Enzyme Reaction Buffer

Unit Definition: One unit is defined as the amount of mRNA Decapping Enzyme required to convert 50% of a 500 nM m7G-capped substrate to a 5´-monophosphorylated form in a total reaction volume of 20 µl in 1 hour at 37°C.

Concentration: 100,000 units/ml

cDNA Synthesis Selection Chart

cDNA Synthesis	NEB #	Features	Size
Kits and Mixes			
LunaScript RT SuperMix Kit	E3010S E3010L	• Ideal for cDNA synthesis of targets up to 3 kb (two-step RT-qPCR, amplicon sequencing) • Single tube supermix contains random hexamer and oligo-dT primers, dNTPs, Murine RNase Inhibitor, and Luna Reverse Transcriptase • Visible blue tracking dye for easy reaction setup • Fast 13-minute protocol	25 reactions 100 reactions
LunaScript RT Master Mix Kit (Primer-free)	E3025S E3025L	• Ideal for first strand cDNA synthesis • Compatible with random primers, oligo dT primers and gene-specific primers for maximum cDNA synthesis flexibility • 5X master mix contains dNTPs, Murine RNase Inhibitor and Luna Reverse Transcriptase • Visible blue tracking dye for easy reaction setup • Fast 13-minute protocol	25 reactions 100 reactions
ProtoScript® II First Strand cDNA Synthesis Kit	E6560S E6560L	• Generates cDNA up to 10 kb in length • Contains ProtoScript II Reverse Transcriptase, an enzyme with increased thermostability and reduced RNase H activity • Convenient 2-tube kit • Includes dNTPs, Oligo-dT primer and Random Primer Mix	30 reactions 150 reactions
ProtoScript® First Strand cDNA Synthesis Kit	E6300S E6300L	• Generates cDNA at least 5 kb in length • Contains M-MuLV Reverse Transcriptase • Convenient 2-tube kit • Includes dNTPs, Oligo-dT primer and Random Primer Mix	30 reactions 150 reactions
Template Switching RT Enzyme Mix	M0466S M0466L	• Incorporates a universal adaptor sequence at the 3´ end of cDNA during the RT reaction • Enzyme mix and buffer are optimized for efficient template switching • RT enzyme mix includes RNase Inhibitor • High sensitivity for cDNA amplification – enables transcriptome analysis by RNA-seq from single cells or as low as 2 pg of human total RNA • Robust and simple workflow for 5´ Rapid Amplification of cDNA Ends (RACE) • Retains the complete 5´ end of transcripts for 2nd Strand cDNA Synthesis	20 reactions 100 reactions
Standalone Reagents			
Induro® Reverse Transcriptase	M0681S M0681L	• Fast and processive intron-encoded RT for generating long transcripts (> 12 kb in under 10 mn.) • Increased reaction temperatures (50–60°C) • Increased inhibitor tolerance	4,000 units 10,000 units
ProtoScript® II Reverse Transcriptase An alternative to SuperScript® II	M0368S M0368L M0368X	• RNase H– mutant of M-MuLV Reverse Transcriptase with increased thermostability and reduced RNase H activity • Increased reaction temperatures (37–50°C)	4,000 units 10,000 units 40,000 units
M-MuLV Reverse Transcriptase	M0253S M0253L	• Robust reverse transcriptase for a variety of templates • Standard reaction temperatures (37–45°C)	10,000 units 50,000 units
AMV Reverse Transcriptase	M0277S M0277L	• Robust reverse transcriptase for a broad temperature range (37–52°C) • Can be used for templates requiring higher reaction temperatures	200 units 1,000 units
WarmStart® RTx Reverse Transcriptase	M0380S M0380L	• Permits room temperature reaction setup • Increased reaction temperatures (50–65°C) • Optimized for RT-LAMP isothermal detection	50 reactions 250 reactions

For RT-PCR and RT-qPCR kits, see DNA Polymerases and Amplification Technologies.

ProtoScript® II Reverse Transcriptase

NEBU RR 42° 65°

#M0368S 4,000 units
#M0368L 10,000 units
#M0368X 40,000 units

Companion Products:

RNase H
#M0297S 250 units
#M0297L 1,250 units

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

- *Efficient reverse transcription from different starting RNA amounts*
- *Increased thermostability*
- *Generates cDNA up to 10 kb or more*

Description: ProtoScript II Reverse Transcriptase is a recombinant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. It can be used to synthesize first strand cDNA at higher temperatures than the wild type M-MuLV. The enzyme is active up to 50°C, providing higher specificity, higher yield of cDNA and more full length cDNA product, up to 12 kb in length. This product was formerly known as M-MuLV Reverse Transcriptase RNase H^-.

Source: The gene encoding a mutant M-MuLV Reverse Transcriptase (RNase H^-) is expressed in *E. coli* and purified to near homogeneity.

Reaction Conditions: ProtoScript II Reverse Transcriptase Reaction Buffer, 42°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in a total reaction volume of 50 µl in 10 minutes at 37°C using poly(rA)•oligo$(dT)_{18}$ as template.

Concentration: 200,000 units/ml

LunaScript® RT SuperMix Kit & LunaScript RT Master Mix Kit (Primer-free)

NEW

LunaScript RT SuperMix

#M3010L	100 reactions
#M3010X	500 reactions
#M3010E	2,500 reactions

LunaScript RT SuperMix Kit

#E3010S	25 reactions
#E3010L	100 reactions

LunaScript RT Master Mix Kit (Primer-free)

#E3025S	25 reactions
#E3025L	100 reactions

LunaScript RT SuperMix Kit is an optimized master mix containing all the necessary components for first strand cDNA synthesis in the context of a two-step RT-qPCR workflow. It features the thermostable Luna Reverse Transcriptase, which supports cDNA synthesis at elevated temperatures. Murine RNase Inhibitor is also included to protect template RNA from degradation. The LunaScript RT SuperMix Kit contains random hexamer and poly-dT primers, allowing for even coverage across the length of the RNA targets.

LunaScript RT Master Mix Kit (Primer-free) is the same formulation as the LunaScript RT SuperMix Kit but does not contain primers. It includes all components needed for carrying out first strand cDNA synthesis except for RNA template and user-supplied primers. It provides flexible options for using different primers (dT primers, random primers, gene-specific primers, modified primers, etc.) for first strand cDNA synthesis.

M-MuLV Reverse Transcriptase

NEB U RR 42° 65°

#M0253S	10,000 units
#M0253L	50,000 units

Companion Product:

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

- *cDNA synthesis*
- *RNA Sequencing*
- *RT-PCR*

Description: Moloney Murine Leukemia Virus (M-MuLV) Reverse Transcriptase is an RNA-directed DNA polymerase. This enzyme can synthesize a complementary DNA strand initiating from a primer using either RNA (cDNA synthesis) or single-stranded DNA as a template. M-MuLV Reverse Transcriptase lacks 3´→ 5´ exonuclease activity.

Source: The gene encoding M-MuLV Reverse Transcriptase is expressed in *E. coli* in a vector that results in 16 additional amino acids at the N-terminus and 13 amino acids at the C-terminus. This construct results in a fully functional Reverse Transcriptase protein with a functional RNase H domain.

Reaction Conditions: M-MuLV Reverse Transcriptase Reaction Buffer, 42°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in a total reaction volume of 50 µl in 10 minutes at 37°C using poly(rA)•oligo(dT) as template primer with 50 mM Tris-HCl (pH 8.3), 6 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM [^{3}H]-dTTP and 0.4 mM poly(rA)•oligo(dT)12-18.

Concentration: 200,000 units/ml

AMV Reverse Transcriptase

NEB U 42° 85°

#M0277S	200 units
#M0277L	1,000 units

Companion Product:

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

- *cDNA synthesis*
- *RNA Sequencing*
- *RT-PCR*

Description: Avian Myeloblastosis Virus (AMV) Reverse Transcriptase is an RNA-directed DNA polymerase. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA (cDNA synthesis) or single-stranded DNA as a template.

Source: Insect cells (Sf21) infected with *baculovirus* containing the pol gene of AMV.

Reaction Conditions: AMV Reverse Transcriptase Reaction Buffer, 42°C. Heat inactivation: 85°C for 5 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into an acid-insoluble form in 10 minutes at 37°C using poly(rA)-oligo(dT) as template primer.

Concentration: 10,000 units/ml

Note: Storage: Once thawed, store at -20°C. Repeated freeze thaw cycles will inactivate the enzyme. Aliquots can be stored for longer periods at -70°C.

WarmStart® RTx Reverse Transcriptase

NEB U RR 80°

#M0380S	50 reactions
#M0380L	250 reactions

Companion Product:

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

- *RT-LAMP*
- *cDNA Synthesis*
- *RT reactions requiring room temperature setup*

Description: WarmStart RTx Reverse Transcriptase is a unique *in silico*-designed, RNA-directed DNA polymerase coupled with a reversibly-bound aptamer that inhibits RTx activity below 40°C. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA (cDNA synthesis) or single-stranded DNA as a template. RTx is a robust enzyme for RNA detection in amplification reactions and is particularly well suited for use in Loop-mediated Isothermal Amplification (LAMP). The WarmStart property enables high-throughput applications, room-temperature setup, and increases the consistency and specificity of amplification reactions. RTx contains intact RNase H activity.

Reaction Conditions: 1X Isothermal Amplification Buffer, template, primer, dNTPs and 0.25-0.5 µl of WarmStart RTx Reverse Transcriptase in a reaction volume of 25 µl. Incubate at 50–55°C for cDNA synthesis or directly at 65°C for One-step RT-LAMP. Heat inactivation: 80°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in a total reaction volume of 50 µl in 20 minutes at 50°C using poly(rA)•oligo(dT)18 as template.

Concentration: 15,000 units/ml

Cloned at NEB · RR Recombinant Enzyme · PCR PCR Enzyme · Hot Start/WarmStart · SAM Requires SAM · 37° Incubation Temperature · 65° Heat Inactivation

NEW

Induro® Reverse Transcriptase

#M0681S 4,000 units
#M0681L 10,000 units

Companion Products:

Oligo $d(T)_{23}$ VN
#S1327S 1 A260 units

Random Primer Mix
#S1330S 100 µl

Deoxynucleotide (dNTP) Solution Mix
#N0447S 8 µmol
#N0447L 40 µmol

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

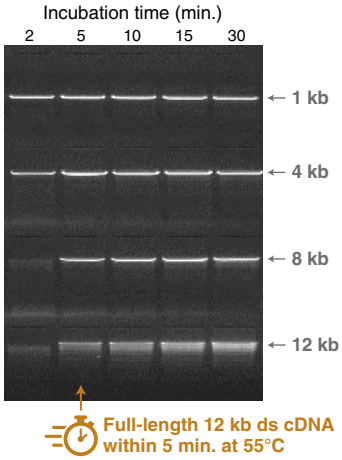
- *Rapidly generate high yields of long cDNA*
- *Strong inhibitor tolerance enables robust cDNA synthesis performance*
- *Support direct RNA sequencing and long read cDNA sequencing workflows*
- *Generate cDNA at higher temps, which is ideal for challenging sample types*
- *Experience comparable fidelity to retroviral RTs*

Description: Induro Reverse Transcriptase is a group II intron-encoded RT that exhibits high processivity, increased thermostability, and increased tolerance of inhibitors for the synthesis of cDNA from long transcripts (>8 kb), RNAs with strong secondary structures, and RNA samples with inhibitors. With improved 5´ sequencing coverage of long transcripts, Induro Reverse Transcriptase can enable RNA-seq applications such as direct RNA and long read cDNA sequencing workflows.

Reagents Supplied:

- Induro RT Reaction Buffer

Concentration: 200,000 units/ml



Induro Reverse Transcriptase exhibits high processivity, permitting rapid cDNA synthesis. *Induro Reverse Transcriptase can synthesize a full-length 12 kb cDNA product within 5 min. at 55°C. In vitro transcribed poly(A) RNA templates (1 kb, 4 kb, 8 kb or 12 kb) were used to investigate full-length cDNA synthesis. After first-strand cDNA synthesis, RNA was hydrolyzed immediately by NaOH. Subsequently, an aliquot of the cDNA products was used to make full-length ds cDNA in the presence of a 5´ specific primer. Equal volume of ds cDNA was analyzed on an agarose gel.*

Template Switching RT Enzyme Mix

#M0466S 20 reactions
#M0466L 100 reactions

Companion Products:

Q5 Hot Start High-Fidelity 2X Master Mix
#M0494S 100 reactions
#M0494L 500 reactions
#M0494X 500 reactions

NEBNext High-Fidelity 2X PCR Master Mix
#M0541S 50 reactions
#M0541L 250 reactions

NEB PCR Cloning Kit
#E1202S 20 reactions

LongAmp Hot Start *Taq* 2X Master Mix
#M0533S 100 reactions
#M0533L 500 reactions

- *Incorporates a universal adaptor sequence at the 3´ end of cDNA during the RT reaction*
- *High sensitivity for cDNA amplification – enables transcriptome analysis by RNA-seq from single cells or as low as 2 pg of human total RNA*
- *Robust and simple workflow for 5´-Rapid Amplification of cDNA Ends (RACE)*
- *Retains the complete 5´ end of transcripts for 2nd Strand cDNA Synthesis*

Description: Template switching reverse transcription (RT) incorporates a universal adaptor sequence to the 3´-end of cDNA. This convenient feature can be utilized in several downstream applications:

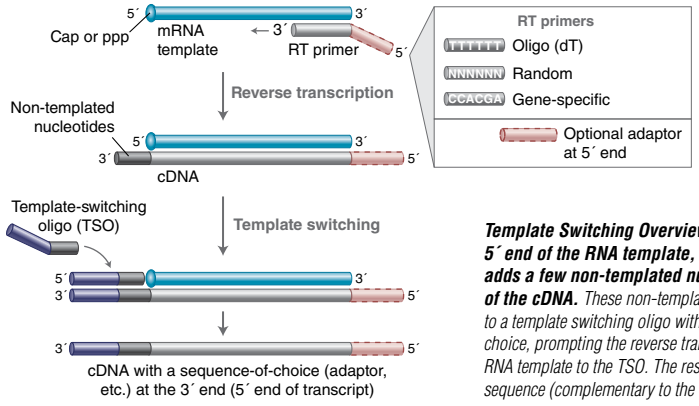
- cDNA synthesis and amplification in a one-tube reaction
- 5´ Rapid Amplification of cDNA Ends (RACE)
- 2nd strand cDNA synthesis that keeps the 5´ end of the transcripts intact

The Template Switching RT Enzyme Mix is optimized for efficient template switching during the RT reaction. The enzyme mix contains RNase Inhibitor in a specially formulated buffer, making reactions easy to setup with no additives needed. It is highly sensitive and specific and can generate RNA-seq libraries from as little as 2 pg of human total RNA or 5´-RACE from 10 ng of total RNA, both with minimal background.

Reagents Supplied:

- Template Switching RT Buffer

Concentration: 10 X



Template Switching Overview Upon reaching the 5´ end of the RNA template, the reverse transcriptase adds a few non-templated nucleotides to the 3´ end of the cDNA. *These non-templated nucleotides can anneal to a template switching oligo with a known sequence handle of choice, prompting the reverse transcriptase to switch from the RNA template to the TSO. The resulting cDNA contains a universal sequence (complementary to the TSO sequence) at the 3´ end.*

Watch our webinar on Induro Reverse Transcriptase.

Primers for cDNA Synthesis

Oligo d(N)n primers are used for the priming and sequencing of mRNA adjacent to the 3´-poly A tail or tailed cDNA.
Note: #S1316 does not contain a 5´-phosphate.

Product	NEB #	Size
Random Primer 6 (5´d(N6)3´) ~14.6 nmol	#S1230S	1 A_{260} units
Random Primer 9 (5´d(N9)3´) ~11.6 nmol	#S1254S	1 A_{260} units
Oligo d(T)$_{23}$ VN	#S1327S	1 A_{260} units
Random Primer Mix	#S1330S	100 µl (60 µM)
Oligo d(T)$_{18}$ mRNA Primer	#S1316S	5 A_{260} units

ProtoScript® II First Strand cDNA Synthesis Kit

#E6560S 30 reactions
#E6560L 150 reactions

Companion Products:

Magnetic mRNA Isolation Kit
#S1550S 25 isolations

RNase Inhibitor, Murine
#M0314S 3,000 units
#M0314L 15,000 units

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

Random Primer Mix
#S1330S 100 µl

Oligo d(T)$_{23}$ VN
#S1327S 1 A_{260} units

- *Enzyme and reaction mixes add flexibility to reaction setup*
- *Suitable for any PCR format*
- *Efficient reverse transcription from different starting RNA amounts*
- *Generates cDNA at least 10 kb*

Description: ProtoScript II First Strand cDNA Synthesis Kit features two optimized mixes, ProtoScript II Enzyme Mix and ProtoScript II Reaction Mix. The enzyme mix combines ProtoScript II Reverse Transcriptase and Murine RNase Inhibitor, while the reaction mix contains dNTPs and an optimized buffer. ProtoScript II Reverse Transcriptase is a recombinant M-MuLV reverse transcriptase with reduced RNase H activity and increased thermostability. It can be used to synthesize first strand cDNA at higher temperatures than the wild type M-MuLV. The enzyme is active up to 50°C, providing higher specificity and higher yield of cDNA.

The kit also provides two optimized primers for reverse transcription and nuclease-free water. An anchored Oligo-dT primer [d(T)$_{23}$VN] forces the primer to anneal to the beginning of the poly(A) tail. The optimized Random Primer Mix provides random and consistent priming sites covering the entire RNA templates including both mRNAs and non-polyadenylated RNAs. The first strand cDNA product generated is up to 10 kb.

Kit Includes:

- ProtoScript II Reaction Mix
- ProtoScript II Enzyme Mix
- Random Primer Mix
- Nuclease-free Water
- Oligo d(T)$_{23}$ VN

For robust amplification of a wide range of DNA templates, we recommend One*Taq*® or Q5® High-Fidelity DNA Polymerases.

ProtoScript® First Strand cDNA Synthesis Kit

#E6300S 30 reactions
#E6300L 150 reactions

Companion Products:

Magnetic mRNA Isolation Kit
#S1550S 25 isolations

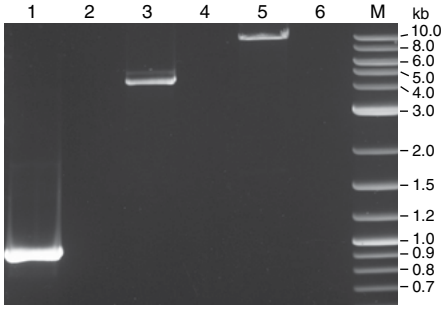
Monarch Total RNA Miniprep Kit
#T2010S 50 preps

- *Enzyme and reaction mixes add flexibility to reaction setup*
- *Suitable for any PCR format*
- *Efficient reverse transcription from different starting RNA amounts*
- *Generates cDNA at least 5 kb*

Description: ProtoScript First Strand cDNA Synthesis Kit features two optimized mixes, ProtoScript Enzyme Mix and Protoscript Reaction Mix. Protoscript Enzyme Mix combines M-MuLV Reverse Transcriptase and RNase Inhibitor, Murine, while ProtoScript Reaction Mix contains dNTPs and an optimized buffer. The kit also contains two optimized primers for reverse transcription and nuclease-free water. An anchored oligo-dT primer [d(T)$_{23}$VN] forces the primer to anneal to the beginning of the polyA tail. The optimized Random Primer Mix provides random and consistent priming sites covering the entire RNA template including both mRNAs and non-polyadenylated RNAs. The first strand cDNA product generated is over 13.0 kb. This product was formerly known as M-MuLV First Strand cDNA Synthesis Kit.

Kit Includes:

- M-MuLV Enzyme Mix
- M-MuLV Reaction Mix
- Random Primer Mix
- Oligo d(T)$_{23}$ VN
- Nuclease-free Water



First Strand cDNA Synthesis with the ProtoScript Kit. *Reactions were performed at 42°C using 2 µg of human spleen total RNA. Negative control reactions (–RT) were carried out with 1X ProtoScript Reaction Mix. A fraction of the first strand cDNA product was used to amplify sequences specific for three different messenger RNAs using 1X LongAmp® Taq 2X Master Mix (NEB #M0287). Lane 1: 1.1 kb of beta-actin gene. Lane 2: –RT control of 1.1 kb of beta-actin gene. Lane 3: 4.7 kb of Xrn-1 gene. Lane 4: –RT control of 4.7 kb of Xrn-1 gene. Lane 5: 9.8 kb of guanine nucleotide exchange factor p532. Lane 6: -RT control of 9.8 kb of guanine nucleotide exchange factor p532. Marker M is 1 kb Plus DNA Ladder (NEB #N3200).*

RT-PCR & RT-qPCR Kits

Luna Universal One-Step RT-qPCR Kit
#E3005S 200 reactions
#E3005L 500 reactions
#E3005X 1,000 reactions
#E3005E 2,500 reactions

Luna Universal Probe One-Step RT-qPCR Kit
#E3006S 200 reactions
#E3006L 500 reactions
#E3006X 1,000 reactions
#E3006E 2,500 reactions

Luna Probe One-Step RT-qPCR Kit (No ROX)
#E3007E 2,500 reactions

Luna Cell Ready One-Step RT-qPCR Kit
#E3030S 100 reactions

Luna Cell Ready Probe One-Step RT-qPCR Kit
#E3031S 100 reactions

Luna Probe One-Step RT-qPCR 4X Mix with UDG
#M3019S 200 reactions
#M3019L 500 reactions
#M3019X 1,000 reactions
#M3019E 2,000 reactions

Luna Probe One-Step RT-qPCR 4X Mix with UDG (No ROX)
#M3029S 200 reactions
#M3029L 500 reactions
#M3029E 2,000 reactions

Luna SARS-CoV-2 RT-qPCR Multiplex Assay Kit
#E3019S 96 reactions
#E3019L 480 reactions

LunaScript Multiplex One-Step RT-PCR Kit
#E1555S 50 reactions
#E1555L 250 reactions

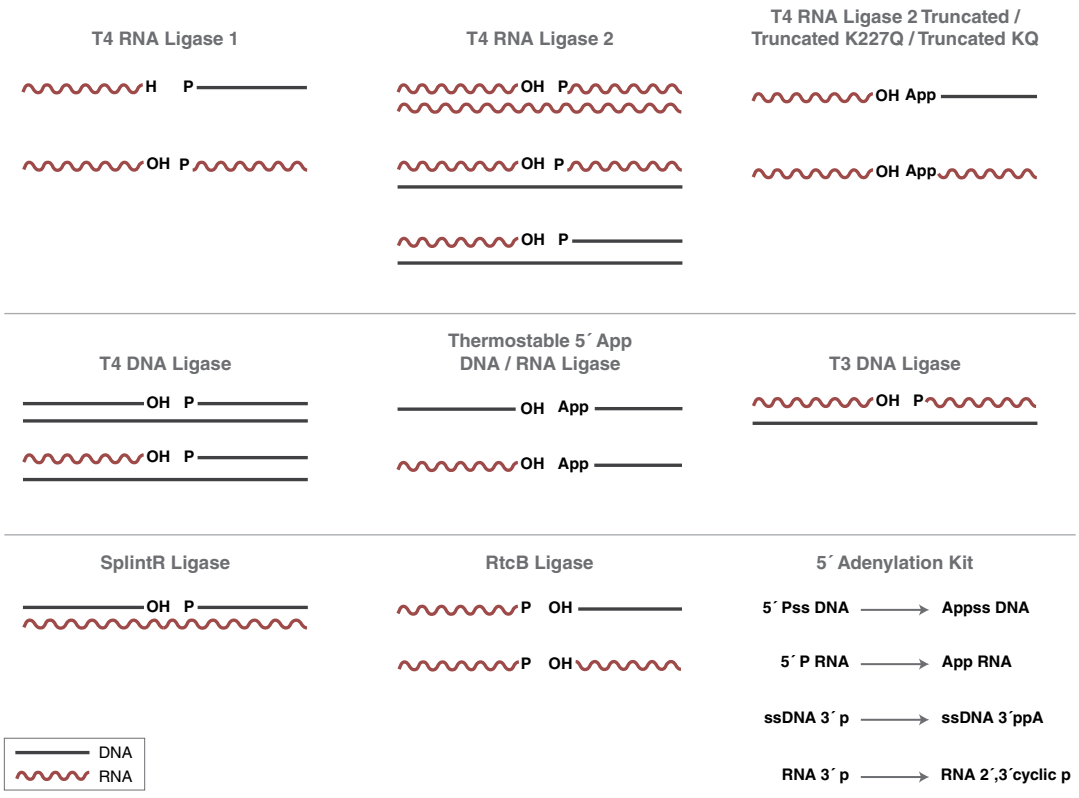
One*Taq* One-Step RT-PCR Kit
#E5315S 30 reactions

One*Taq* RT-PCR Kit
#E5310S 30 reactions

RNA Ligase Activity Chart

NEB offers a variety of ligases for DNA and RNA research. The chart below highlights reported activities of our T4 ligases. For more information, see the substrate-based ligase selection chart at www.neb.com.

Reported Activities and Applications for T4 Ligases



RNA Ligase Selection Chart

	T4 RNA Ligase 1	T4 RNA Ligase 2	T4 RNA Ligase 2 Truncated	T4 RNA Ligase 2, Truncated K227Q	T4 RNA Ligase 2, Truncated KQ	Thermostable 5' AppDNA/ RNA Ligase	5' Adenylation Kit	SplintR® Ligase	RtcB Ligase
RNA Applications									
Nicks in dsRNA		★★★							
Labeling of 3´ termini of RNA	★★★		★	★	★	★			
Ligation of ssRNA to ssRNA	★★★								
Ligation of preadenylated adaptors to RNA	★★		★★	★★	★★★	★★			
5´ Adenylation							★★★		
Ligation of 3'P and 5'OH of ssRNA									★★★
DNA Applications									
Ligation of preadenylated adaptors to ssDNA						★★★			
DNA/RNA Applications									
Joining of RNA & DNA in a ds-structure		★★							
ssDNA Ligation with RNA splint								★★★	
Ligation of RNA and DNA with 3'P and 5'OH									★★
NGS Applications									
NGS Library Prep ssRNA-ssDNA (ligation)	▲		▲	▲	▲				
NGS Library Prep ssRNA-ds-Adaptor splinted ligation		▲							
Features									
Thermostable						●	●		
Recombinant	●	●	●	●	●	●	●	●	●

KEY			
★★★ Optimal, recommended ligase for selected application	★★ Works well for selected application	★ Will perform selected application, but is not recommended	▲ Please consult the specific NGS protocol to determine the optimal enzyme for your needs

T4 RNA Ligase 1 (ssRNA Ligase)

T4 RNA Ligase 1 (ssRNA Ligase)
#M0204S 1,000 units
#M0204L 5,000 units

T4 RNA Ligase 1 (ssRNA Ligase), High Concentration
#M0437M 5,000 units

Companion Products:

Adenosine 5'-Triphosphate (ATP)
#P0756S 1 ml
#P0756L 5 ml

Universal miRNA Cloning Linker
#S1315 5 µg

- *Ligation of ss-RNA and DNA*
- *Labeling of 3´-termini of RNA with 5´-[^{32}P] pCp*
- *Inter- and intramolecular joining of RNA and DNA molecules*
- *Synthesis of ss-oligodeoxyribonucleotides?*
- *Incorporation of unnatural amino acids into proteins*

Description: Catalyzes ligation of a 5´ phosphoryl terminated nucleic acid donor to a 3´ hydroxyl-terminated nucleic acid acceptor through the formation of a 3´→ 5´ phosphodiester bond with hydrolysis of ATP to AMP and PPi. Substrates include single-stranded RNA and DNA as well as dinucleoside pyrophosphates.

Source: An *E. coli* strain that carries the T4 RNA Ligase 1 gene

Reaction Conditions: 1X T4 RNA Ligase Reaction Buffer, 25°C. Supplement with 1 mM ATP (included). Heat Inactivation: 65°C for 15 minutes.

Notes on Use: Addition of DMSO to 10% (v/v) is required for pCp ligation.

Reagents Supplied:
- 10X T4 RNA Ligase Reaction Buffer
- 10 mM ATP (with NEB #M0204) or 100 mM ATP (with NEB #M0437)
- 50% PEG 8000

Unit Definition: One unit is defined as the amount of enzyme required to convert 1 nmol of 5´-[^{32}P] rA16 into a phosphate resistant form in 30 minutes at 37°C.

Concentration: 10,000 or 30,000 units/ml

T4 RNA Ligase 2 (dsRNA Ligase)

#M0239S 150 units
#M0239L 750 units

- *Cohesive-end adaptor ligation*
- *Best choice for ligating nicks in dsRNA*
- *Suitable for ligating 3´ OH of RNA to 5´ phosphate of DNA in a DNA/RNA hybrid*

Description: T4 RNA Ligase 2, also known as T4 Rnl2 (gp24.1), has both intermolecular and intramolecular RNA strand-joining activity. Unlike T4 RNA Ligase 1 (NEB #M0204), T4 RNA Ligase 2 is much more active joining nicks on double stranded RNA than on joining the ends of single stranded RNA. The enzyme requires an adjacent 5´ phosphate and 3´ OH for ligation. The enzyme can also ligate the 3´ OH of RNA to the 5´ phosphate of DNA in a double stranded structure.

Source: An *E. coli* strain that carries the T4 RNA Ligase 2 gene.

Reaction Conditions: T4 Rnl2 Reaction Buffer, 37°C. Heat inactivation: 80°C for 5 minutes.

Reagents Supplied:
- T4 Rnl2 Reaction Buffer

Unit Definition: One unit is defined as the amount of enzyme required to ligate 0.4 µg of an equimolar mix of a 23-mer and 17-mer RNAs in a total reaction volume of 20 µl in 30 minutes at 37°C.

Concentration: 10,000 units/ml

T4 RNA Ligase 2, truncated

#M0242S 2,000 units
#M0242L 10,000 units

Companion Product:

Universal miRNA Cloning Linker
#S1315S 5 µg

- *Ligate a pre-adenylated DNA or RNA sequence tag to any RNA 3´ end*
- *Join a single-stranded adenylated primer to small RNAs for cDNA library creation*
- *Join a single-stranded adenylated primer to RNA for strand-specific cDNA library construction*

Description: T4 RNA Ligase 2, truncated (T4 Rnl2tr) specifically ligates the pre-adenylated 5´ end of DNA or RNA to the 3´ end of RNA. The enzyme does not require ATP, but does need the pre-adenylated substrate. T4 Rnl2tr is expressed from a plasmid in *E. coli* which encodes the first 249 amino acids of the full-length T4 RNA Ligase 2. Unlike the full-length ligase, T4 Rnl2tr cannot ligate the phosphorylated 5´ end of RNA or DNA to the 3´ end of RNA. This enzyme, also known as Rnl2 (1–249), has been used for optimized linker ligation for the cloning of microRNAs. This enzyme reduces background ligation, because it can only use pre-adenylated linkers.

Source: An *E. coli* strain that carries the cloned truncated T4 RNA Ligase 2 gene.

Reaction Conditions: T4 RNA Ligase Reaction Buffer , 25°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied:
- T4 RNA Ligase Reaction Buffer
- PEG 8000

Unit Definition:

200 units is defined as the amount of enzyme required to give 80% ligation of a 31-mer RNA to the pre-adenylated end of a 17-mer DNA Universal miRNA Cloning Linker (#S1315) in a total reaction volume of 20 µl in 1 hour at 25°C.

5´-FAM-rArGrUrCrGrUrArGrCrCrUrUrUrArUrCrCrGrArGrArUrUrCrArGrCrArArUrA-3´

5´-rAppCTGTAGGCACCATCAAT–NH2-3´

Molarity = 14 µM

Concentration: 200,000 units/ml

T4 RNA Ligase 2, truncated K227Q and truncated KQ

RR 25° 65°

T4 RNA Ligase 2, truncated K227Q
#M0351S 2,000 units
#M0351L 10,000 units

T4 RNA Ligase 2, truncated KQ
#M0373S 2,000 units
#M0373L 10,000 units

Companion Product:

Universal miRNA Cloning Linker
#S1315S 5 µg

- *Ligate a pre-adenylated DNA or RNA sequence tag to any RNA 3´ end*
- *Join a single stranded adenylated primer to small RNAs for cDNA library creation*
- *Join a single stranded adenylated primer to RNA for strand-specific cDNA library construction*

Description: T4 RNA Ligase 2, K227Q and truncated KQ (T4 Rnl2tr KQ) specifically ligate the pre-adenylated 5´ end of DNA or RNA to the 3´ OH end of RNA. The enzymes do not use ATP for ligation, but requires preadenylated linkers.

Mutation of K227 in T4 RNA Ligase 2 reduces enzyme lysyl adenylation. K227Q reduces the formation of undesired ligation products (concatemers and circles) by T4 Rnl2tr, by reducing the trace activity of T4 Rnl2tr in transfer of adenylyl groups from linkers to the 5´-phosphates of input RNAs. T4 Rnl2tr KQ is a double-point mutant of T4 RNA Ligase 2, truncated (NEB #M0242). Mutation of R55K in T4 Rnl2tr K227Q increases the ligation activity of the enzyme to levels similar to T4 Rnl2tr.

The exclusion of ATP, use of pre-adenylated linkers, and the reduced enzyme lysyl adenylation activity provide the lowest possible background in ligation reactions. These enzymes have been used for optimized linker ligation for high-throughput sequencing library construction of small RNAs.

Source: Expressed as an MBP fusion from a plasmid in E. coli which encodes the first 249 amino acids of the full length T4 RNA Ligase 2. T4 Rnl2tr K227Q has a lysine to glutamine mutation at position 227. T4 Rnl2tr KQ has an arginine to lysine and lysine to glutamine mutation at positions 55 and 227, respectively.

Reaction Conditions: 1X T4 RNA Ligase Reaction Buffer, 25°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied:

- 10X T4 RNA Ligase Reaction Buffer
- 50% PEG 8000

Unit Definition: 200 units is defined as the amount of enzyme required to give 80% ligation of a 31-mer RNA to the pre-adenylated end of a 17-mer DNA [Universal miRNA Cloning Linker (NEB #S1315)] in a total reaction volume of 20 µl in 1 hour at 25°C.

Concentration: 200,000 units/ml

RtcB Ligase

NEB RR 37°

#M0458S 25 reactions

- *Ligate ssRNA or ssDNA with a 3´-phosphate or a 2´,3´-cyclic phosphate to the 5´-OH of ssRNA*
- *Circularization of ssRNA with compatible ends*

Description: RtcB Ligase from *E. coli* joins single stranded RNA with a 3´-phosphate or 2´,3´-cyclic phosphate to another RNA with a 5´ -hydroxyl. Ligation requires both GTP and $MnCl_2$ and proceeds through a 3´-guanylate intermediate. With substrates having a 2´,3´-cyclic phosphate end, hydrolysis to a 3´-phosphate precedes 3´ end activation with GMP and ligation.

Source: RtcB Ligase is expressed as a His-tagged fusion in *E. coli*.

Reaction Conditions: RtcB Reaction Buffer, 37°C. Supplement with 0.1 mM GTP and 1 mM $MnCl_2$.

Reagents Supplied:

- RtcB Reaction Buffer
- $MnCl_2$
- GTP

Concentration: 15 µM

This is an **Enzyme for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

Thermostable 5´ App DNA/RNA Ligase

NEB RR 65° Mg

#M0319S 10 reactions
#M0319L 50 reactions

Companion Product:

Universal miRNA Cloning Linker
#S1315S 5 µg

- *Ligation of ssDNA to an adenylated DNA linker for NGS library construction*
- *Ligation of an adenylated linker to RNA at elevated temperatures for small RNA NGS library construction*

Description: Thermostable 5´ App DNA/RNA Ligase is a point mutant of catalytic lysine of RNA ligase from *Methanobacterium thermoautotrophicum*. This enzyme is ATP independent. It requires a 5´ pre-adenylated linker for ligation to the 3´-OH end of either RNA or single stranded DNA (ssDNA). The enzyme is also active in ligation of RNA with 2´-*O*-methylated 3´ end to 5´-adenylated linkers. The optimal temperature for ligation reaction is 60–65°C. The mutant ligase is unable to adenylate the 5´-phosphate of RNA or ssDNA, which reduces the formation of undesired ligation products (concatemers and circles).

The ability of the ligase to function at 65°C might reduce the constraints of RNA secondary structure in RNA ligation experiments.

Source: Thermostable 5´ App DNA/RNA Ligase is expressed as His-tag fusion in *E. coli*.

Reaction Conditions: NEBuffer 1, 65°C.

Reagents Supplied:

- NEBuffer 1
- $MnCl_2$

Concentration: 20 µM

Note: For optimal ligation of ssDNA to adenylated DNA linkers, we recommend using NEBuffer 1 supplemented with manganese. For ligation of ssRNA to adenylated DNA linkers, just use NEBuffer 1.

5´ DNA Adenylation Kit

#E2610S 10 reactions
#E2610L 50 reactions

- *Enzymatic 5´ adenylation of ss-DNA linkers for next gen sequencing*
- *One-step reaction gives quantitative adenylation*
- *Simpler than existing chemical and enzymatic methods*
- *Reduces need for purification of reaction product*

Description: The 5´ DNA Adenylation Kit is a simple and efficient enzymatic method for generating 5´-adenylated DNA oligonucleotides using *Mth* RNA ligase, ATP and single stranded 5´-phosphorylated DNA. The kit is optimized to produce the adenylated DNA intermediate with or without 3´ terminator. The 5´ DNA adenylation kit routinely generates greater than 95% conversion of pDNA to AppDNA. This highly efficient process eliminates the need for gel isolation of the product and increases overall yield.

Kit Includes:

- Mth RNA Ligase
- 5' DNA Adenylation Reaction Buffer
- Adenosine 5' Triphosphate

Note: The low turnover of the enzyme requires an approximately equimolar concentration of the enzyme and the oligonucleotide substrate. Adenylated DNA linkers can be used for 3´-end ligation of RNA in cDNA library preparation for Next Generation sequencing protocols.

SplintR® Ligase

#M0375S 1,250 units
#M0375L 6,250 units

- *Ligation of adjacent, single-stranded DNA splinted by a complimentary RNA*
- *Characterization of miRNAs and mRNAs, including SNPs*

Description: SplintR Ligase, also known as PBCV-1 DNA Ligase or *Chlorella* virus DNA Ligase, efficiently catalyzes the ligation of adjacent, single-stranded DNA oligonucleotides splinted by a complementary RNA strand. This previously unreported activity may enable novel approaches for characterization of miRNAs and mRNAs, including SNPs. SplintR is ideally suited for many target enrichment workflows with applications in next-generation sequencing and molecular diagnostics. The robust activity of the enzyme and its affinity for RNA-splinted DNA substrates (apparent Km = 1 nM) enable sub-nanomolar detection of unique RNA species within a complex mixture, making SplintR ligase a superior choice for demanding RNA detection technologies.

Source: An *E. coli* strain that carries a recombinant gene encoding PBCV-1 DNA Ligase.

Reaction Conditions: SplintR Ligase Reaction Buffer, 25°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied:

- SplintR Ligase Reaction Buffer

Unit Definition: One unit is defined as the amount of enzyme needed to ligate (to 50% completion) 2 picomoles of a tripartite FAM-labeled DNA:RNA hybrid substrate in a 20 µl reaction at 25°C in 15 minutes in 1X SplintR Ligase Reaction Buffer.

Concentration: 25,000 units/ml

RNA 5' Pyrophosphohydrolase (RppH)

#M0356S 200 units

- *Conversion of 5´-triphosphate RNA to monophosphate RNA*
- *Preparation of 5´-phosphate RNA for ligation*
- *Characterization of RNA 5´ ends*

Description: The bacterial RNA 5´ Pyrophosphohydrolase (RppH) removes pyrophosphate from the 5´ end of triphosphorylated RNA to leave a 5´ monophosphate RNA. The RppH protein was also known as NudH/YgdP which can split diadenosine penta-phosphate to ADP and ATP.

Source: An *E. coli* strain containing a clone of the *E. coli* RppH gene.

Reaction Conditions: NEBuffer 2, 37°C.

Reagents Supplied:

- NEBuffer 2

Unit Definition: One unit is the amount of enzyme that converts 1 µg 300 mer RNA transcript into a XRN-1 digestible RNA in 30 minutes at 37°C.

Concentration: 5,000 units/ml

5´ Deadenylase

NEB1 RR 30° 70°

#M0331S 2,500 units

- *Deadenylation of 5´ end of DNA and RNA*
- *Aprataxin-dependent DNA repair assay*
- *Analysis of dinucleoside tetraphosphate*

Description: Yeast 5´ Deadenylase is a member of the HIT (histidine triad) family of proteins and specifically a member of the Hint branch. It is the yeast orthologue of aprataxin. Mutations in human aprataxin have been known to be involved in the neurological disorder known as ataxia oculomotor apraxia-1. The human protein has been shown to resolve abortive ligation intermediates by removing the AMP at the 5´ end of DNA (AMP-DNA hydrolase activity). It also repairs DNA damage at 3´ ends by removing 3´-phosphate and 3´-phosphoglycolate. Human aprataxin acts on small molecules, such as nucleotide polyphosphates diadenosine tetraphosphate (AppppA) and lysyl-AMP.

The 5´ Deadenylase is encoded by the *HNT3* gene of *S. cerevisiae*. NEB has shown this protein is capable of deadenylation from 5´ end of DNA and RNA, leaving the phosphate at 5´ end. It also cleaves AppppA into ATP and AMP. Its activity on lysyl-AMP is not detectable.

Source: Purified from an *E. coli* strain carrying a plasmid encoding 5´ Deadenylase from S. *cerevisiae*.

Reaction Conditions: NEBuffer 1, 30°C. Heat inactivation: 70°C for 20 minutes.

Reagents Supplied:

• NEBuffer 1

Unit Definition: One unit is defined as the amount enzyme required to remove 10 pmoles of AMP from a 5'-adenylated DNA oligo in 10 minutes at 30°C.

Concentration: 50,000 units/ml

RNase I_f

NEB3 RR 37° 70°

#M0243S 5,000 units
#M0243L 25,000 units

- *Eliminates RNA from DNA and protein preparations*
- *Degradation of single-stranded RNA to mono-, di- and trinucleotides*
- *Used in ribonuclease protection assays*

Description: Ribonuclease I_f (RNase I_f) is an RNA endonuclease which will cleave at all RNA dinucleotide bonds leaving a 5´ hydroxyl and 2´, 3´ cyclic monophosphate. It has a preference for single-stranded RNA over double-stranded RNA. RNase I_f is a recombinant protein fusion of RNase I (from *E. coli*) and maltose-binding protein. It has identical activity to RNase I.

Source: An *E. coli* strain containing a genetic fusion of the *E. coli* RNase I gene (2nd) and the gene coding for maltose binding protein (MBP).

Reaction Conditions: NEBuffer 3, 37°C. Heat inactivation: 70°C for 20 minutes.

Reagents Supplied:

• NEBuffer 3

Unit Definition: One unit is defined as the amount of enzyme required to fully digest 1 picomole of synthetic ssRNA 33-mer in a total reaction volume of 10 µl in 15 minutes in 1X NEBuffer 3 as visualized on a 20% acrylamide gel.

Concentration: 50,000 units/ml

Note: RNase I_f will not degrade DNA. It has a preference for single-stranded RNA over double-stranded RNA.

RNase H

NEBU RR 37° 65°

#M0297S 250 units
#M0297L 1,250 units

Description: Ribonuclease H (RNase H) is an endoribonuclease which specifically hydrolyzes the phosphodiester bonds of RNA which is hybridized to DNA. This enzyme does not digest single- or double-stranded DNA.

Source: An *E. coli* strain that carries the cloned RNase H gene (rnh) from *Escherichia coli*.

Reaction Conditions: RNase H Reaction Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied:

• RNase H Reaction Buffer

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of ribonucleotides from 20 picomoles of a fluorescently labelled 50 base pair RNA-DNA hybrid in a total reaction volume of 50 µl in 20 minutes at 37°C.

Concentration: 5,000 units/ml

Thermostable RNase H

NEBU RR 50° NO

#M0523S 250 units

- *Higher stringency RNA structure mapping and site-specific RNA cleavage*
- *Removal of poly(A) tails from mRNA hybridized to oligo(dT)*
- *Removal of mRNA during second strand cDNA synthesis*
- *Component of isothermal amplification methods*

Description: Thermostable RNase H specifically recognizes and cleaves the phosphodiester bonds of an RNA strand in an RNA-DNA hybrid while leaving the DNA strand intact. This thermostable nuclease exhibits the same enzymatic properties as *E. coli* RNase H, but is active at much higher temperatures.

Source: An *E. coli* strain carrying a codon optimized plasmid encoding RNase H from the extreme thermophile *Thermus thermophilus*.

Reaction Conditions: RNase H Reaction Buffer, ≥50°C.

Reagents Supplied:

• RNase H Reaction Buffer

Unit Definition: One unit is defined as the amount of enzyme required to produce 1 nmol of ribonucleotides from 40 pmol of a fluorescently labeled 25 base pair RNA:DNA hybrid in a total reaction volume of 50 µl in 20 minutes at 50°C.

Concentration: 5,000 units/ml

RNase HII

NEBU RR 37° M

#M0288S 250 units
#M0288L 1,250 units

- *Nicking of products generated with a polymerase that will incorporate ribonucleotides*
- *Generation of a double-stranded break at the site of an incorporated ribonucleotide when used with T7 Endo I*
- *Degradation of the RNA portion of Okazaki fragments or other RNA-DNA hybrids*

Description: Ribonuclease HII (RNase HII) is an endoribonuclease that preferentially nicks 5´ to a ribonucleotide within the context of a DNA duplex. The enzyme leaves 5´ phosphate and 3´ hydroxyl ends. RNase HII will also nick at multiple sites along the RNA portion of an Okazaki fragment.

Source: An *E. coli* strain containing a genetic fusion of the RNase HII gene (rnhB) from *E. coli* and the gene coding for maltose binding protein (MBP). Following affinity chromatography, RNase HII is cleaved from the fusion construct by Factor Xa and then purified away from both MBP and Factor Xa. RNase HII cleaved from MBP has four additional amino acids at its N-terminus (Ile-Ser-Glu-Phe).

Reaction Conditions: ThermoPol Reaction Buffer, 37°C.

Reagents Supplied:

• ThermoPol Reaction Buffer

Unit Definition: One unit is defined as the amount of enzyme required to yield a fluorescence signal consistent with the nicking of 100 picomol of synthetic double-stranded DNA substrate containing a single ribonucleotide near the quencher of a fluorophore/quencher pair in 30 minutes at 37°C in 1X ThermoPol Buffer.

Concentration: 5,000 units/ml

Note: Incubation with 0.1% SDS is sufficient to inactivate RNase HII.

Phosphorylation and Dephosphorylation

Quick CIP
#M0525S 1,000 units
#M0525L 5,000 units

Antarctic Phosphatase
#M0289S 1,000 units
#M0289L 5,000 units

Shrimp Alkaline Phosphatase (rSAP)
#M0371S 500 units
#M0371L 2,500 units

T4 Polynucleotide Kinase
#M0201S 500 units
#M0201L 2,500 units

NEB offers a selection of products for phosphorylation and dephosphorylation of DNA and RNA. Full product details can be found in the DNA Modifying Enzymes & Cloning Technologies chapter, or at www.neb.com.

ShortCut® RNase III

NEBU RR 37° M

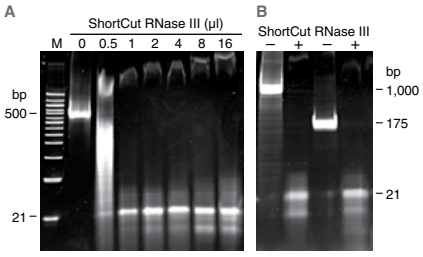
#M0245S 200 units
#M0245L 1,000 units

- *Generates siRNAs for RNA interference studies*
- *Gene silencing*
- *Target validation*
- *Removal of long dsRNAs*

Description: ShortCut RNase III converts long double-stranded RNA into a heterogeneous mix of short (18–25 bp) interfering RNAs (siRNA) suitable for RNA interference in mammalian cells. 1.5 units (1 µl) of ShortCut RNase III is sufficient to convert 1 µg of dsRNA into siRNA.

Source: An *E. coli* strain containing a genetic fusion of the *E. coli* RNase III gene (rnc) and the gene coding for maltose binding protein (MBP).

Reaction Conditions: ShortCut Reaction Buffer, 37°C.



siRNA production by ShortCut RNase III. *A. Varying amounts of ShortCut RNase III were incubated with 2 µg of a 500 bp dsRNA for 20 minutes. B. dsRNA fragments (1 kb and 175 bp) were digested with ShortCut RNase III. Digests were analyzed by 20% TBE polyacrylamide gel electrophoresis.*

Reagents Supplied:

• ShortCut Reaction Buffer
• 10X EDTA
• $MnCl_2$
• Glycogen RNase-Free

Unit Definition: One unit is the amount of enzyme required to digest 1 µg of dsRNA to siRNA in 20 minutes at 37°C in a total reaction volume of 50 µl.

Concentration: 2,000 units/ml

XRN-1

NEB3 RR 37°

#M0338S 20 units
#M0338L 100 units

- *Removal of RNA containing 5´ monophosphate from an RNA mixture*

Description: XRN-1 is a highly processive 5´ to 3´ exoribonuclease, requiring a 5´ monophosphate. It also acts on 5´ monophosphate ssDNA with reduced efficiency.

Source: Purified from *E. coli* carrying a plasmid overexpressing the yeast XRN-1 gene (1).

Reaction Conditions: NEBuffer 3, 37°C.

Reagents Supplied:

• NEBuffer 3

Unit Definition: One unit is defined as the amount of enzyme that digests 1 µg of phosphorylated yeast RNA in 60 minutes at 37°C.

Concentration: 1,000 units/ml

Exonuclease T

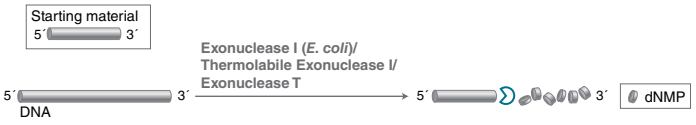
NEB4 RR 25° 65°

#M0265S 250 units
#M0265L 1,250 units

- *Generate blunt ends in DNA or RNA with 3´ overhangs*

Description: Exonuclease T (Exo T), also known as RNase T, is a single-stranded RNA or DNA specific nuclease that requires a free 3´ terminus and removes nucleotides in the 3´→ 5´ direction. Exo T can be used to generate blunt ends from RNA or DNA having 3´ extensions.

Source: Exonuclease T is overexpressed and purified as a C-terminal fusion to maltose-binding protein (MBP). MBP is removed from Exonuclease T by Factor Xa cleavage and Exonuclease T is then purified away from Factor Xa and MBP. Exonuclease T cleaved from MBP has an additional amino acid on the N-terminus and a Phe instead of a Met (i.e., Glu-Phe-Exo T instead of Met-Exo T).



Reaction Conditions: NEBuffer 4, 25°C. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to release 1 nmol of single dT nucleotides from 50 pmol of Fam-labeled polythymidine substrate in 30 minutes at 25°C.

Concentration: 5,000 units/ml

Note: Exonuclease T has a different activity on RNA vs. DNA. For RNA, 1 unit of Exonuclease T is required to completely digest 1.0 pmol of rA20 under standard reaction conditions as measured by gel electrophoresis.

Nucleoside Digestion Mix

NEBU 37°

#M0649S 50 reactions

- *Convenient one-step protocol*
- *Digests both DNA and RNA to single nucleosides*
- *Low-glycerol formulation significantly reduces glycerol-induced ion suppression during MS analysis*

Description: The Nucleoside Digestion Mix is a mixture of enzymes that provides a convenient one-step method to generate single nucleosides from DNA or RNA. Optimized for quantitative analysis by liquid chromatography-mass spectrometry (LC-MS), this reagent eliminates the need for sequential multi-step, time-consuming digestion protocols. The Nucleoside Digestion Mix digests ssDNA, dsDNA, DNA/RNA hybrids and RNA (except mRNA cap structures) containing epigenetically modified (m5C, hm5C, f5C, ca5C, m4C, m6A, etc.), unnatural, or damaged bases. Moreover, the low-glycerol formulation (< 1%) significantly reduces glycerol-induced ion suppression during mass spectrometry analysis.

Reaction Conditions: Nucleoside Digestion Mix Reaction Buffer, 37°C

DNase I (RNase-Free)

NEBU RR 37° 75°

NEW
DNase I-XT

NEBU RR 37° No

DNase I (RNase-free)
#M0303S 1,000 units
#M0303L 5,000 units

NEW
DNase I-XT
#M0570S 1,000 units
#M0570L 5,000 units

- *Removal of contaminating genomic DNA from RNA samples*
- *Degradation of DNA templates in transcription reactions*

Description: DNase I is a DNA-specific endonuclease that degrades ds- and ss-DNA to release short oligos with 5´ phosphorylated and 3´-hydroxylated ends.

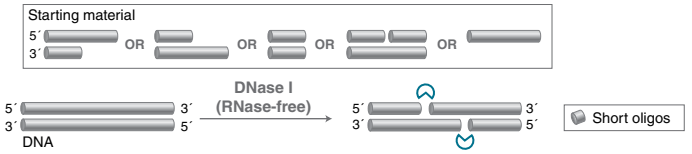
DNase I-XT is a salt tolerant enzyme that exhibits optimal activity between 50-100 mM salt and retains 65% and ~40% activity in 200 and 300 mM salt, respectively. Increased salt tolerance makes it ideal for DNA template removal from an *in vitro* transcription (IVT) reaction.

Both enzymes are RNase-free, allowing for complete removal of DNA from RNA preps, while maintaining RNA integrity.

Reaction Conditions: 1X DNase I Reaction Buffer, 37°C. DNase I can be heat inactivated at 75°C for 10 minutes, while DNase I-XT cannot.

Reagents Supplied:

- DNase I Reaction Buffer (NEB #M0303)
- DNase I-XT Reaction Buffer (NEB #M0570)



Unit Definition: DNase I – One unit is defined as the amount of enzyme which will completely degrade 1 µg of pBR322 DNA in a total reaction volume of 50 µl in 10 minutes at 37°C. DNase I-XT – One unit is defined as the amount of enzyme required to release 210 pmol of FAM from a 35-mer FAM-BHQ1 labeled hairpin oligonucleotide in 1 minute at 30°C in a 50 µl reaction volume.

Concentration: 2,000 units/ml

Notes: DNase I-XT is supplied with an optimized reaction buffer. Use with supplied buffer, and not DNase I Reaction Buffer. Likewise, due to the sub-optimal salt concentration, do not use DNase I-XT Buffer with DNase I. When using DNase I, EDTA should be added to a final concentration of 5 mM to protect RNA from being degraded during enzyme inactivation.

RNase Inhibitor, Murine

RR

#M0314S 3,000 units
#M0314L 15,000 units

- *Inhibits common eukaryotic RNases*
- *Compatible with Taq Polymerase, AMV or M-MuLV Reverse Transcriptases*
- *cDNA synthesis & RT-PCR*
- *In vitro transcription/translation*
- *Enzymatic RNA labeling reaction*

RNase Inhibitor, Murine has significantly **improved resistance to oxidation** compared to human & porcine RNase inhibitors.

Description: RNase Inhibitor, Murine is a 50 kDa recombinant protein of murine origin. It specifically inhibits RNases A, B and C by binding noncovalently in a 1:1 ratio with high affinity. It is not effective against RNase 1, RNase T1, S1 Nuclease, RNase H or RNase from *Aspergillus*. No inhibition of polymerase activity is observed when used with *Taq* DNA Polymerase, AMV or M-MuLV Reverse Transcriptases, or Phage RNA Polymerases (SP6, T7, or T3).

Recombinant RNase Inhibitor, Murine does not contain the pair of cysteines identified in the human version that are very sensitive to oxidation and lead to inactivation of the inhibitor. As a result, RNase Inhibitor, Murine has significantly improved resistance to oxidation compared to the human/porcine RNase inhibitors, and is stable at low DTT concentrations (< 1 mM). This makes it ideal for reactions where high concentration DTT is adverse to the reaction (e.g., RT-qPCR).

Source: An E. coli strain that carries the Ribonuclease Inhibitor gene from mouse.

Unit Definition: One unit is defined as the amount of Murine RNase Inhibitor required to inhibit the activity of 5 ng of RNase A by 50%. Activity is measured by the inhibition of hydrolysis of cytidine 2', 3'-cyclic monophosphate by RNase A.

Concentration: 40,000 units/ml

RNase Inhibitor, Human Placenta

RR

#M0307S 2,000 units
#M0307L 10,000 units

- *Inhibits common eukaryotic RNases*
- *Compatible with Taq Polymerase, AMV or M-MuLV Reverse Transcriptases*
- *Active over a broad pH range (pH 5–8)*
- *cDNA synthesis reactions*
- *In vitro transcription/translation*

Description: RNase Inhibitor, Human Placenta is a recombinant human placental protein which specifically inhibits ribonucleases (RNases) A, B and C. It is not effective against RNase 1, RNase T1, S1 Nuclease, RNase H or RNase from *Aspergillus*. In addition, no inhibition of polymerase activity is observed when RNase Inhibitor, Human Placenta is used with *Taq* DNA Polymerase, AMV or M-MuLV Reverse Transcriptases, or Phage RNA Polymerases (SP6, T7, or T3).

The 50 kDa protein inhibits RNases by binding noncovalently in a 1:1 ratio with an association constant greater than 10^{14}.

Source: An *E. coli* strain that carries the Ribonuclease Inhibitor gene from human placenta.

Unit Definition: One unit is defined as the amount of RNase Inhibitor, Human Placenta required to inhibit the activity of 5 ng of RNase A by 50%. Activity is measured by the inhibition of hydrolysis of cytidine 2, 3'-cyclic monophosphate by RNase A.

Concentration: 40,000 units/ml

Ribonucleoside Vanadyl Complex

#S1402S 10 ml

Description: Vanadium complexes are used in mRNA purifications as exogenous ribonuclease inhibitors. They are compatible with cell lysis techniques and with sucrose gradient fractionation of cytoplasmic components.

Ribonucleoside Vanadyl Complex is an equimolar mixture of all four ribonucleosides, complexed with oxovanadium IV by a modification of the procedures by Berger. Each lot of the complex is assayed for oxovanadium V content and inhibition of ribonuclease activity.

Source: This vanadyl complex is prepared from a modification of procedures by Lienhard using all four ribonucleosides (4).

Concentration: 200 mM

Cathy, Tim and Toby are members of the NEB Production Department. Together they have 95 years of experience at NEB. Their dedication has resulted in the dependability of NEB products in customer's hands.

NEBNext Reagents for RNA Library Preparation

NEBNext Ultra II Directional RNA Library Prep Kit for Illumina
#E7760S 24 reactions
#E7760L 96 reactions

NEBNext Ultra II Directional RNA Library Prep with Sample Purification Beads
#E7765S 24 reactions
#E7765L 96 reactions

NEBNext Ultra II RNA Library Prep Kit for Illumina
#E7770S 24 reactions
#E7770L 96 reactions

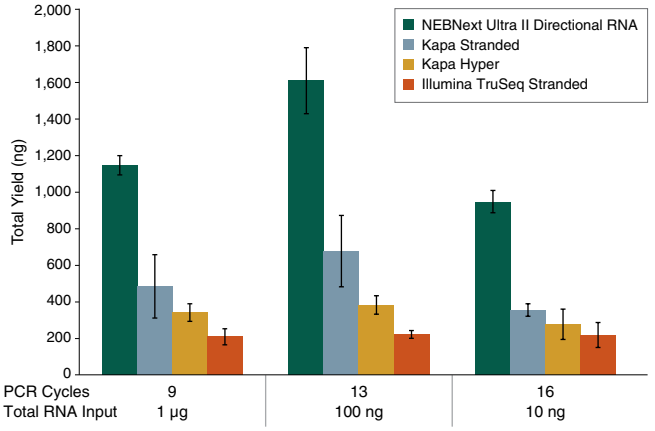
NEBNext Ultra II RNA Library Prep with Sample Purification Beads
#E7775S 24 reactions
#E7775L 96 reactions

NEBNext Single Cell/Low Input RNA Library Prep Kit for Illumina
#E6420S 24 reactions
#E6420L 96 reactions

NEBNext Single Cell/Low Input cDNA Synthesis & Amplification Module
#E6421S 24 reactions
#E6421L 96 reactions

Kits for **rRNA depletion** from a variety of samples, including human, mouse, rat and bacteria are available, as well as a **customizable option** for any sample type. Kits for **Small RNA** library construction are also available. See our **NEBNext reagents** for library preparation.

NEBNext Kits for RNA sample preparation for next generation sequencing keep pace with the use of ever-decreasing input amounts and sub-optimal sample quality, along with the need for superior performance, reliability, and automation compatibility. The fast and streamlined Ultra II Workflow is at the heart of our RNA library prep kits, including our NEBNext Single Cell/ Low Input Library Prep Kit for Illumina, and these are all available in flexible, user-friendly formats. Our NEBNext RNA products also include kits for Small RNA Library Prep, and multiple options for removal of abundant RNAs as well as a growing range of adaptors and primers.



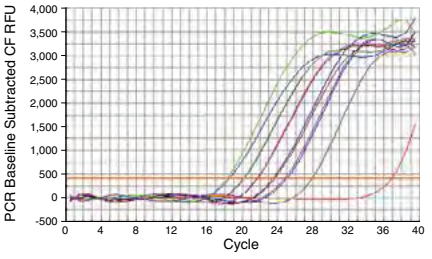
NEBNext Ultra II Directional RNA produces the highest yields, from a range of input amounts. *Poly(A)-containing mRNA was isolated from 10 ng, 100 ng and 1 ?g of Universal Human Reference RNA (Agilent #740000) and libraries were made using the NEBNext Ultra II Directional RNA kit, Kapa™ Stranded mRNA-Seq kit, Kapa mRNA HyperPrep kit and Illumina® TruSeq® Stranded mRNA Kit. The input RNA amount and number of PCR cycles are indicated. Library yields from an average of three replicates are shown. Error bars indicate standard deviation. Library yields were assessed using the Agilent® Bioanalyzer®.*

Magnetic mRNA Isolation Kit

#S1550S 25 isolations

- *Suitable for automated high-throughput applications*
- *Eliminates need for organic solvents*
- *No need to precipitate poly(A)+ transcripts in eluent*
- *Obtain intact poly(A)+ RNA in < 1 hour*
- *Negligible gDNA contamination*

Description: The New England Biolabs Magnetic mRNA Isolation Kit is designed to isolate intact poly(A)$^+$ RNA from cells and tissue without requiring phenol or other organic solvents. The technology is based on the coupling of Oligo d(T)$_{25}$ to 1 µm paramagnetic beads which are then used as the solid support for the direct binding of poly(A)$^+$ RNA. Thus, the procedure permits the manual processing of multiple samples and can be adapted for automated high-throughput applications. Additionally, magnetic separation technology permits elution of intact mRNA in small volumes eliminating the need for precipitating the poly(A)$^+$ transcripts in the eluent. Intact poly(A)$^+$ RNA which is fully representative of the mRNA population of the original sample can be obtained in less than one hour. Oligo d(T)$_{25}$ Magnetic Beads can be reused up to three times and the researcher has the option of eluting the isolated mRNA or using the bound dT DNA as a primer in a first-strand cDNA reaction.



Consistency and wide isolation range are demonstrated by poly(A)+ RNA isolation from duplicate samples of decreasing numbers of HEPG2 cells (5 x 10^5 to 1 x 10^3) by direct lysis/binding in microtiter plates followed by mRNA isolation with the magnetic method. *1/10th of isolated mRNA is converted to oligo (dT) primed?cDNA using ProtoScript M-MuLV First Strand cDNA Synthesis Kit (NEB #E6300) and qPCR done with validated primers for the peptidylpropyl isomerase, a low-abundance housekeeping gene.*

Oligo d(T)$_{25}$ Magnetic Beads

#S1419S 5 ml

Companion Product:

96-Well Microtiter Plate Magnetic Separation Rack
#S1511S 96 wells

- *Small-scale purification or immunoprecipitation of IgG species*
- *No centrifugation required*
- *Regenerate matrix without binding capacity loss*

Description: An affinity matrix for the small-scale isolation of mRNA from crude cell lysates and tissue. The isolation occurs through the hybridization of covalently coupled oligo d(T)$_{25}$ to the poly(A) region present in most eukaryotic mRNAs. Applications include direct mRNA isolation following lysis and second-round purification of previously isolated total RNA. The magnetic separation technology is scalable and permits elution of intact mRNA in small volumes eliminating the need for precipitation of the isolated mRNA. Beads can be reused up to three times, and the researcher has the option of eluting the isolated mRNA or using the bound d(T)$_{25}$ as a primer in a first-strand cDNA reaction.

Beads are supplied as a 5 mg/ml suspension in phosphate buffer (PBS) (pH 7.4), containing 0.05% Tween-20 and 0.05% NaN_3.

Concentration: 5 mg/ml

Support Matrix: 1 µm nonporous superparamagnetic microparticles.

Binding Capacity: 1 mg of Oligo d(T)$_{25}$ Beads will bind 10 µg of poly(A)$^+$ RNA.

Streptavidin Magnetic Beads

#S1420S 5 ml

Companion Products:

6-Tube Magnetic Separation Rack
#S1506S 6 tubes

96-Well Microtiter Plate Magnetic Separation Rack
#S1511S 96 wells

Description: Streptavidin Magnetic Beads are 1 µm superparamagnetic particles covalently coupled to a highly pure form of streptavidin. The beads can be used to capture of biotin labeled substrates including antigens, antibodies and nucleic acids. The strength of the biotin-streptavidin interaction coupled with low non-specific binding permits captured substrates to be useful as ligands in subsequent experiments including mRNA isolation and the capture of primary or secondary antibodies.

Beads are supplied as a 4 mg/ml suspension in phosphate buffer (PBS) (pH 7.4) containing 0.1% BSA, 0.05% Tween-20 and 0.05% NaN_3.

Concentration: 4 mg/ml

Support Matrix: 1 µM nonporous superparamagnetic microparticle

Binding Capacity: The beads will bind greater than 1000 pmol of free biotin per mg and greater than 500 pmol of single-stranded 25 bp biotinylated oligonucleotide per mg.

EpiMark® N6-Methyladenosine Enrichment Kit

#E1610S 20 reactions

- *Enrichment for m6A modified RNA in immunoprecipitation protocols*
- *Enriched RNA can be used directly for next gen sequencing or RT-qPCR*

Description: The EpiMark N6-Methyladenosine Enrichment Kit contains a rabbit monoclonal antibody specific for N6-Methyladenosine (m6A). The kit also contains two control RNAs, one with m6A modification (*Gaussia* luciferase) and one without (*Cypridina* luciferase) to monitor enrichment and depletion. The GLuc RNA control was transcribed in the presence of 20% m6ATP and 80% ATP.

This kit can be used to enrich m6A modified RNA in immunoprecipitation protocols for downstream analysis by next-generation RNA sequencing or RT-qPCR.

Modified RNA is isolated from a fragmented RNA sample by binding to the N6-Methyladenosine antibody attached to Protein G Magnetic Beads. After multiple wash and clean-up steps, the enriched RNA is eluted in nuclease-free water and is ready for further analysis.

Kit Includes:

- N6-Methyladenosine Antibody
- m6A Control RNA
- Unmodified Control RNA

N6-Methyladenosine Antibody is produced by Cell Signaling Technology, Inc. and sold by New England Biolabs, Inc.

David joined NEB in 2002 and is currently Senior Web Master of our Marketing Technologies Team. David is an avid gardener and a member of the NEB gardening club, which tend raised-bed gardens at our Ipswich campus.

Monarch Kits for Cleanup & Isolation

Monarch Total RNA Miniprep Kit
#T2010S 50 preps

Monarch RNA Cleanup Kit (10 µg)
#T2030S 10 preps
#T2030L 100 preps

Monarch RNA Cleanup Kit (50 µg)
#T2040S 10 preps
#T2040L 100 preps

Monarch RNA Cleanup Kit (500 µg)
#T2050S 10 preps
#T2050L 100 preps

Companion Products:

Monarch RNA Purification Columns
#T2007L 100 columns

Monarch gDNA Removal Columns
#T2017L 100 columns

Monarch Collection Tubes II
#T2018L 100 tubes

Monarch DNA/RNA Protection Reagent
#T2011L 56 ml

Monarch RNA Lysis Buffer
#T2012L 100 ml

Monarch Total RNA Miniprep Enzyme Pack
#T2019L 1 Pack

Monarch RNA Priming Buffer
#T2013L 56 ml

Monarch RNA Wash Buffer
#T2014L 50 ml

Nuclease-free Water
#B1500S 25 ml
#B1500L 100 ml

- *Use with a wide variety of sample types*
- *Purify RNA of all sizes, including miRNA & small RNAs >20 nucleotides*
- *Includes DNase I, gDNA removal columns, Proteinase K, and a stabilization reagent*
- *Efficiently remove contaminating genomic DNA*
- *Compatible with Qiacube® and Kingfisher® Flex automation platforms*
- *Protocols available for RNA fractionation and RNA cleanup*
- *Save money with value pricing for an all-in-one kit*

The Monarch Total RNA Miniprep Kit is a comprehensive solution for sample preservation, cell lysis, gDNA removal, and purification of total RNA from a wide variety of biological samples, including cultured cells, blood, and mammalian tissues. Additionally, tough-tolyse samples, such as bacteria, yeast, and plant, can be processed with additional steps that enhance lysis. Total RNA, including viral RNA, can also be extracted from clinically-relevant samples like saliva, buccal swabs and nasopharyngeal swabs. Cleanup of enzymatic reactions or purification of RNA from TRIzol® -extracted samples is also possible using this kit. Purified RNA has high quality metrics, including $A_{260/280}$ and $A_{260/230}$ ratios ≥ 1.8, high RIN scores, and minimal residual gDNA. Captured RNA ranges in size from full-length rRNAs down to intact miRNAs. Additionally, differential binding conditions allow selective capture or exclusion of the sub-200 nucleotide RNA pool that includes miRNA, 5S rRNA, and tRNA. Purified RNA is suitable for downstream applications, such as RT-qPCR, cDNA synthesis, RNA-seq, Northern blot analysis, etc.

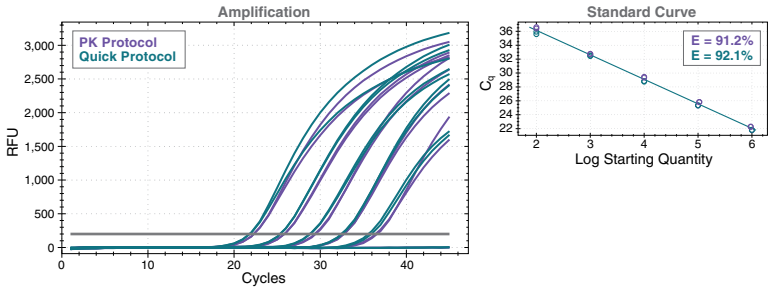
The Monarch RNA Cleanup Kits provide a fast and simple silica spin column-based solution for RNA cleanup and concentration after any enzymatic reaction (including *in vitro* transcription, DNase I treatment, capping and labeling) and after other purification methods such as phenol/chloroform extraction. These kits can also be used to extract total RNA from cells, saliva and buccal/ nasopharyngeal swabs. The Monarch RNA Cleanup Kits are available in 3 different binding capacities (10 µg, 50 µg and 500 µg). Each kit contains unique columns, all designed to prevent buffer retention and ensure no carryover of contaminants, enabling low-volume elution of highly-pure RNA. Following the standard protocol, RNA ≥ 25 nt is purified with this kit; however, a modified protocol is available to enable the binding of RNA as small as 15 nt (including miRNAs).

The Monarch Total RNA Miniprep Kit Includes:

- gDNA Removal Columns
- RNA Purification Columns
- Collection Tubes II
- DNA/RNA Protection Reagent (2X)
- RNA Lysis Buffer
- Proteinase K and associated buffers
- DNase I & associated reaction buffer
- RNA Priming Buffer
- RNA Wash Buffer (5X)
- Nuclease-free Water

The Monarch RNA Cleanup Kits Include:

- RNA Cleanup Columns (10, 50 or 500 µg)
- RNA Cleanup Binding Buffer
- RNA Cleanup Wash Buffer (5X)
- Collection Tubes II
- Nuclease-free Water



The Monarch Total RNA Miniprep Kit successfully purifies synthetic SARS-CoV-2 viral RNA from saliva samples. *The Monarch Total RNA Miniprep Kit Proteinase K and Quick Protocols were used to isolate total RNA from saliva samples containing 10-fold serial dilutions of synthetic SARS-CoV-2 N-gene RNA. Purified RNA was eluted in 100 µl nuclease-free water to yield 50 to 500,000 copies of viral RNA/µl. Using the Luna Universal Probe One-Step RT-qPCR Kit (NEB #E3006), titers as low as 50 copies (the lowest input tested) were detected and linear, quantitative recovery of the SARS-CoV-2 N-gene was observed over a 5-Log range.*

RNA Markers & Ladders

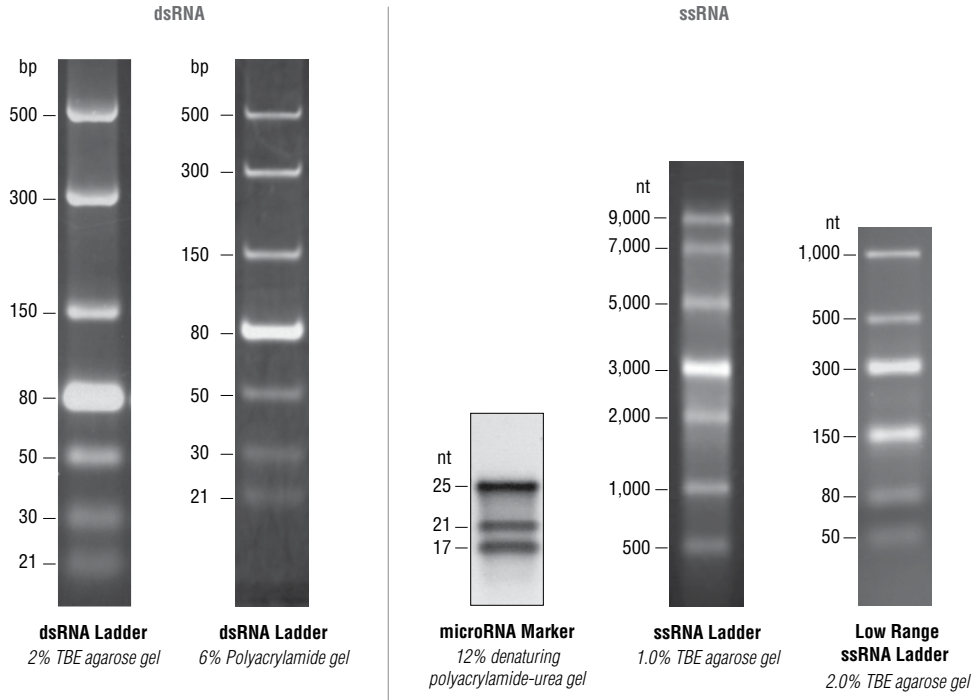
dsRNA Ladder
#N0363S 25 gel lanes

microRNA Marker
#N2102S 100 gel lanes

ssRNA Ladder
#N0362S 25 gel lanes

Low Range ssRNA Ladder
#N0364S 100 gel lanes

NEB offers several RNA Markers and Ladders with a size range from 17 to 9,000 bases. The ssRNA ladders are suitable for use as RNA size standards on denaturing or native gels. Both are supplied with 2X Loading Buffer and feature a higher intensity fragment to serve as a reference band. The microRNA Marker, supplied in a ready to-load denaturing solution, is ideally used as a size marker on polyacrylamide gels or Northern blots and is best visualized stained with ssRNA fluorescent dyes. It is supplied with a 3´-biotinylated 21-mer oligonucleotide probe that can also be labeled with [φ-^{32}P] ATP and T4 PNK (NEB #M0201). The dsRNA Ladder is suitable for use as a size standard in dsRNA analysis on both polyacrylamide and agarose gels.



dsRNA Ladder
2% TBE agarose gel

dsRNA Ladder
6% Polyacrylamide gel

microRNA Marker
12% denaturing polyacrylamide-urea gel

ssRNA Ladder
1.0% TBE agarose gel

Low Range ssRNA Ladder
2.0% TBE agarose gel

RNA Loading Dye, (2X)

#B0363S 4 ml

Description: The RNA Loading Dye, (2X) is a premixed loading dye for use with denaturing and non-denaturing PAGE/agarose gels.

RNA Loading Dye Composition: 1X RNA Loading Dye: 47.5% Formamide, 0.01% SDS, 0.01% Bromophenol Blue, 0.005% xylene cyanol and 0.5 mM EDTA.

Universal miRNA Cloning Linker

#S1315S 5 µg

Companion Product:

T4 RNA Ligase 2, truncated KQ
#M0373S 2,000 units
#M0373L 10,000 units

This 5-adenylated, 3´-blocked oligoribonucleotide can be used for cloning short RNAs according to the procedure of Bartel (1). RNA ligase recognizes the "activated" adenylated oligo and covalently links (ligates) its 5´ end to the 3´ OH of a second single-stranded sequence in the absence of ATP. In a mixture of nucleic acids use of the 5´ adenylated, 3´ blocked oligo with T4 RNA Ligase 2, truncated, T4 RNA Ligase 2, truncated K227Q or T4 RNA Ligase 1 (w/o ATP) results in ligation of the target oligo only.

The sequence of the adenylated DNA oligo is 5´-rAppCTGTAGGCACCATCAAT-NH_2 3´

(1) Lau et al. (2001) *Science*, 294, 858–856.

New and old.
Credit: Stephen, Adobe Stock

Offsetting CO_2 emissions through reforestation

Every time we drive our cars, the combustion of gasoline releases carbon dioxide (CO_2) into the air. However, cars are just a small contribution to the carbon that is produced by humans around the globe. In 2022, the National Oceanic and Atmospheric Administration (NOAA) estimated that 37.4 billion tons of carbon was released into the air primarily from burning coal, oil and gas. About 10% of this released CO_2 makes it into the atmosphere and functions as a greenhouse gas, which means that it absorbs thermal heat radiating from the Earth and releases this heat slowly over time. Greenhouse gases help Earth maintain consistent temperatures, keeping Earth's surface from dipping into freezing temperatures when the sun goes down. However, increased amounts of greenhouse gases lead to increased thermal heat absorption in the atmosphere, and ultimately, increased temperatures on Earth's surface. And this is the basis of global warming.

So how do we balance carbon emissions with carbon sequestration, or capturing methods? The answer lies in nature. The natural world has supplied us with a carbon-capturing technology that has an unlimited lifespan, low overhead cost, and a leafy canopy – trees. But for centuries, even before cars and factories were spewing CO_2 into the air, we have been cutting down trees. In fact, we have been clearing whole forests. Sometimes to make way for roads or buildings, sometimes to cultivate other crops or to create grazing grounds for cattle or other farmed animals. It is estimated that agriculture is responsible for approximately 80% of tropical forest loss.

Reforestation refers to planting trees in areas where a forest was previously cut down. Forests can be cut down for many reasons: to sell the wood, to grow food, or to make space for cattle pasture. But what are the effects of deforestation? There are several worth noting: it disrupts ecosystems and migratory patterns, it leads to a change in land, and the cutting of trees releases CO_2 into the air. And as always, a portion of this CO_2 makes it into the atmosphere. The Global Carbon Project projected land use changes to contribute 3.9 $GtCO_2$ in 2022. Deforestation remains the main driver of land-use emissions – the good news is that reforestation efforts now counterbalance approximately half the deforestation emissions.

Reforestation offers several advantages over carbon credits: it recovers the shade that cools land temperatures, it creates or extends ecosystems for wildlife, and it can also provide farmers with an economically sound crop. Reforest the Tropics (RTT) is a non-profit organization based out of Mystic, Connecticut, U.S. that has been planting sustainable forests in Costa Rica since 1996. RTT provides permanent carbon capture and storage for U.S. CO_2 emitters through the funding of these reforestation efforts. Over 50 years of research in tropical forestry has led RTT to a specialized mixture of fast growing and hardwood trees that offers significant carbon capture storage between 3-5 years after planting. And importantly, it creates a sustainable forest model allowing farmers to profit from timber harvested during thinning processes necessary to maintain the forest.

In August of 2019, NEB entered into agreement with RTT to fund the planting of 100 hectares of tropical forest in Costa Rica to offset carbon emissions. And in June of 2022, we added another 100 hectares of forest to this project. This forest is conservatively estimated to sequester over 100,000 tons of CO_2 in the first 25 years and could sequester 100,000 tons more over the following 25 years.

Listen to an excerpt from our Reforest the Tropics podcast.

Protein Expression & Purification

NEB offers an array of solutions for robust expression of your target protein.

At first glance, recombinant protein expression looks quite simple. Essentially, DNA encoding a target protein is cloned downstream of a promoter in an expression vector. This vector is then introduced into a host cell, and the cell's protein synthesis machinery produces the desired protein. In practice, however, protein expression can be very challenging, because so many factors may influence the process. For example, each protein folds in its own unique manner, a process that may be influenced by the choice of expression host. Similarly, some proteins require post-translational modifications or proper insertion into a biological membrane. Finally, some proteins may have an activity that is detrimental to the host. Thus, no single solution exists for successful production of all recombinant proteins. Instead, it is beneficial to have access to a wide range of expression tools, and a willingness to explore multiple approaches to better one's chances for success.

NEB offers an array of expression systems offering different advantages, enabling you to choose the strategy that best suits your protein expression and purification needs. Many share a compatible polylinker, enabling the gene of interest to be easily shuffled between systems. Additionally, a selection of competent cells is available for *in vitro* expression of difficult-to-express proteins.

Featured Products

231 NEBExpress® Cell-free *E. coli* Protein Synthesis System

232 PURExpress® *In Vitro* Protein Synthesis Kit

238 NEBExpress Ni Spin Columns

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349 Protein Expression with T7 Express Strains

To learn more about NEB's portfolio of products for protein expression and purification, Visit **www.neb.com/Protein Expression** to learn more.

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Cell-Free Expression

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PURExpress Δ Ribosome Kit 232
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Yeast

K. lactis Protein Expression Kit 235

Competent Cells for Protein Expression

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Protein Expression Overview

Experts in protein expression and purification

Protein expression can be a very complex, multi-factorial process. Each protein requires a specific intracellular environment to correctly achieve its secondary and tertiary structures. Proteins may also require post-translational modifications or insertion into a cellular membrane for proper function. Other proteins, once expressed, may be toxic to the host. Thus, no single solution exists for the successful production of all recombinant proteins, and a broad range of expression tools is needed to ensure the successful expression of your target protein.

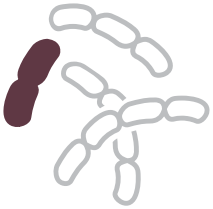
Our NEBExpress portfolio of products includes solutions for expression and purification, and is supported by access to scientists with over 40 years of experience in developing and using recombinant protein technologies in *E. coli*. We use these solutions in our own research and manufacturing processes, and know that quality and performance are critical – all of our products are stringently tested so that you can be sure they will work optimally for your solution, just as we rely on them to work in ours.

Generate analytical amounts of protein with our cell-free expression systems

- NEBExpress Cell-free *E. coli* Protein Synthesis System, our novel *E. coli* cell-extract based transcription/translation system, is designed to synthesize proteins under the control of T7 RNA Polymerase at high yields for a wide variety of proteins
- The PURExpress® *In Vitro* Protein Synthesis Kit, our novel cell-free transcription/translation system, enables protein expression in approximately two hours and is ideal for high throughput technologies

Generate and purify high yields of recombinant proteins

- The NEBExpress MBP Fusion and Purification System utilizes a pMAL vector and the *malE* gene for the expression of MBP-fusion proteins, which can be isolated by a two-step affinity purification
- The IMPACT™ Kit utilizes engineered protein splicing elements (inteins) to purify recombinant proteins in a single step
- For analysis, try our protein standards, which are available unstained, prestained, or with two colors for easy identification



Express a variety of proteins with our competent cells

- Our popular BL21 and BL21(DE3) Competent *E. coli* strains are available for routine expression
- Lemo21(DE3) Competent *E. coli* offers tunable T7 expression for difficult targets
- For expression of His-tagged proteins, we offer NiCo21(DE3) Competent *E. coli*
- SHuffle® strains are available for the expression of proteins with multiple disulfide bonds

Purify tagged proteins with our magnetic beads, columns and resins

- Nickel spin columns, magnetic beads and resin enable rapid purification of His-tagged proteins
- Amylose resins for purification of MBP-tagged proteins are available in a variety formats (standard, high flow and magnetic)
- Chitin resin allows for rapid purification of CBD-tagged proteins
- Remove affinity tags following your purification with TEV Protease

NEBExpress® Cell-free *E. coli* Protein Synthesis System

#E5360S	10 reactions
#E5360L	100 reactions

Companion Product:

NEBExpress GamS Nuclease Inhibitor	
#P0774S	75 µg

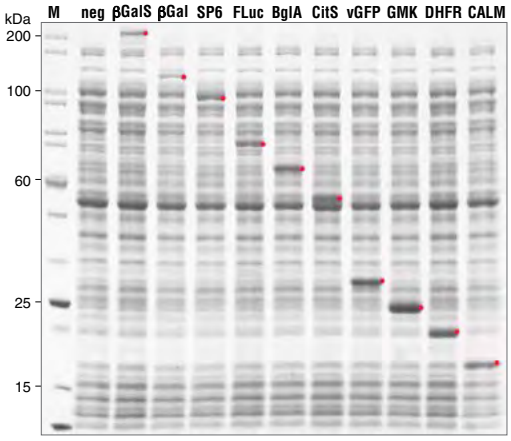
- *Synthesize high yields of protein (typically 0.5 mg/ml)*
- *Protein can be synthesized and visualized in approximately 2–4 hours*
- *Synthesize target proteins ranging from 17 to 230 kDa*
- *Templates can be plasmid DNA, linear DNA, or mRNA*
- *RNase contamination can be inhibited by the supplied RNase inhibitor, eliminating clean-up steps*
- *Flexible reaction conditions achieve maximum yield; protein synthesis can be sustained for 10 hours at 37°C or up to 24 hours at lower temperatures*
- *Reactions can be miniaturized or scaled up to yield milligram quantities of protein*

Description: The NEBExpress Cell-free *E. coli* Protein Synthesis System is a coupled transcription/translation system designed to synthesize proteins encoded by a DNA or mRNA template under the control of a T7 RNA Polymerase promoter. The system offers high expression levels, the ability to produce high molecular weight proteins, scalability, and is cost-effective for high throughput expression applications. The speed and robustness of the system facilitates protein synthesis in applications such as protein engineering, mutagenesis studies and enzyme screening.

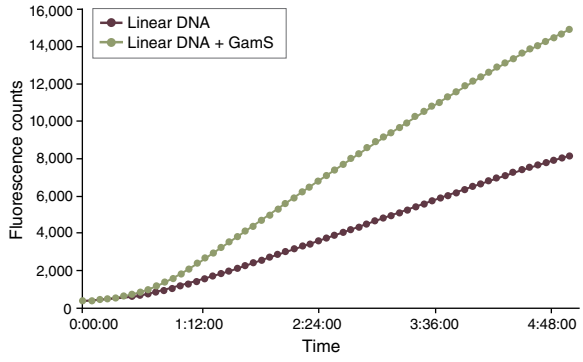
The NEBExpress Cell-free *E. coli* Protein Synthesis System contains all the components required for protein synthesis, except for the target template DNA. It is a combination of a highly active cell extract from a genetically engineered strain, a reaction buffer, and an optimized T7 RNA Polymerase, which together yield robust expression of a wide variety of protein targets ranging from 17 to 230 kDa.

Applications:

- High throughput screening and liquid handling
- Mutation studies: effect of point mutations, deletions and insertions, rapidly identify active domains and functional residues
- Epitope mapping and protein folding
- Expression of toxic proteins



The NEBExpress Cell-free E. coli Protein Synthesis System can be used to express a wide range of proteins. *50 µl reactions containing 250 ng template DNA were incubated at 37°C for 3 hours. The red dot indicates the protein of interest. M = Unstained Protein Standard, Broad Range (NEB #P7717), "neg" = negative control, no DNA.*



NEBExpress GamS Nuclease Inhibitor enhances synthesis of linear DNA. *GamS inhibits Exonuclease V (RecBCD) activity and stabilizes linear DNA templates in E. coli based in vitro protein synthesis reactions. 50 µl reactions containing 100 ng linear template DNA, the components of the NEBExpress Cell-free E. coli Protein Synthesis System and 1.5 µg NEBExpress GamS Nuclease Inhibitor incubated for 5 hours at 37°C were monitored for activity as determined by fluorescence signal.*

PURExpress® *In Vitro* Protein Synthesis Kits

PURExpress *In Vitro* Protein Synthesis Kit
#E6800S 10 reactions
#E6800L 100 reactions

PURExpress Δ Ribosome Kit
#E3313S 10 reactions

PURExpress Δ (aa, tRNA) Kit
#E6840S 10 reactions

PURExpress Δ RF123 Kit
#E6850S 10 reactions

Companion Products:

PURExpress Disulfide Bond Enhancer
#E6820S 50 reactions

E. coli Ribosome
#P0763S 1 mg

- *Generation of analytical amounts of proteins for further characterization*
- *Confirmation of open reading frames*
- *Generation of truncated proteins to identify active domains and functional residues*
- *Introduction of modified, unnatural or labeled amino acids (NEB #E6840, #E6850)*
- *tRNA structure and function studies (NEB #E6840)*
- *Ribosome structure and function studies (NEB #E3313, #P0763)*
- *Release factor function studies/ribosome display (NEB #E6850)*
- *Epitope mapping*

PURExpress is based on the PURE system technology originally developed by Dr. Takuya Ueda at the University of Tokyo and commercialized as the PURESYSTEM™ by Biocomber (Tokyo, Japan).

PURESYSTEM™ is a trademark of Post Genome Institute.

Description: A rapid method for gene expression analysis, PURExpress is a novel cell-free transcription/translation system reconstituted from the purified components necessary for *E. coli* translation. The nuclease-free and protease-free nature of the PURExpress platform preserves the integrity of DNA and RNA templates/complexes and results in proteins that are free of modification and degradation. Transcription and translation are carried out in a one-step reaction, and require the mixing of only two tubes. With results available in a few hours, PURExpress saves valuable laboratory time and is ideal for high throughput technologies.

Advantages:

- Suitable for circular or linear DNA template
- Visualize synthesized protein directly on a Coomassie stained gel
- Protein expression in approximately 2 hours
- Transcription/translation components can be removed by affinity chromatography

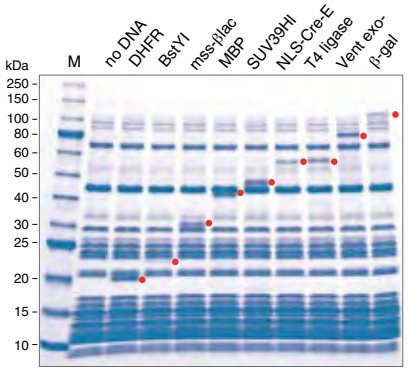
PURExpress Disulfide Bond Enhancer: This proprietary blend of proteins and buffer components is designed to correctly fold target proteins with multiple disulfide bonds produced in PURExpress reactions or NEBExpress *E. coli* S30 extracts. Added at the beginning of a reaction, the components assist with the oxidation of cysteine thiols and correcting mis-oxidized substrates, increasing yield of soluble and functionally active protein.

PURExpress Δ Ribosome Kit: The ribosomes are removed from this kit, allowing users to add their own modified ribosomes for studies on protein translation. Control ribosomes (sufficient for 2 reactions) are provided in a separate tube.

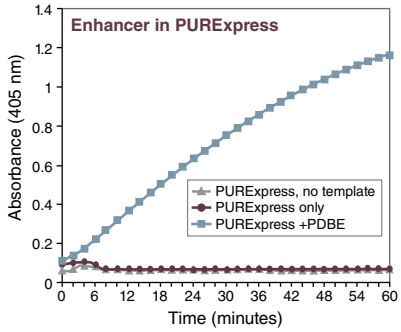
PURExpress Δ RF123 Kit: Release factors are involved in termination of protein translation by recognizing the stop codons in an mRNA sequence. In a ribosome display experiment using PURExpress, lack of release factors could stabilize the ternary complex of mRNAribosome-nascent protein. As a result, the cDNA recovery could be higher. In this kit, the three release factors are supplied separately, allowing users to perform a protein synthesis reaction/ribosome display experiment with/ without release factors of their choice.

PURExpress Δ (aa, tRNA) Kit: The tRNA and amino acids are supplied separately in this kit, allowing users to run a protein synthesis reaction by adding modified amino acids and tRNA mixtures to the reaction.

***E. coli* Ribosome:** The 70S *E. coli* Ribosome consists of a small subunit (30S) and a large subunit (50S). This preparation of ribosomes is highly active in NEB's PURExpress Protein Synthesis Kit (NEB #E6800), and can be used in ribosome structure and function studies, as a target for drug screening and as starting material for isolation of native ribosomal RNAs (5S, 16S, 23S).



Protein expression using the PURExpress In Vitro Protein Synthesis Kit. *25 µl reactions containing 250 ng template DNA and 20 units RNase Inhibitor were incubated at 37°C for 2 hours. 2.5 µl of each reaction was analyzed by SDS-PAGE using a 10–20% Tris-glycine gel. The red dot indicates the protein of interest. Marker M is the Protein Standard.*



PURExpress Disulfide Bond Enhancer. *(PDBE) promotes proper folding of active vtPA. Reactions were set up according to PURExpress specifications with the vtPA template DNA. After a 2 hour incubation at 37°C, 5 µl of each reaction was used in an activity assay and cleavage of the chromogenic substrate was monitored for one hour.*

PURExpress Kit Components

PURExpress *In Vitro* Protein Synthesis Kit NEB #E6800	PURExpress Δ Ribosome Kit NEB #E3313	PURExpress Δ (aa, tRNA) Kit NEB #E6840	PURExpress Δ RF123 Kit NEB #E6850
• Solution A • Solution B • Control (DHFR) template	• Solution A • Factor Mix • Control (DHFR) template • Control Ribosomes	• Solution A (minus aa and tRNA) • Solution B • Control (DHFR) template • Amino Acid Mixture • *E. coli* tRNA	• Solution A • Solution B (minus RF1, RF2 and RF3) • Control (DHFR) template • RF1, RF2 and RF3

NEBExpress® MBP Fusion and Purification System

#E8201S 1 set

Companion Products:

TEV Protease
#P8112S 1,000 units

Amylose Resin
#E8021S 15 ml
#E8021L 100 ml

Anti-MBP Monoclonal Antibody
#E8032S 0.05 ml
#E8032L 0.25 ml

pMAL-c6T Vector
#N0378S 10 µg

- *Reliable E. coli expression: substantial yields (up to 100 mg/L)*
- *Fusion to MBP significantly enhances proper folding of target proteins*
- *Two-step purification: amylose elution followed by TEV Protease cleavage and Ni resin isolation results in a highly pure tag-free target protein*
- *Gentle elution with maltose: no detergents or harsh denaturants required*

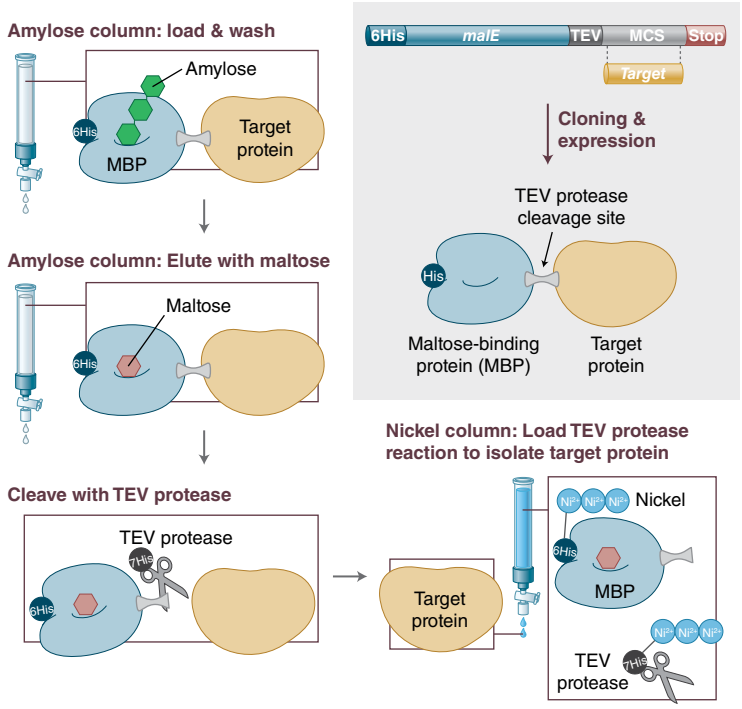
Description: In the NEBExpress MBP Fusion and Purification System, the pMAL-c6T vector provides a method for expressing and purifying a protein produced from a cloned gene or open reading frame. The cloned gene is inserted downstream from and in frame with the *malE* gene of *E. coli*, which encodes maltose-binding protein (MBP); this construct results in the expression of an MBP fusion protein. The pMAL-c6T vector expresses the *N*-terminal hexahistidine tagged *malE* gene (lacking its secretory signal sequence and engineered for tighter binding to amylose) followed by a multiple cloning site containing a TEV protease recognition sequence and stop codons in all three frames. The pMAL-c6T vector expresses the MBP fusion in the cytoplasm. The method uses the strong "tac" promoter and the *malE* translation initiation signals to yield high-level expression of the cloned sequences. The fusion protein is then purified by a one-step purification method using amylose resin and MBP's affinity for maltose.

Following amylose purification, the target protein can be cleaved from the MBP-tag using TEV Protease, without adding any vector-derived residues to the protein. Both the MBP-tag and TEV Protease are polyhistidine-tagged for easy removal from the reaction. Loading the digest onto NEBExpress Ni Resin (NEB #S1428) sequesters both the MBP-tag and TEV Protease, thereby isolating the target protein in the column flow through. The target protein yield can be up to 100 mg/L, with typical yields in the range of 10–40 mg/L.

References: References for properties and applications of this product can be found at www.neb.com.

Kit Includes:

- pMAL-c6T Vector
- MBP6 Protein
- MBP6-TEV-Paramyosin ΔSal
- TEV Protease
- TEV Protease Reaction Buffer
- Anti-MBP Monoclonal Antibody
- *E. coli* ER2523 (NEB Express) (Glycerol Stock)
- Amylose Resin



Schematic illustration of the NEBExpress MBP Fusion and Purification System.

IMPACT™ Kit

#E6901S 1 set

Companion Products:

Anti-CBD Monoclonal Antibody
#E8034S 0.05 ml

pTWIN1 Vector
#N6951S 10 µg

pTXB1 Vector
#N6707S 10 µg

pTYB21 Vector
#N6709S 10 µg

Chitin Resin
#S6651S 20 ml
#S6651L 100 ml

- *Single-column purification without the use of proteases*
- *Produce target protein without vector-derived amino acids*
- *Fusion to either N- or C-terminus of target protein*
- *Ligation and labeling of recombinant proteins*
- *Isolation of proteins with or without N-terminal methionine*

Restriction maps for pTXB1 and pTYB21 can be found in the technical reference section or at **www.neb.com.**

Description: The IMPACT (Intein Mediated Purification with an Affinity Chitin-binding Tag) Kit utilizes engineered protein splicing elements (inteins) to purify recombinant proteins by a single column (Figure 1). This kit distinguishes itself from other protein fusion systems by its ability to separate a recombinant protein from the affinity tag without the use of a protease.

The IMPACT Kit allows fusion of a tag consisting of an intein and the chitin binding domain (CBD) from *Bacillus circulans*, to either the C-terminus (pTXB1) or the N-terminus (pTYB21) of a target protein (Figure 2). In the presence of thiols, such as DTT, the intein undergoes specific self-cleavage which releases the target protein. The pTXB1 vector can also be used to express a protein with a C-terminal thioester for use in Intein-mediated Protein Ligation (IPL). The IPL reaction, also referred to as expressed protein ligation, allows for ligation of a peptide or a protein with a N-terminal cysteine to a bacterially expressed protein with a C-terminal thioester through a native peptide bond for use in protein labeling and semisynthesis. For more information on the IMPACT System and IPL, visit www.neb.com.

pTXB1 is a *E. coli* expression vector that utilizes a mini-intein from the *Mycobacterium xenopi gyrA* gene [*Mxe* GyrA intein; 22 kDa]. This intein has been modified and combined with the CBD to create an affinity tag which can be bound to chitin beads (NEB #S6651). Release of the target protein is induced by thiol-reagents such as DTT or 2-mercaptoethanesulfonic acid (for ligation).

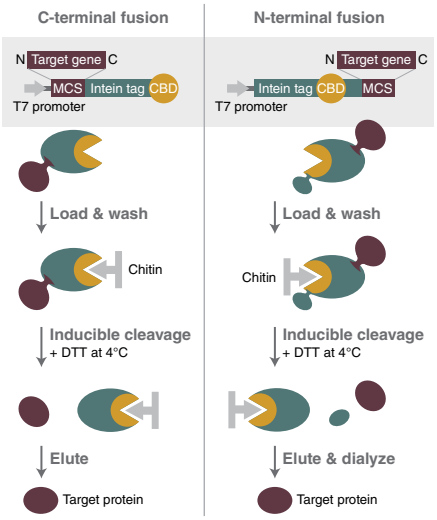
The pTYB21 vector allows for the fusion of the intein tag containing the *Saccharomyces cerevisiae* (*Sce*) *VMA*1 intein and CBD to the N-terminus of the target protein.

pTWIN1 Vector is available separately and enables isolation of proteins with an N-terminal cysteine and/or a C-terminal thioester. The polylinker is designed for the in-frame fusion of a target gene between the modified *Ssp* DnaB and *Mxe* GyrA inteins. The presence of the CBD facilitates purification.

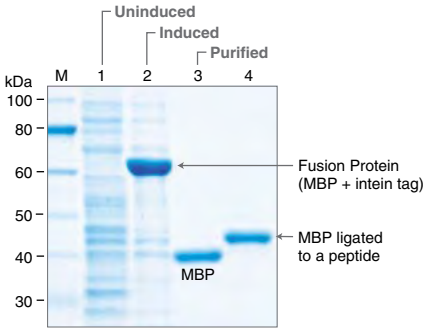
References: References for properties and applications of this product can be found at www.neb.com.

Kit Includes:

- *E. coli* ER2566
- Blue Protein Loading Dye
- pTXB1 Vector
- pMXB10 Control Plasmid
- pTYB21 Vector
- Anti-CBD Monoclonal Antibody
- DTT
- Chitin Resin



Schematic of the IMPACT System.



Purification of Maltose Binding Protein (MBP) in a single affinity purification step. *Lane 1: uninduced cell extract. Lane 2: induced cell extract showing expressed fusion protein. Lane 3: MBP fraction eluted after inducing cleavage overnight at 4°C. Lane 4: MBP ligated to a peptide containing an N-terminal cysteine. Marker M is the protein ladder.*

Guide to IMPACT vectors and applications

Vectors	Site of Target Protein Fusion	Intein Tag (kDa)	Recommended Cloning Sites[a]	Preferred Residues at Cleavage Site[b]	Method of Cleavage[c,d]	Applications
pTXB1	C-terminus	Mxe GyrA intein (28)	NdeI-SapI/SpeI	Y, F, O, N, T, K, A, H, M Unfavorable residues: S, P, D, G	DTT (or MESNA) pH 8.0-8.5, 4°C	Purification; C-terminal thioester for ligation and modification
pTYB21	N-terminus	Sce VMA1 intein (56)	SapI/BsmI/ NdeI-PstI	A, Q, M, G, L, N, W, F, Y Unfavorable residues: P, S, C, T, R	DTTd pH 8.0-8.5, 25°C	Purification
pTWIN1	C-terminus (Intein 2)	Mxe GyrA intein (28)	NdeI-SapI/SpeI	M, Y, F, LEM Unfavorable residues: S, P, E, D	DTT (or MESNA) pH 8.0-8.5, 4°C	Purification; C-terminal thioester for ligation and modification

[a] NEBuilder HiFi DNA Assembly Cloning Kit (NEB #E5520) can be used to generate construct without the use of restriction enzymes.
[b] Actual cleavage efficiency is dependent on the adjacent residues as well as the folding of the fusion protein.
[c] Dithiothreitol (DTT) is used only for protein purification. 2-mercaptoethanesulfonic acid (MESNA) is used for isolation of proteins possessing a C-terminal thioester for ligation, labeling and cyclization.
[d] Cysteine can be used in the place of DTT.

K. lactis Protein Expression Kit

#E1000S 1 set

Companion Products:

SacII
#R0157S 2,000 units
#R0157L 10,000 units

Yeast Carbon Base Medium Powder
#B9017S 12 g

K. lactis GG799 Competent Cells
#C1001S 5 reactions

BstXI
#R0113S 1,000 units
#R0113L 5,000 units

Enterokinase, light chain
#P8070S 480 units
#P8070L 2,560 units

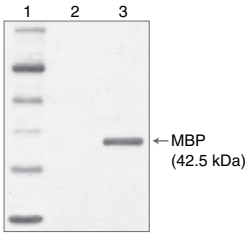
- *Clone and express genes toxic to E. coli*
- *Simultaneous expression of multiple genes*
- *No expensive antibiotics or methanol required*
- *Easy-to-use protocols for those inexperienced with yeast systems*
- *Attractive commercial sublicensing*

A restriction map for pKLAC2 can be found in the technical reference section or at **www.neb.com.**

Description: The *K. lactis* Protein Expression Kit provides an easy method for expressing a gene of interest in the yeast *Kluyveromyces lactis*. The gene is cloned into the integrative pKLAC-series of vectors, and may be expressed intracellularly or secreted. The *K. lactis* system offers several advantages over other yeast and bacterial expression systems. Abundant overexpression of protein is achieved through high culture densities as well as the ability to integrate multiple copies of the vector. The pKLAC-series of vectors use a strong *LAC4* promoter, which has been modified to lack expression in *E. coli*, making this system useful for expressing toxic genes. For the selection of transformants, no expensive antibiotics are required. In addition, no methanol is required in growth media. Finally, the *K. lactis* system can express post-translationally modified proteins, making it a useful alternative to bacterial expression systems.

pKLAC2 is a general purpose expression vector. Using this vector, proteins may be produced intracellularly or may be fused to the *K. lactis* α-mating factor sequence for secreted expression. Vector pKLAC2 contains an MCS that is compatible with other expression systems available from NEB.

GG799 competent cells are provided in the *K. lactis* Protein Expression Kit. GG799 cells are characterized by very high cell density growth and efficient expression of foreign proteins. GG799 cells have no genetic modifications.

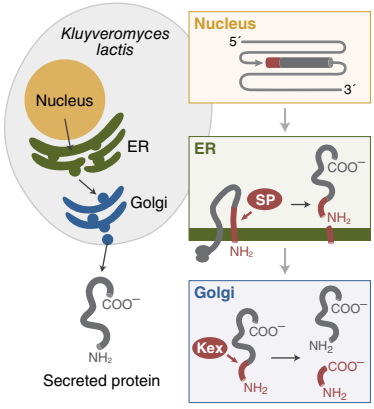


Protein Expression in K. lactis. *SDS-polyacrylamide gel electrophoresis separation of secreted recombinant maltose-binding protein (MBP) detected directly in peptone rich growth medium by Coomassie staining. Lane 1: Protein Molecular Weight Markers. Lane 2: spent culture medium (15 µl) from wild-type K. lactis cells. Lane 3: spent culture medium (15 µl) from K. lactis cells harboring an integrated expression cassette containing the E. coli malE gene.*

References: References for properties and applications of these products can be found at www.neb.com.

Kit Includes:

- SacII
- pKLAC1-*malE* Control Plasmid
- pKLAC2 Vector
- rCutSmart
- Yeast Carbon Base Medium Powder (12 g)
- Acetamide solution (sterile) (10 ml)
- Integration Primer 2
- Integration Primer 1
- Integration Primer 3
- *K. lactis* GG799 Competent Cells
- NEB Yeast Transformation Reagent (5 ml)



Secreted protein processing. *In the nucleus, an integrated expression vector encoding a fusion between the α-MF domain (blue) and a desired protein (black) is expressed. A signal peptide in the α-MF domain directs entry of the fusion protein into the endoplasmic reticulum (ER) and is removed by signal peptidase (SP). The fusion protein is transported to the Golgi apparatus where the Kex protease removes the α-MF domain. The protein of interest is then secreted from the cell.*

Jagruti and Gary are both Talent Acquisition Partners on the NEB Human Resources team at NEB. Jagruti joined NEB in 2021 and enjoys cooking and painting in her free time. Gary joined NEB in 2022 and enjoys cheering on local Patriots and Celtics teams and spending time with family and friends.

Competent Cells for Protein Expression

NEB offers a wide selection of competent cell strains ideal for expression of a variety of proteins. Proteins with multiple disulfide bonds are correctly oxidized to significantly higher yields with SHuffle® strains. Tunable T7 expression is achieved with Lemo21(DE3), an ideal strain for difficult targets including membrane proteins. NiCo21(DE3) is designed for the expression and purification of His-tagged proteins. NEBExpress and T7 Express are offered with varying levels of control. Only NEB offers exceptional control of T7 expression by the *lysY* gene, which is ideal for proteins that are difficult to express or toxic to the cell. Each strain is provided with a protocol for optimal expression.

Strain	NEB #	Characteristics	Size
NEBExpress Competent *E. coli* (High Efficiency)*	C2523H C2523I	• Versatile non-T7 expression strain • Protease deficient	20 x 0.05 ml 6 x 0.2 ml
NEBExpress *Iq* Competent *E. coli* (High Efficiency)	C3037I	• Control of IPTG induced expression from Plac , Ptac and Ptrc • Protease deficient	6 x 0.2 ml
T7 Express Competent *E. coli* (High Efficiency)	C2566H C2566I	• Most popular T7 expression strain • Protease deficient	20 x 0.05 ml 6 x 0.2 ml
T7 Express *lysY* Competent *E. coli* (High Efficiency)	C3010I	• T7 expression • Protease deficient • Better reduction of basal expression	6 x 0.2 ml
T7 Express *lysY/Iq* Competent *E. coli* (High Efficiency)	C3013I	• T7 expression • Protease deficient • Highest level of expression control	6 x 0.2 ml
SHuffle Express Competent *E. coli*	C3028J	• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • Protease deficient/B strain	12 x 0.05 ml
SHuffle T7 Express Competent *E. coli*	C3029J	• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression • Protease deficient/B strain	12 x 0.05 ml
SHuffle T7 Express *lysY* Competent *E. coli*	C3030J	• T7 expression • Protease deficient/B strain • Tightly controlled expression of toxic proteins • Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm	12 x 0.05 ml
SHuffle T7 Competent *E. coli*	C3026J	• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression/K12 strain	12 x 0.05 ml
BL21 Competent *E. coli*	C2530H	• Routine expression for non-T7 Vectors • Protease deficient	20 x 0.05 ml
BL21(DE3) Competent *E. coli*	C2527H C2527I	• Routine T7 Expression • Protease deficient	20 x 0.05 ml 6 x 0.2 ml
Lemo21(DE3) Competent *E. coli*	C2528J	• Tunable T7 Expression for difficult targets • Protease deficient	12 x 0.05 ml
NiCo21(DE3) Competent *E. coli*	C2529H	• Expression and purification of His-tagged proteins • Protease deficient	20 x 0.05 ml

Note: Store Competent Cells at –80°C. Once thawed, do not refreeze. Storage at –20°C will result in a significant decrease in transformation efficiency. Cells lose efficiency whenever they are warmed above –80°C, even if they do not thaw.

* NEB Express is the recommended strain for the NEBExpress MBP Fusion and Purification System.

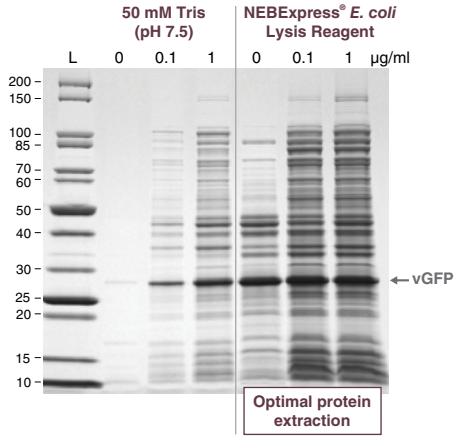
NEW

NEBExpress® T4 Lysozyme

#P8115S 200 µg
#P8115L 1000 µg

- *200-fold more active than chicken egg white lysozyme*
- *Lysis reactions are scalable and compatible with high throughput workflows*
- *Lysis efficiency increases 2-fold when used in combination with NEBExpress E. coli Lysis Reagent*
- *Fast and non-mechanical bacterial lysis; the lysate is ready to use and compatible with affinity resins.*
- *Recombinant, animal free and REACH compliant*

NEBExpress T4 Lysozyme is a recombinant murein hydrolase that breaks down the bacterial cell wall by hydrolyzing the β-1,4 linkage between N-acetylmuramic acid and N-acetylglucosamine in the peptidoglycan of prokaryotic cells (gram-negative and some gram-positive bacteria). It can be used to extract soluble proteins, membrane proteins, DNA, RNA or metabolites.



Optimal protein extraction with NEBExpress T4 Lysozyme in the presence of NEBExpress E. coli Lysis Reagent. *T7 Express E. coli expressing vGFP was lysed with NEBExpress T4 Lysozyme (T4L) in 50 mM Tris-HCl pH 7.5 or in NEBExpress E. coli Lysis Reagent (NEB #P8116S): 4 UOD600 of cell pellet were resuspended in 200 µl of Tris buffer or NEBExpress E. coli Lysis Reagent and lysed for 5 minutes at room temperature in the presence of NEBExpress T4 Lysozyme at 0, 0.1 or 1 ug per 1 ml of cell suspension. The soluble proteins were harvested by centrifugation and analyzed on SDS-PAGE.*

NEW

NEBExpress® *E. coli* Lysis Reagent

25°

#P8116S 100 ml
#P8116L 500 ml

- *Scalable lysis reactions from small to large bacterial cell pellets and compatible with high throughput workflows*
- *Compatible with analyses such as SDS-PAGE, Western blots, activity assay, immunoprecipitation, and downstream purification*

NEBExpress® *E. coli* Lysis Reagent is a chemical lysis solution composed of a proprietary mix of non-ionic and zwitterionic detergents and Tris-based buffer. It allows disruption of *E. coli* cells without denaturing soluble proteins. It is ideal for extracting proteins, especially thermosensitive proteins vulnerable to mechanical lysis procedures, and can disrupt most Gram-negative bacterial cells. Provided as a ready-to-use liquid that is stable at room temperature.

Purification Beads, Columns and Resin Selection Chart

Isolation of pure substrates or proteins for downstream experiments is a common, yet time consuming, task. New England Biolabs offers a variety of resins and magnetic beads that are easy-to-use, highly specific, and available in several different formats for rapid isolation and purification of proteins, nucleic acids and immunoglobulins. NEB's magnetic beads are ideally suited for applications involving high-throughput proteomic screening, small-scale protein isolation, immunomagnetic isolations or cell separation experiments. With magnetic beads, affinity purification of tagged proteins, antigens, antibodies and nucleic acids can be done conveniently and quickly. Immobilized substrates remain biologically active and can be eluted in small volumes or serve as ligands in subsequent pull-down or target interaction experiments involving DNA or proteins. NEB's resins enable simple, one-step purification strategies for tagged proteins, and result in a high yield of highly pure substrate. For the full list of products available for protein expression and purification, visit **www.neb.com/ ProteinExpression**.

Product	Protein Purification	Large-scale Purifications	Use in Automated Chromatography	High-throughput	Biotinylated Substrate Binding	Protein Pull-down	Nucleic Acid Pull-down	mRNA Purification/ Pull-down	Immuno-precipitation
NEBExpress Ni-NTA Magnetic Beads (NEB #S1423)	● His-tag			●		●			
NEBExpress Ni Spin Columns (NEB #S1427)	● His-tag			●		●			
NEBExpress® Ni Resin (NEB #S1428)	● His-tag	●	●			●			
Amylose Resin (NEB #E8021)	● MBP	●				●			
Amylose Resin High Flow (NEB #E8022)	● MBP	●	●			●			
Amylose Magnetic Beads (NEB #E8035)	● MBP			●		●			
Anti-MBP Magnetic Beads (NEB #E8037)	● MBP			●		●			
Chitin Resin (NEB #S6651)	● Intein-CBD tag	●				●			
Chitin Magnetic Beads (NEB #E8036)	● Intein-CBD tag			●		●			
Oligo $d(T)_{25}$ Magnetic Beads (NEB #S1419)				●			●	●	
Streptavidin Magnetic Beads (NEB #S1420)				●	●	● Biotinylated bait	● Biotinylated bait		
Hydrophilic Streptavidin Magnetic Beads (NEB #S1421)				●	●	● Biotinylated bait	● Biotinylated bait		
Protein A Magnetic Beads (NEB #S1425)				●					●
Protein G Magnetic Beads (NEB #S1430)				●					●
Magnetic mRNA Isolation Kit (NEB #S1550)				●				●	

Polyhistidine-tagged Protein Purification

NEBExpress Ni-NTA Magnetic Beads
#S1423S 1 ml
#S1423L 5 ml

NEBExpress Ni Resin
#S1428S 25 ml

NEBExpress Ni Spin Columns
#S1427S 10 Each
#S1427L 25 Each

TEV Protease
#P8112S 1,000 units

NEBExpress Ni-NTA Magnetic Beads: An affinity matrix for the small-scale isolation and purification of polyhistidine-tagged (His-tagged) fusion proteins in manual or automated formats. High specific binding yields purities of > 95% in a single-purification step. Matrix tolerates a wide range of conditions, including the presence of denaturants and detergents. Compatible with commercially available detergent-based cell lysis reagents. Elution can be achieved by protonation, ligand exchange (with imidazole) or extraction of the metal ion by a strong chelator (e.g., EDTA).

- Support Matrix: Spherical, agarose based superparamagnetic microparticles ranging in size from 20-100 µm.
- Binding Capacity: Varies with target, typically ≥ 7.5 mg His-tagged fusion protein/ml bed volume.

NEBExpress Ni Resin: NEBExpress Ni Resin is an affinity matrix for the isolation and purification of polyhistidine-tagged (His-tagged) fusion proteins. It is intended for use in gravity or pressure flow columns and batch purifications, and high specific binding yields purities of > 95% in a single-purification step. NEBExpress Ni Resin is comprised of a highly uniform and chemical-tolerant resin that is pre-charged with nickel ions on the matrix surface. It is resistant to a wide range of chemicals, including NaOH, EDTA, and commonly used reducing agents such as TCEP, DTT, and β-mercaptoethanol. Can be used under native or denaturing conditions.

- Support Matrix: Spherical, agarose based microparticles ranging in size from 10-100 µm.
- Binding Capacity: 1 ml of NEBExpress Ni Resin will bind ≥ 10 mg of His-tagged fusion protein.

NEBExpress Ni Spin Columns: NEBExpress Ni Spin columns are pre-packed with agarose-based microparticles ranging in size from 10-100 µm for the small-scale isolation and purification of polyhistidine-tagged (His-tagged) fusion proteins. Purification can be performed under native or denaturing conditions, including conditions in which EDTA or reducing reagents are required, yielding highly pure target protein in a single purification step. This enables screening of expression conditions and streamlines the functional and structural characterization of the target protein.

- Support Matrix: Spherical, agarose based microparticles ranging in size from 10-100 µm.
- Binding Capacity: Varies with target, ≥ 1 mg His-tagged fusion protein per column.

TEV Protease: TEV Protease, also known as Tobacco Etch Virus (TEV) Protease, is a highly specific cysteine protease that recognizes the amino-acid sequence Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser) and cleaves between the Gln and Gly/Ser residues. It is often used for the removal of affinity purification tags such as maltose-binding protein (MBP) or poly-histidine from fusion proteins. TEV Protease has a 7XHis-tag for easy removal from a reaction using nickel affinity resins and has been engineered to improve thermal stability and decrease autolysis.

Maltose Binding Protein (MBP) Purification

Amylose Resin
#E8021S 15 ml
#E8021L 100 ml

Amylose Resin High Flow
#E8022S 15 ml
#E8022L 100 ml

Amylose Magnetic Beads
#E8035S 25 mg

Anti-MBP Magnetic Beads
#E8037S 10 mg

Anti-MBP Monoclonal Antibody
#E8032S 0.05 ml
#E8032L 0.25 ml

Amylose Resin and Amylose Resin High Flow: Affinity matrix used for isolation of proteins fused to maltose-binding protein. It is a composite amylose/agarose bead. Amylose Resin High Flow is a more rigid bead, suitable for use in automated chromatography systems.

- Binding Capacity: Amylose Resin and Amylose Resin High Flow: > 4 mg MBP5-paramyosin ΔSal fusion protein/ml of bed volume.

Amylose Magnetic Beads and Anti-MBP Magnetic Beads: Affinity matrices for the small-scale isolation and purification of MBP-fusion proteins. Amylose or monoclonal Anti-MBP are covalently coupled to a paramagnetic particle through a linkage that is stable and leak resistant over a wide pH range.

- Support Matrix: Amylose Magnetic Beads – 10 µM superparamagnetic particles; Anti-MBP Magnetic Beads – 1 µM nonporous super paramagnetic particle.
- Binding Capacity: 1 mg of Amylose Magnetic Beads will bind ≥ 10 µg of MBP-fusion protein. 1 mg of Anti-MBP Magnetic Beads will bind 5 µg of MBP-paramyosin ΔSal fusion protein.

Anti-MBP Monoclonal Antibody: Anti-MBP Monoclonal Antibody is a murine anti-maltose-binding protein antibody, isotype IgG2a. It is purified from tissue culture supernatant by protein A affinity chromatography.

Chitin Binding Domain (CBD) Purification

Chitin Resin
#S6651S 20 ml
#S6651L 100 ml

Chitin Magnetic Beads
#E8036S 5 ml

Anti-CBD Monoclonal Antibody
#E8034S 0.05 ml

Chitin Resin: An affinity matrix for the isolation of target proteins fused on an intein-chitin binding domain (CBD). Strong specific binding enables purification of highly pure protein from crude lysates in on step. Removal of CBD-tag during elutin typically yields highly pure, native protein without the use of a protease.

- Support Matrix: Approximately 50-70 µm microparticles
- Binding Capacity: 2.0 mg maltose-binding protein/ ml bed volume released from the resin after cleavage of the fusion protein expressed from pMYB5.

Chitin Magnetic Beads: An affinity matrix for the small-scale isolation of target proteins fused to a chitin binding domain (CBD). Chitin beads have been prepared with encapsulated magnetite, thereby permitting the magnetic isolation of CBD-fusion proteins from cell culture supernatants. Removal of CBD-tag during elution typically yields highly pure, native protein.

- Support Matrix: Approximately 50-70 µm paramagnetic microparticles
- Binding Capacity: 2 mg chitin binding domain protein / ml bed volume released

Anti-CBD Monoclonal Antibody: Anti-CBD Monoclonal Antibody is a murine anti-chitin binding domain (CBD) antibody, isotype IgG1. It has high purity and specificity for chitin binding domain tag, and is verified for use in both Western blotting and ELISA.

Magenetic Bead Purification Products

Oligo $d(T)_{25}$ Magnetic Beads
#S1419S 5 ml

Magnetic mRNA Isolation Kit
#S1550S 25 isolations

Streptavidin Magnetic Beads
#S1420S 5 ml

Hydrophilic Streptavidin Magnetic Beads
#S1421S 5 ml

Protein A Magnetic Beads
#S1425S 1 ml

Protein G Magnetic Beads
#S1430S 1 ml

Companion Product:

96-Well Microtiter Plate Magnetic Separation Rack
#S1511S 96 wells

- *Small-scale purification or immunoprecipitation of IgG species*
- *No centrifugation required*
- *Regenerate matrix without binding capacity loss*

Oligo $d(T)_{25}$ Magnetic Beads: These beads enable small-scale isolations of mRNA from a variety of samples, including *in vitro* transcribed mRNA, total RNA, crude cell lysates and tissue. The selectivity for mRNA results from the annealing of bead-linked oligo $d(T)_{25}$ to the poly(A) region present in most eukaryotic mRNAs.

- Support Matrix: 1 µm nonporous superparamagnetic microparticles
- Binding Capacity: ≥ 5 µg rA_{30} per mg of beads

Magnetic mRNA Isolation Kit: The Magnetic mRNA Isolation Kit is designed to isolate intact poly(A)$^+$ RNA from cells and tissue without requiring phenol or other organic solvents. The technology is based on the coupling of Oligo $d(T)_{25}$ to 1 µm paramagnetic beads, which is then used as the solid support for the direct binding of poly(A)$^+$ RNA.

Streptavidin Magnetic Beads: The beads provide fast magnetic response times and reaction kinetics, and they have high binding capacity and sensitivity while retaining their physical integrity. They can be used to capture biotin-labeled substrates including DNA, RNA, peptides, antigens, antibodies and other proteins of interest in manual or automated workflows. These beads typically exhibit lower non-specific binding of proteins.

- Support Matrix: 1 µm nonporous superparamagnetic microparticles
- Binding Capacity: ≥ 30 µg biotinylated antibody per mg of beads or > 500 pmol of single-stranded 25 bp biotinylated oligonucleotide per mg of beads

Hydrophilic Streptavidin Magnetic Beads: The beads provide rapid magnetic response times and reaction kinetics, and they have high binding capacity and sensitivity while retaining their physical integrity. They can be used to capture biotin-labeled substrates including DNA, RNA, peptides, antigens, antibodies and other proteins of interest in manual or automated workflows. These beads typically exhibit lower non-specific binding of nucleic acids.

- Support Matrix: 2 µM non-porous superparamagnetic microparticles
- Binding Capacity: > 400 pmol of single-stranded 25 bp biotinylated oligonucleotide per mg of beads

Protein A and Protein G Magnetic Beads: The beads allow for isolation of most mammalian immunoglobulins (IgGs) and are amenable to immunoprecipitation. Predominant Fc-binding allows optimal IgG orientation upon binding to the outer surface of the Protein A and Protein G Magnetic Beads allowing Fab regions to efficiently bind antigen. These beads can be used to immunoprecipitate target proteins from crude cell lysates using a selected primary antibody. In addition, specific antibodies can be chemically cross-linked to the Protein A- or Protein G-coated surface to create a reusable immunoprecipitation bead, thereby avoiding the co-elution of antibody with the target antigen.

- Support Matrix: 2 µm nonporous superparamagnetic microparticles
- Binding Capacity: > 280 µg of Human IgG per ml of beads

Magnetic Separation Racks

Product	Application	Magnets	Capacity	Convenience
6-Tube Magnetic Separation Rack (NEB #S1506)	Designed for small-scale separations using magnetic particles	Neodymium rare earth permanent magnets	6 tubes (1.5 ml)	Use with magnetic particle-based affinity purification for rapid, small-scale purifications
50 ml Magnetic Separation Rack (NEB #S1507)	Designed for small-scale separations using magnetic particles	Neodymium rare earth permanent magnets	4 tubes (50 ml)	Use with magnetic particle-based affinity purification for rapid, streamlined purifications
12-Tube Magnetic Separation Rack (NEB #S1509)	Designed for small-scale separations using magnetic particles	Neodymium rare earth permanent magnets	12 tubes (1.5 ml)	Use with magnetic particle-based affinity purification for rapid, small-scale purifications
96-Well Microtiter Plate Magnetic Separation Rack (NEB #S1511)	Designed for use with commercially available high-flanged 100 µl to 300 µl flat-bottom 96-well microplates	24 side-pull magnetic pins attract magnetic beads from solution to the side walls of four adjacent wells	96-well	The orientation of the magnetic field ensures complete removal of the magnetic beads from solution during pipetting steps, thereby minimizing sample loss
NEBNext Magnetic Separation Rack (NEB #S1515)	Designed for rapid and effective small-scale separations of magnetic particles	Anodized aluminum rack with Neodymium Iron Boron (NdFeB) rare earth magnets	24 tubes (0.2 ml)	Next generation sequencing library preparation workflows include magnetic bead-based purification and size-selection steps. It is important for library yield and quality that bead separation be highly efficient and fast, and this is enabled by the powerful fixed magnet cores in this rack.

Balancing wind energy production with wildlife protection

Wind power is expanding fast, but the urgency to produce clean energy must be balanced with wildlife protection. Weighing the environmental cost of wide-scale implementation of clean energy technologies requires research, planning and observation to ensure we are not destroying ecosystems in order to reduce carbon emissions.

Wind power is the fastest-growing form of renewable energy. In 2021, it produced 55% more electric power than it did in 2020, but it needs to be scaled up drastically and quickly to have a significant impact in phasing out fossil fuels and reducing carbon emissions. There is a greater investment in the development of onshore wind turbine projects, which are currently present in 115 countries, than offshore wind projects, which are present in only 19 countries. Both on- and offshore wind projects show incredible potential, but are not without challenges.

The impact of wind turbines on marine and avian species, and through a ripple effect, entire ecosystems, has raised public concern. When debate arises about the suitability of onshore wind turbines as an alternative clean energy source, speculation about the danger they pose to bird life inevitably becomes a part of the conversation. Extensive research shows that wind farms kill far fewer birds than cats, communication towers, cars, building windows and power lines. However, it is raptors (birds of prey) and large seabirds that are disproportionally affected. This is because they use the same wind resources (updraughts and thermals) as wind turbines to power their flight. Another critical factor is that birds of prey produce few young, and this amplifies the impact on the population of even a small number of collisions.

Noise related to wind turbines also has impacts. Offshore turbines are larger and taller, and their construction is no small feat. Noise pollution resulting from offshore wind project construction has caused species to relocate. Then, during operation, the low-frequency noise of the turbines not only stresses birds and other marine life, but can also hinder their communication, which is used to find prey or a mate. All of the observed wildlife threats associated with wind power projects foster community discourse. For example, a wind farm on Prince Edward Island has sparked debate because it is in wetlands that serve as nesting grounds for vulnerable bird species. Another proposed project in Tasmania, Australia that threatens the endangered, orange-bellied parrot was approved on the condition that the turbines are shut down for a lengthy five months a year during the migratory period, bringing into question the viability of the project.

Careful planning of the placement and operation of wind farms can help avoid disturbing sensitive ecosystems. Slow moving blades can reduce the number of blade-strike injuries and reduce bird or bat collisions. Turbine blade designs with larger surface areas do not need to spin as fast to produce the same amount of power as fast-moving turbines with narrower blades. Offshore wind projects have the advantage of overcoming the intermittency of wind, as winds at sea are more constant and stronger, making the power supply more consistent. Offshore wind projects can positively affect species like mussels that adhere to the underwater platforms. These projects can be a haven for some marine life because fishing and bottom trawling are not permitted in the region of wind farms.

Climate change is already changing the migration patterns of many species making it hard for them to find suitable environments. A strategic approach that considers ecosystem protection will allow wind farms to remain a significant clean energy source in our arsenal of tools to fight climate change.

Explore wind turbines in 3D.

Wind turbines.
Credit: majeczka, Adobe Stock

Competent Cells

NEB® has a competent cell strain for your needs.

Choose the right cells for your cloning and protein expression applications from NEB's portfolio of high efficiency competent cell strains.

For cloning experiments, choose from a variety of formats, including chemical and electrocompetent. These *E. coli* strains are T1 phage resistant and are Endonuclease I-deficient for high-quality plasmid preparations. Additionally, all competent cells from NEB are free of animal products.

NEB also offers a wide variety of competent cell strains ideal for many protein expression applications. These strains address the needs of difficult protein expression control, toxic protein expression and cytoplasmic disulfide bond formation. NEBExpress®, T7 Express and SHuffle® strains are available with varying levels of control. I^q strains feature added control from increased supply of Lac repressor (*lacI*^{q}). Only NEB offers the exceptional control of expression from the *lysY* gene that reduces basal expression from T7 strains without inhibiting IPTG-induced expression. Our Lemo21(DE3) strain features tunable T7 expression for difficult targets. Each strain is provided with a detailed protocol for optimal expression.

NO DRY ICE CHARGES
with Competent Cells from NEB

Featured Products

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Visit **www.neb.com** to find additional online tools, including our Competitor Cross-reference Tool for comparing NEB strains to other commercially available strains.

Find tips for successful transformation.

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Cloning Strains

NEB Cloning Competent *E. coli* Sampler 246
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Protein Expression Strains

BL21 Competent *E. coli* 249
BL21(DE3) Competent *E. coli* 249
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NEBExpress Competent *E. coli* (High Efficiency) 250
NEBExpress I^q Competent *E. coli* (High Efficiency) 250
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SHuffle Express Competent *E. coli* 252
SHuffle T7 Express Competent *E. coli* 252
SHuffle T7 Competent *E. coli* 253
SHuffle T7 Express *lysY* Competent *E. coli* 253

Competent Cell Strain Properties

There are many properties to consider when choosing a strain for your experiments. Requirements such as plasmid preparation, blue/white screening, proper disulfide bond formation and fast colony growth necessitate specific strain choices. The following selection chart highlights the characteristics of NEB's strains to help select the optimal strain for a particular experiment.

CAUTION: Chemically Competent *E. coli* contain DMSO, a hazardous material. Review the MSDS before handling.

Cloning Strain Properties

Strain Properties	Features	Transformation Efficiency (cfu/µg)		Available Formats [7]	Outgrowth Medium & Control Plasmid Included?	Strain Background	Library Construction
		Chemical	Electrocompetent				
dam⁻/dcm⁻	• Dam/Dcm methyltransferase free plasmid growth	1-3 x 10^6	N/A	50, 200	●	K12	
NEB Turbo (High Efficiency)	• Fastest growth – colonies visible after 6.5 hours • Plasmid preparation after 4 hours	1-3 x 10^9	N/A	50, 200	●	K12	●
NEB 5-alpha (High Efficiency)	• Versatile cloning strain • DH5α™ derivative	1-3 x 10^9 [8]	N/A	50, 200, 96, 384, Strips	●	K12	●
NEB 5-alpha F´ *I*^{q} (High Efficiency)	• Toxic gene cloning • F´ strain with extremely high transformation efficiency	1-3 x 10^9	N/A	50, 200	●	K12	●
NEB 10-beta (High Efficiency)	• Large plasmid and BAC cloning • DH10B™ derivative	1-3 x 10^9 [9]	> 2 x 10^{10}	50, 200, 96	●	K12	●
NEB Stable (High Efficiency)	• Cloning unstable inserts • Isolating and propagating retroviral/ lentiviral clones	1-3 x 10^9	N/A	50, 200	●	K12	●
NEB 5-alpha (Subcloning Efficiency)	• Ideal for subcloning efficiency transformations, such as plasmid transformation or routine subcloning	> 1 x 10^6	N/A	400		K12	

Protein Expression Strain Properties

Strain Properties	Features	Chemical Transformation Efficiency (cfu/µg)	Available Formats [7]	Outgrowth Medium & Control Plasmid Included?	Strain Background	Library Construction
NEBExpress	• Versatile non-T7 expression strain • Protease deficient	0.6-1 x 10^9	50, 200	●	B	●
BL21(DE3)	• Routine T7 expression	1-5 x 10^7	50, 200	●	B	
Lemo21(DE3)	• Tunable T7 expression for difficult targets	1-3 x 10^7	50	● [1]	B	
NiCo21(DE3)	• Improved purity of target proteins isolated by IMAC	1-5 x 10^7	50	●	B	
BL21	• Routine non-T7 expression	1-5 x 10^7	50	●	B	
T7 Express	• Most popular T7 expression strain • Protease deficient	0.6-1 x 10^9	50, 200	●	B	●
T7 Express *lysY*	• T7 expression • Protease deficient • Better reduction of basal expression	0.6-1 x 10^9	200		B	●
T7 Express *lysY/I*^{q}	• T7 expression • Protease deficient • Highest level of expression control	0.6-1 x 10^9	200		B	●
SHuffle T7	• T7 expression/K12 strain • Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm	1 x 10^6	50		K12	
SHuffle Express	• Protease deficient/B strain • Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm	1 x 10^7	50		B	
SHuffle T7 Express	• T7 expression • Protease deficient/B strain • Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm	1 x 10^7	50		B	
SHuffle T7 Express *lysY*	• T7 expression • Protease deficient/B strain • Tightly controlled expression of toxic proteins • Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm	1 x 10^7	50		B	
NEBExpress *I*^{q}	• Control of IPTG induced expression from P_{lac}, P_{lac}, P_{trc} and $T5_{lac}$ • Protease deficient	0.6-1 x 10^9	200		B	●

	lacI^q	F´	*endA*$^-$ (2)	*recA*$^-$	Blue/White Screening	Drug Resistance (5)	Methylation Phenotype
			•			cam, str, nit	*Dam*$^-$, *Dcm*$^-$, M. EcoKI$^+$
	•	•	•		•	nit	*Dam*$^+$, *Dcm*$^+$, M. EcoKI$^-$
			•	•	•	none	*Dam*$^+$, *Dcm*$^+$, M. EcoKI$^+$
	•	•	•	•	•	tet	*Dam*$^+$, *Dcm*$^+$, M. EcoKI$^+$
			•	•	•	str	*Dam*$^+$, *Dcm*$^+$, M. EcoKI$^-$
	•	•	•	•	•	tet, str	*Dam*$^+$, *Dcm*$^+$, M. EcoKI$^-$
			•	•	•	none	*Dam*$^+$, *Dcm*$^+$, M. EcoKI$^+$

(1) Rhamnose solution is provided instead of SOC; control plasmid is included.
(2) Important for high-quality plasmid preparation.
(3) Lacks Lon and OmpT protease activity.
(4) Constitutively expresses a chromosomal copy of the disulfide bond isomerase DsbC.
(5) nit = nitrofurantoin, tet = tetracycline, cam = chloramphenicol, str = streptomycin, spec = spectinomycin
(6) Resistance to low levels of streptomycin may be observed.
(7) 50 = 50 µl tubes
200 = 200 µl tubes
96 = 96 well plate
384 = 384 well plate
strips = 96 tube strips (50 µl/tube)
400 = 400 µl tubes
(8) 1-5 x 10^8 for R-format.
(9) 1-3 x 10^8 for P-format.

	lacI^q	F´	*endA*$^-$	*lysY*	Protease Deficient (3)	T7 RNA Polymerase	Cytoplasmic Disulfide Bond Formation (4)	Drug Resistance (5)	Methylation Phenotype
			•		•			nit	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
					•	•		none	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
				•	•	•		cam	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
					•	•		none	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
					•			none	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
			•		•	•		nit	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
			•	•	•	•		cam, nit	*Dam*$^+$, *Dcm*$^-$, M. EcoKI$^-$
	•		•	•	•	•		cam, nit	*Dam*$^+$, Dcm$^-$, M. EcoKI$^-$
	•	•				•	•	str, spec, nit	*Dam*$^+$, *Dcm*$^+$, M. EcoKI$^+$
	•		•		•		•	spec(6), nit	Dam$^+$, *Dcm*$^-$, M. EcoKI$^-$
	•		•		•	•	•	spec(6), nit	Dam$^+$, *Dcm*$^-$, M. EcoKI$^-$
	•		•	•	•	•	•	cam, spec(6), nit	Dam+, *Dcm*$^-$, M. EcoKI–
	•		•		•			cam, nit	Dam$^+$, *Dcm*$^-$, M. EcoKI$^-$

Competitor Cross Reference

Using another competent cell strain? Try our **Competitor Cross Reference Tool** to find out which NEB strain is compatible.

NEBcloner®

For help with choosing the right competent cell strain, try **NEBcloner at NEBcloner.neb.com**.

Learn how to perform a transformation.

NEB Cloning Competent *E. coli* Sampler

#C1010S 8 x 0.05 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Outgrowth medium and control plasmid included*
- *Value pricing*
- *Free of animal products*

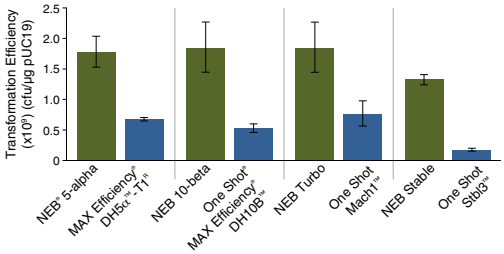
Description: A sample pack of four cloning strains of *E. coli* suitable for high efficiency transformation.

Please refer to the individual datacards for each reagent's recommended use and storage conditions.

Transformation Efficiency: 1-3 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 *(fhuA2)*

Sensitivity: Nit, Spec



Benefit from high transformation efficiencies. *Transformation efficiencies were compared using manufacturers' recommended protocols. Values shown are the average of triplicate experiments.*

Kit Includes:

- NEB 5-alpha Competent *E. coli* (High Efficiency)
- NEB 10-beta Competent *E. coli* (High Efficiency)
- NEB Stable Competent *E. coli* (High Efficiency)
- NEB Turbo Competent *E. coli* (High Efficiency)
- SOC Outgrowth Medium
- NEB 10-beta/Stable Outgrowth Medium
- pUC19 Vector

NEB® Turbo Competent *E. coli* (High Efficiency)

#C2984H 20 x 0.05 ml
#C2984I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Tight expression control ($lacI^q$)*
- *Colonies visible after 6.5 hours*
- *Isolate DNA after 4 hrs growth*
- *5 minute transformation protocol with Amp^R plasmids*
- *Free of animal products*

Description: *E. coli* cells featuring fast colony growth (6.5 hours) and tight expression control.

Genotype: F' *$proA^+B^+$ $lacI^q$ ΔlacZM15 / fhuA2 Δ(lac-proAB) glnV galK16 galE15 R(zgb-210::Tn10)*Tet^S *endA1 thi-1 Δ(hsdS-mcrB)5*

Features:

- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Suitable for blue/white screening

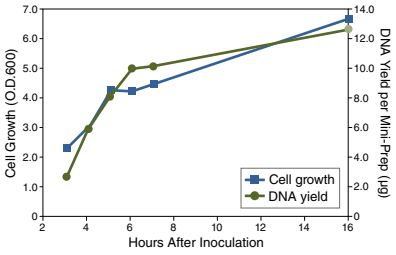
Transformation Efficiency:
1 - 3 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 *(fhuA2)*, Nit

Sensitivity: Amp, Cam, Kan, Spec, Str, Tet

Reagents Supplied:

- pUC19 Vector
- SOC Outgrowth Medium



Miniprep DNA can be prepared from a single overnight colony after inoculation and only 3 hours growth. *DNA yield doubles after an additional hour of growth.*

NEB Turbo Transformation: *With NEB Turbo, colonies are visible after only 8 hours. Ligation products were transformed into 50 µl of NEB Turbo Competent E. coli and plated on LB/Amp. Plates were incubated for 8 hours, 10 hours and 12 hours at 37°C. NEB Turbo features fast colony growth and blue/white selection to simplify cloning experiments.*

What is the difference between chemical transformation and electroporation?

NEB 10-beta Competent *E. coli*

NEB 10-beta Competent *E. coli* (High Efficiency)
#C3019H 20 x 0.05 ml
#C3019I 6 x 0.2 ml
#C3019P 1 96-well plate

NEB 10-beta Electrocompetent *E. coli*
#C3020K 6 x 0.1 ml

Companion Product:
NEB 10-beta/Stable Outgrowth Medium
#B9035S 100 ml

- *Clone large plasmids and BACs*
- *DH10B derivative*
- *Free of animal products*

Description: A DH10B derivative suitable for a wide range of applications, including large plasmid and BAC cloning.

Genotype: *Δ(ara-leu) 7697 araD139 fhuA ΔlacX74 galK16 galE15 e14- Φ80dlacZΔM15 recA1 relA1 endA1 nupG rpsL (Str^R) rph spoT1 Δ(mrr-hsdRMS-mcrBC)*

Features:

- Efficient transformation of methylated DNA derived from eukaryotic sources or unmethylated DNA derived from PCR, cDNA and many other sources
- Suitable for blue/white screening without IPTG
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Reduced recombination of cloned DNA (*recA1*)

Transformation Efficiency: High Efficiency: 1–3 x 10^9 cfu/µg pUC19 DNA (NEB #C3019H, #C3019I); 1–3 x 10^8 cfu/µg pUC19 DNA (NEB #C3019P)

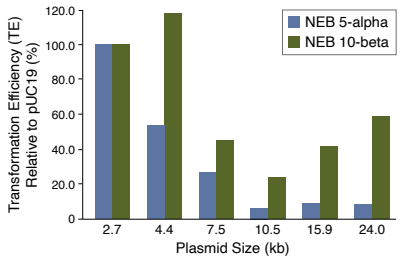
Electrocompetent: > 2 x 10^{10} cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 (*fhuA*), Str

Sensitivity: Amp, Cam, Kan, Nit, Spec, Tet

Reagents Supplied:

- NEB 10-beta/Stable Outgrowth Medium
- pUC19 Control DNA



Effect of Plasmid Size on Transformation Efficiency. *NEB 10-beta chemically competent cells are more efficiently transformed with large plasmids than NEB 5-alpha cells. The difference in TE between the two cell lines increases with the size of the plasmid being transformed.*

NEB 5-alpha Competent *E. coli*

NEB 5-alpha Competent *E. coli* (High Efficiency)
#C2987H 20 x 0.05 ml
#C2987I 6 x 0.2 ml
#C2987P 1 96-well plate
#C2987R 1 384-well plate
#C2987U 96 x 0.05 ml

NEB 5-alpha Competent *E. coli* (Subcloning Efficiency)
#C2988J 6 x 0.4 ml

Companion Product:
SOC Outgrowth Medium
#B9020S 100 ml

- *DH5α derivative*
- *Free of animal products*

Description: A DH5a derivative and versatile *E. coli* cloning strain.

Genotype: *fhuA2Δ(argF-lacZ) U169 phoA glnV44 Φ80Δ(lacZ) M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*

Features:

- Efficient transformation of unmethylated DNA derived from PCR, cDNA and many other sources (*hsdR*)
- Suitable for blue/white screening
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Reduced recombination of cloned DNA (*recA1*)

Transformation Efficiency: High Efficiency: 1–3 x 10^9 cfu/µg pUC19 DNA (NEB #C2987H, #C2987I, #C2987P, #C2987U); 1–5 x 10^8 cfu/µg pUC19 DNA (NEB #C2987R)

Subcloning Efficiency: > 1 x 10^6 cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 (*fhuA2*)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:

SOC Outgrowth Medium

pUC19 Control DNA

* NEB 5-alpha Competent *E. coli* (Subcloning Efficiency) is not supplied with SOC Outgrowth Medium or pUC19 Control DNA.

NEB® 5-alpha F'I^q Competent *E. coli* (High Efficiency)

#C2992H 20 x 0.05 ml
#C2992I 6 x 0.2 ml

Companion Product:
SOC Outgrowth Medium
#B9020S 100 ml

- *Tight expression control (lacI^q)*
- *F´ Strain with extremely high TE*
- *DH5α derivative*
- *Free of animal products*

Description: An F´ *E. coli* strain with extremely high transformation efficiency suitable for toxic gene cloning.

Genotype: F´ *proA$^+$B$^+$ lacI^q Δ(lacZ)M15 zzf::Tn10 (Tet^R) / fhuA2Δ(argF-lacZ)U169 phoA glnV44 Φ80Δ(lacZ) M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17*

Features:

- Efficient transformation of unmethylated DNA derived from PCR, cDNA and many other sources (*hsdR*)
- Suitable for blue/white screening
- Activity of nonspecific endonuclease I (*endA1*) eliminated for highest quality plasmid preparations
- Reduced recombination of cloned DNA (*recA1*)
- Suitable for propagation of M13 clones

Transformation Efficiency:
1-3 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 *(fhuA2),* Tet

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str

Reagents Supplied:

- SOC Outgrowth Medium
- pUC19 Vector

NEB® Stable Competent *E. coli* (High Efficiency)

#C3040H 20 x 0.05 ml
#C3040I 6 x 0.2 ml

Companion Product:

NEB 10-beta/Stable Outgrowth Medium
#B9035S 100 ml

- *T1 phage resistance (fhuA)*
- *Free of animal products*
- *Carries endA mutation (isolated plasmids are free of EndoI)*
- *Ideal for cloning unstable inserts, as well as isolating and propagating retroviral/ lentiviral clones*
- *Compatible with DNA assembly reactions and ligation reactions*

Description: Chemically competent *E. coli* cells suitable for high efficiency transformation and isolation of plasmid clones containing repeat elements.

Genotype: F' *proA*$^{+}$*B*$^{+}$ *lacI*^{q} *Δ(lacZ)M15 zzf::Tn10* (Tet^{R})*/Δ(ara-leu) 7697 araD139 fhuA ΔlacX74 galK16 galE15 e14- Φ80dlacZΔM15 recA1 relA1 endA1 nupG rpsL* (Str^{R}) *rph spoT1 Δ(mrr-hsdRMS-mcrBC)*

Features:

- Activity of nonspecific endonuclease I (*endA1*) abolished for highest quality plasmid preparations
- Reduced recombination of cloned DNA (*recA1*)

Transformation Efficiency:
1-3 x 10^{9} cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 *(fhuA),* Str, Tet

Sensitivity: Amp, Cam, Kan, Nit, Spec

Reagents Supplied:

- NEB 10-beta/Stable Outgrowth Medium
- pUC19 Vector

dam^{-}*/dcm*$^{-}$ Competent *E. coli*

#C2925H 20 x 0.05 ml
#C2925I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Isolate plasmids free of Dam and Dcm methylation*
- *Free of animal products*

Description: Methyltransferase deficient *E. coli* cells suitable for growth of plasmids free of *Dam* and *Dcm* methylation.

Genotype: *ara-14 leuB6 fhuA31 lacY1 tsx78 glnV44 galK2 galT22 mcrA dcm-6 hisG4 rfbD1 R(zgb210::Tn10)* Tet^{S} *endA1 rspL136* (Str^{R}) *dam13::Tn9* (Cam^{R}) *xylA-5 mtl-1 thi-1 mcrB1 hsdR2*

Features:

- Allows for propagation of plasmids free of *Dam* and *Dcm* methylation
- Activity of nonspecific endonuclease I (*endA1*) abolished for highest quality plasmid preparations

Transformation Efficiency:
1-3 x 10^{6} cfu/µg pUC19 DNA

Resistance: Phage T1 resistant (*fhuA31*), Cam, Nit, Str

Sensitivity: Amp, Kan, Spec, Tet

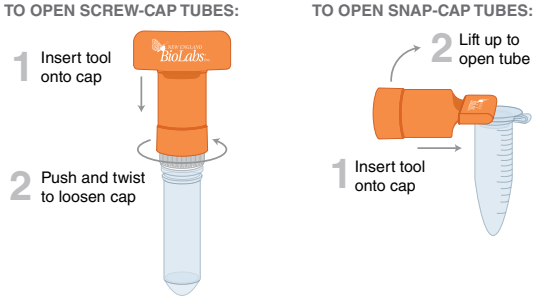
Reagents Supplied:

- pUC19 Vector
- SOC Outgrowth Medium

NEB Tube Opener

#C1008S 2 Each

Description: Use to open a variety of microcentrifuge tubes. Can be used for snap-on caps or screw-on caps.



BL21 Competent *E. coli*

#C2530H 20 x 0.05 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Ideal for P_{lac}, P_{tac}, P_{trc}, ParaBAD expression vectors*
- *Resistance to phage T1 (fhuA2)*
- *Protease deficient B strain*
- *Free of animal products*

Description: Widely used non-T7 expression *E. coli* strain. Suitable for transformation and protein expression. This strain does not express the T7 RNA Polymerase.

Genotype: *fhuA2 [lon] ompT gal [dcm] ΔhsdS*

Features:

- Deficient in proteases Lon and OmpT

Transformation Efficiency:
1–5 x 10^7 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 (*fhuA2*)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:

- pUC19 Vector
- SOC Outgrowth Medium

BL21(DE3) Competent *E. coli*

#C2527H 20 x 0.05 ml
#C2527I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Routine T7 expression*
- *Resistance to phage T1 (fhuA2)*
- *Protease deficient B strain*
- *Free of animal products*

Description: Widely used T7 expression *E. coli* strain.

Genotype: *fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5*

Features:

- Deficient in proteases Lon and OmpT
- Resistant to phage T1 (*fhuA2*)

Transformation Efficiency:
1–5 x 10^7 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 (*fhu*A2)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:

- SOC Outgrowth Medium
- pUC19 Vector

Lemo21(DE3) Competent *E. coli*

#C2528J 12 x 0.05 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Expression of difficult targets*
- *Membrane protein expression*
- *Ideal for periplasmic expression*
- *Expression of toxic proteins*
- *Proteins with solubility issues*

Description: Lemo21(DE3) Competent *E. coli* is a tunable T7 expression strain designed for the expression of challenging proteins. A derivative of BL21(DE3), Lemo21(DE3) offers the host features of this popular expression strain, with the added benefit of being able to control expression levels by varying the level of T7 lysozyme (*lysY*), the natural inhibitor of T7 RNA Polymerase. The fine control of expression makes Lemo21(DE3) ideal for membrane proteins, toxic proteins, secreted proteins and proteins prone to insoluble expression.

Genotype: *fhuA2 [lon] ompT gal (λ DE3) [dcm] ΔhsdS/* pLemo(Cam^R) *λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5*
pLemo = pACYC184-*PrhaBAD-lysY*

Features:

- Enhanced BL21(DE3) derivative
- Fine control of expression
- Greatest range of expression of any T7 strain (0-2,000 µM rhamnose)
- Potential elimination of inclusion body formation

Transformation Efficiency:
1–3 x 10^7 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 (fhuA2), Cam

Sensitivity: Amp, Kan, Nit, Spec, Str, Tet

Reagents Supplied:

- pUC19 Vector
- L-rhamnose solution

NiCo21(DE3) Competent *E. coli*

#C2529H 20 x 0.05 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Superior alternative to BL21(DE3) for routine protein expression*
- *Improved purity of target proteins isolated by IMAC*
- *Free of animal products*

Description: Poly-histidine tagged recombinant proteins that are isolated by immobilized metal affinity chromatography (IMAC) are often contaminated with significant amounts of endogenous *E. coli* metal binding proteins. The protein expression strain NiCo21(DE3) has been engineered to minimize *E. coli* protein contamination of IMAC fractions: GlmS is mutated to eliminate binding to IMAC resins and three other proteins (SlyD, ArnA and Can) are tagged to enable rapid removal by chitin affinity chromatography.

Genotype: *can::CBD fhuA2 [lon] ompT gal (λ DE3) [dcm] arnA::CBD slyD::CBD glmS6Ala ΔhsdS λ DE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5*

Features:
- Identical growth characteristics as BL21(DE3)
- Deficient in proteases Lon and OmpT

Transformation Efficiency:
1–5 x 10^7 cfu/µg pUC19

Resistance: Resistant to phage T1 (*fhuA2*)

Sensitivity: Amp, Cam, Kan, Nit, Spec, Str, Tet

Reagents Supplied:
- pUC19 Vector
- SOC Outgrowth Medium

NEBExpress® Competent *E. coli* (High Efficiency)

#C2523H 20 x 0.05 ml
#C2523I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Enhanced BL21 derivative ideal for P_{lac}, P_{tac}, P_{trc} expression vectors*
- *Fast growth from colonies*
- *Free of animal products*
- *Protease deficient*

Description: A versatile non-T7 expression *E. coli* strain. NEBExpress is the recommended host strain for the NEBExpress MBP Protein Fusion and Purification System (NEB #E8201).

Genotype: *fhuA2 [lon] ompT gal sulA11 R(mcr-73::miniTn10--Tet^S)2 [dcm] R(zgb-210::Tn10--Tet^S) endA1 Δ(mcrC-mrr)114::IS10*

Features:
- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA

Transformation Efficiency:
0.6-1 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 *(fhuA2),* Nit

Sensitivity: Amp, Cam, Kan, Spec, Str, Tet

Reagents Supplied:
- SOC Outgrowth Medium
- pUC19 Vector

NEBExpress® I^q Competent *E. coli* (High Efficiency)

#C3037I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Enhanced BL21 derivative ideal for P_{lac}, P_{tac}, P_{trc}, P_{T5} expression vectors*
- *Better control of IPTG induced expression with non-T7 plasmids*
- *Fast growth from colonies*
- *$lacI^q$ reduces basal expression*
- *Protease deficient*
- *Free of animal products*

Description: *E. coli* cells featuring control of IPTG induced expression with non-T7 plasmids.

Genotype: MiniF *$lacI^q$* (Cam^R) / *fhuA2 [lon] ompT gal sulA11 R(mcr-73::miniTn10--Tet^S)2 [dcm] R(zgb-210::Tn10--Tet^S) endA1 Δ(mcrC-mrr)114::IS10*

Features:
- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA
- Ideal for controlled protein expression from pUC19 and pUC19 derivatives

Transformation Efficiency:
0.6-1 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 *(fhuA2),* Cam, Nit

Sensitivity: Amp, Kan, Spec, Str, Tet

T7 Express Competent *E. coli* (High Efficiency)

#C2566H 20 x 0.05 ml
#C2566I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Enhanced BL21 derivative*
- *Popular T7 expression strain*
- *Fast growth from colonies*
- *Free of animal products*

Description: Enhanced BL21 *E. coli* derivative for T7 expression.

Genotype: *fhuA2 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10--*Tet^S*) endA1 Δ(mcrC-mrr)114::IS10*

Features:
- T7 RNA Polymerase in the *lac* operon - no λ prophage
- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA

Transformation Efficiency:
0.6-1 x 10^9 cfu/µg pUC19

Resistance: Resistant to phage T1 *(fhuA2),* Nit

Sensitivity: Amp, Cam, Kan, Spec, Str, Tet

Reagents Supplied:
- pUC19 Vector
- SOC Outgrowth Medium

T7 Express *lysY* Competent *E. coli* (High Efficiency)

#C3010I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Enhanced BL21 derivative*
- *T7 Lysozyme for expression control*
- *Clone toxic genes*
- *Fast growth from colonies*
- *Free of animal products*

Description: Enhanced BL21 *E. coli* derivative for T7 expression with enhanced reduction of basal expression.

Genotype: MiniF *lysY* (Cam^R) */ fhuA2 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10--*Tet^S*) endA1 Δ(mcrC-mrr)114::IS10*

Features:
- T7 RNA Polymerase in the *lac* operon - no λ prophage
- Control of T7 RNA Polymerase by T7 lysozyme allows potentially toxic genes to be expressed
- LysY is a variant of T7 lysozyme lacking amidase activity, thus cells are not susceptible to lysis during induction
- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA
- No Cam requirement

Transformation Efficiency:
0.6-1 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 *(fhuA2),* Cam, Nit

Sensitivity: Amp, Kan, Spec, Str, Tet

T7 Express *lysY/I^q* Competent *E. coli* (High Efficiency)

#C3013I 6 x 0.2 ml

Companion Product:

SOC Outgrowth Medium
#B9020S 100 ml

- *Enhanced BL21 derivative*
- *Tight control of expression (lacI^q)*
- *Highest level of expression control*
- *Clone toxic genes*
- *Fast growth from colonies*
- *Free of animal products*

Description: Enhanced BL21 *E. coli* derivative with highest level of T7 expression control.

Genotype: MiniF *lysY lacI^q*(Cam^R) */ fhuA2 lacZ::T7 gene1 [lon] ompT gal sulA11 R(mcr-73::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10--*Tet^S*) endA1 Δ(mcrC-mrr) 114::IS10*

Features:
- T7 RNA Polymerase in the *lac* operon - no λ prophage
- Tight control of expression by *lacI^q* allows potentially toxic genes to be cloned
- Control of T7 RNA Polymerase by T7 lysozyme allows toxic genes to be expressed
- LysY is a variant of T7 lysozyme lacking amidase activity, thus cells are less susceptible to lysis during induction
- Deficient in proteases Lon and OmpT
- Does not restrict methylated DNA
- No Cam requirement

Transformation Efficiency:
0.6-1 x 10^9 cfu/µg pUC19 DNA

Resistance: Resistant to phage T1 *(fhuA2),* Cam, Nit

Sensitivity: Amp, Kan, Spec, Str, Tet

Disulfide Bonds

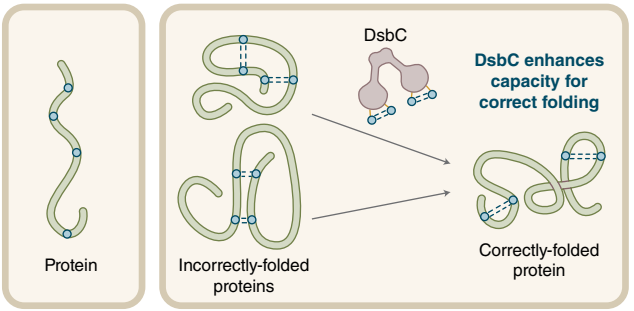
Features of Shuffle® Strains:

- Engineered *E. coli* K12 or B strains promote disulfide bond formation in the cytoplasm
- Constitutively expresses a chromosomal copy of the disulfide bond isomerase DsbC
- DsbC promotes the correction of mis-oxidized proteins into their correct form
- The cytoplasmic DsbC is a chaperone that can also assist in the folding of proteins that do not require disulfide bonds
- Alternative expression strain for proteins that do not fold in wild-type *E. coli*, independent of redox state

SHuffle strains from NEB are engineered *E. coli* strains capable of expressing proteins with increasing disulfide bond complexity in the cytoplasm. SHuffle strains express the disulfide bond isomerase DsbC within the cytoplasm. DsbC isomerizes mis-oxidized substrates into their correctly folded state greatly enhancing the fidelity of disulfide bond formation. Cytoplasmic expression also results in significantly higher protein yields of disulfide bonded proteins when compared to periplasmic expression. SHuffle strains are sensitive to kan, amp, tet and in most cases, cam, which makes them able to express proteins from a wide variety of expression vectors offering greater versatility in experimental design.

References:

References for properties and applications for these products can be found at www.neb.com.



Disulfide bond formation in the cytoplasm of wild type E. coli is not favorable, while SHuffle is capable of correctly folding proteins with multiple disulfide bonds in the cytoplasm.

SHuffle® Express Competent *E. coli*

#C3028J 12 x 0.05 ml

- *Folds disulfide bonded proteins in the cytoplasm*
- *Protease deficient*
- *Enhanced BL21 derivative*
- *Free of animal products*

Description: *E. coli* cells with enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm.

Genotype: *fhuA2 [lon] ompT ahpC gal* λatt::pNEB3-r1-*cDsbC* (Spec^R, *lacI*^{q}) *ΔtrxB sulA11* R(*mcr-73::miniTn10--*Tet^S)*2 [dcm] R(zgb-210::Tn10 --*Tet^S) *endA1 Δgor Δ(mcrC-mrr)114::IS10*

Transformation Efficiency:

1 x 10^7 cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 (*fhuA2*), Nit, Spec and Str*.

*Note that resistance to low levels of streptomycin may be observed.

Sensitivity: Amp, Cam, Kan, Tet

SHuffle® T7 Express Competent *E. coli*

#C3029J 12 x 0.05 ml

- *Folds disulfide bonded proteins in the cytoplasm*
- *T7 expression*
- *Protease deficient B strain*
- *Enhanced BL21 derivative*
- *Free of animal products*

Description: T7 Expression *E. coli* strain with enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm.

Genotype: *fhuA2 lacZ::T7 gene1 [lon] ompT ahpC gal λatt::pNEB3-r1-cDsbC* (Spec^R, *lacI*^{q}) *ΔtrxB sulA11 R(mcr-73::miniTn10--*Tet^S*)2 [dcm] R(zgb-210::Tn10 --*Tet^S*) endA1 Δgor Δ(mcrC-mrr)114::IS10*

Transformation Efficiency:

1 x 10^7 cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 (*fhuA2*), Nit, Spec and Str*.

*Note that resistance to low levels of streptomycin may be observed.

Sensitivity: Amp, Cam, Kan, Tet

What is a disulfide bond?

SHuffle® T7 Competent *E. coli*

#C3026J 12 x 0.05 ml

- *Folds disulfide bonded proteins in the cytoplasm*
- *T7 expression*
- *K12 strain*
- *Free of animal products*

Description: T7 Expression *E. coli* strain with enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm.

Genotype: F´ *lac, pro, lacI*^{q} */ Δ(ara-leu)7697 araD139 fhuA2 lacZ::T7 gene1 Δ(phoA)PvuII phoR ahpC* galE (or U) galK λatt::pNEB3-r1-cDsbC* (Spec^{R}, *lacI*^{q}) *ΔtrxB rpsL150*(Str^{R}) *Δgor Δ(malF)3*

Transformation Efficiency:
1 x 10^{6} cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 *(fhuA2)*, Nit, Str, Spec

Sensitivity: Amp, Cam, Kan, Tet

SHuffle® T7 Express *lysY* Competent *E. coli*

#C3030J 12 x 0.05 ml

- *Express toxic proteins (lysY)*
- *Folds disulfide bonded proteins in the cytoplasm*
- *T7 expression*
- *Protease deficient B strain*
- *Free of animal products*
- *Enhanced BL21 derivative*

Description: *E. coli* strain with tight T7 Expression control and enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm.

Genotype: MiniF *lysY* (Cam^{R}) */ fhuA2 lacZ::T7 gene1 [lon] ompT ahpC gal λatt::pNEB3-r1-cDsbC* (Spec^{R}, *lacI*^{q}) *ΔtrxB sulA11 R(mcr-73::miniTn10--*Tet^{S}*)2 [dcm] R(zgb-210::Tn10 --*Tet^{S}*) endA1 Δgor Δ(mcrC-mrr)114::IS10*

Transformation Efficiency:
1 x 10^{7} cfu/µg pUC19 DNA

Resistance: Resistance to phage T1 (*fhuA2*), Cam, Nit, Spec and Str*.

*Note that resistance to low levels of streptomycin may be observed.

Sensitivity: Amp, Kan, Tet

How do I express my protein in SHuffle cells?

Currently there are two SHuffle cell lines available from NEB; SHuffle (NEB #C3026) based on *E. coli* K12, and SHuffle Express (NEB #C3028, #C3029, #C3030) based on *E. coli* B.

We recommend testing both B and K12 expression strains, as we do see variability in expression depending on the protein of interest (see table). If T7 expression is not necessary, then we recommend comparing NEB #C3026 and #C3028. If T7 expression is necessary, test NEB #C3026 and #C3029. If T7-driven expression of a protein is toxic, switch to a non-leaky lysY version (NEB #C3030). Once the strain is chosen expression conditions should be optimized. This can include temperature as well as auto expression (1).

Reference:
(1) Ke, N. and Berkmen, M. (2014) *Current Protocols Molecular Biology* 16.1B.21.

View our online tutorial for tips on setting up reactions with SHuffle.

Percentage of relative solubility of various proteins using SHuffle (K12 and B strains):

Protein	Relative % Soluble		# Cysteines
	K12	B	
Gluc	65	100	10
Urokinase	60	100	24
vtPA	5	100	12
BSA	100	0	35
Polymerase	100	0	0
Nuclease	100	10	4

Results are determined based on protein levels detected by SDS-PAGE (not shown)

Memo is a Senior Research Scientist and has been with NEB for 16 years. Learn more about Memo's role at NEB and his love of bacterial art in his video reel.

#NEBiographies

Food transport versus food choices

What makes a diet eco-friendly? Where the food originated, or the food type? The science of diet sustainability connects our eating habits to environmental conservation and human health. Research reports on food choice and food transport have both received attention in the public sphere. Regrettably, an either/or fallacy on their use in decision-making can frustrate individuals seeking to adopt a sustainable diet. An integrated approach is perhaps the best choice when making diet decisions.

The slogan “Buy Local” emphasizes food miles in sustainable diets, but it’s not the whole story. Food miles are the geographical distance a food item travels from producer to consumer. It’s been estimated that 19% of overall food-system emissions result from transport alone. Fruit and vegetable transport typically generates greater emissions than from farming itself. However, “Buy Local” is not an absolute principle. It’s a generalization that shorter transports always incur lower greenhouse gas emissions (GHG). Localized food production can sometimes be worse for the environment. Food from a local farm does not necessarily have a lower carbon footprint. Geographically distant farms can compensate for transport emissions with mitigations like winter cover cropping and/or reducing tillage intensity. On balance, how farms operate carries more weight than food miles.

Dietary choices have more influence overall than transport in food carbon footprints. Food systems generate approximately 34% of global GHG emissions. It’s been estimated that beef, milk, rice, maize, wheat, pork and poultry are responsible for 80% of those emissions. Beef is scrutinized for the highest emissions and least calories per agricultural land use. Studies conflict on the environmental superiority between conventional and grass-fed beef.

To provide guidance on diet shifts, the EAT-Lancet Commission reported on how we produce, transport, consume and waste food planet-wide. A positive finding was that nutritious foods were more sustainable. Their recommended diet contains no refined grains, highly processed foods or added sugar. It sets a weekly goal for approximately 250 g of dairy, 200 g of poultry, 200 g of seafood and 100 g of beef, lamb or pork. Vegetables, fruit, grains, legumes, potatoes, and nuts constitute the main daily diet. This diet could help prevent 11 million adult deaths annually based on dietary risk factors. It reduces diet-related emissions dramatically. That said, diet diversity is recognized by researchers. Developed countries have higher meat consumption and therefore greater responsibility for carbon emissions. A striking environmental caveat is that if all countries adopted the EAT-Lancet diet, water use would decrease globally but would increase for nearly 40% of the global population who rely mainly on starchy root diets. Diet recommendations need to integrate local cultures and infrastructure. Food choices based on the EAT-Lancet offer a starting point.

There is considerable concern and confusion about sustainable diets but also enough evidence to discern positive health and environmental food choices. Integrating multifactorial data into conclusions on how food influences sustainability is challenging. News sources present the public with concerns that range from the ecological impacts of red meat to the formidable challenges involved in greening food transport. The food industry is responding with low carbon renewable fueled shipping vessels and agroecosystem sustainability. Notwithstanding the need for further study and debate, there is enough expert guidance available to begin cultivating sustainable diets now.

Explore food transport in 3D.

Potatoes in truck.
Credit: Clement, Adobe Stock

Glycobiology & Protein Analysis Tools

Trust NEB's expertise in enzymology when you need reagents for glycobiology and protein analysis.

Glycobiology

Proteomics, the systematic study of proteins in biological systems, has expanded the knowledge of protein expression, modification, interaction and function. However, in eukaryotic cells, the majority of proteins are post-translationally modified (1). A common post-translational modification, essential for cell viability, is the attachment of glycans. Glycosylation defines the adhesive properties of glycoconjugates, and it is largely through glycan–protein interactions that cell–cell and cell–pathogen (including viruses) contacts occur, a fact that accentuates the importance of glycobiology.

Glycomics, the study of glycan expression in biological systems, relies on effective enzymatic and analytical techniques for the correlation of glycan structure with function. Glycobiology is a small but rapidly growing field in biology, with relevance to biomedicine, biotechnology, biofuels and basic research. Glycan molecules modulate many other processes important for cell and tissue differentiation, metabolic and gene regulation, protein activity, protein clearance, transport and more (2-9).

Protein Analysis Tools

Not only are proteins a major structural component of living systems, they can also be effector molecules whose states determine downstream activities. Therefore, studying the protein complement within a cell can reveal the mechanisms behind many of the cell's responses to its environment. Given the vast number of applications for protein analysis, several tools and methods for its study exist; determining the correct method for your application is paramount to success.

Phage display technology is an *in vitro* screening technique for identifying ligands for proteins and other macromolecules. At the crux of phage display technology is the ability to express peptide or protein sequences as fusions to the coat proteins of a bacteriophage. Libraries of phage-displayed peptides or proteins are thereby physically linked to their encoding nucleic acid, allowing selection of binding partners for myriad target types by iterative rounds of in vitro panning and amplification, followed by DNA sequencing.

All NEB products pass stringent quality control assays to ensure the highest level of functionality and purity.

Featured Products

261 Rapid™ PNGase F

271 O-Glycoprotease (IMPa)

274 Thermolabile Proteinase K

Featured Tools & Resources

Protein Analysis Tools & Glycomics Overview

PNGase F Overview & Selection Chart

Visit **www.NEBglycosidase.com** to view our online tutorial on *N*- and *O*-linked glycosylation.

(1) Khoury G.A. et al. (2011) Scientific Reports 1: 90. PMID: 22034591.
(2) Varki A. (1993) Glycobiology. 3, 97–130. PMID: 8490246.
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RI Recombinant Enzyme

Glycosidases

- *Enabling Novel Technologies*
- *Unique Specifications*
- *Exceptional Value*
- *High Purity*

NEB offers a selection of endoglycosidases and exoglycosidases for glycobiology research. Many of these reagents are recombinant, and all undergo several quality control assays, enabling us to provide products with lower unit cost, high purity, and reduced lot-to-lot variation.

All of our glycosidases are tested for contaminants. Since *p*-nitrophenyl-glycosides are not hydrolyzed by some exoglycosidases, we use only fluorescently-labeled oligosaccharides to screen for contaminating glycosidases.

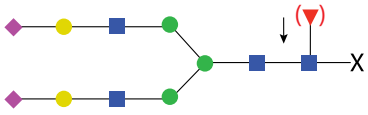
NEB's glycosidases are provided with 10X buffer to ensure optimal activity. Using more than one glycosidase simultaneously is a common timesaving procedure. Selecting the best buffer to provide reaction conditions that optimize enzyme activity is an important consideration.

Reaction Buffer Compositions:
Visit **www.NEBGlycosidase.com** for details.

Endo F2

NEBU Cloned at NEB RR 37° 65°

#P0772S 480 units



- *Removal of complex biantennary N-linked glycans from glycoproteins and glycopeptides*
- *Useful for determining N-glycosylation sites*

Description: Endo F2 is a highly specific recombinant endoglycosidase which cleaves within the chitobiose core of asparagine-linked complex biantennary and high mannose oligosaccharides from glycoproteins and glycopeptides. Endo F2 cleaves biantennary glycans at a rate approximately 20 times greater than high mannose glycans. The activity of Endo F2 is identical on biantennary structures with and without core fucosylation. However, Endo F2 is not active on hybrid or tri- and tetra-antennary oligosaccharides. Endo F2 is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol free for optimal performance in HPLC and MS intensive methods.

Source: Cloned from *Elizabethkingia miricola* (formerly *Flavobacterium meningosepticum*) and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 4, 37°C.
Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
• GlycoBuffer 4

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the carbohydrate from 10 µg Porcine Fibrinogen in 1 hour at 37°C in a total reaction volume of 10 µl..

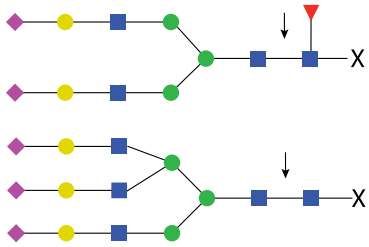
Molecular Weight: 39 kDa.

Concentration: 8,000 units/ml

Endo F3

NEBU Cloned at NEB RR 37° 65°

#P0771S 240 units



- *Removal of complex biantennary and triantennary N-linked glycans from glycoproteins and glycopeptides*
- *Useful for determining N-glycosylation sites*

Description: Endo F3 is a highly specific recombinant endoglycosidase which cleaves within the chitobiose core of asparagine-linked fucosylated-biantennary and triantennary complex oligosaccharides from glycoproteins. Endo F3 is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol free for optimal performance in HPLC and MS intensive methods.

Source: Cloned from *Elizabethkingia miricola* (formerly *Flavobacterium meningosepticum*) and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 4, 37°C.
Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:
• GlycoBuffer 4

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the carbohydrate from 10 µg Porcine Fibrinogen in 1 hour at 37°C in a total reaction volume of 10 µl.

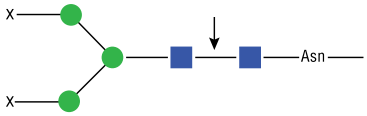
Molecular Weight: 38 kDa.

Concentration: 8,000 units/ml

Find an overview of glycobiology.

Endo D

#P0742S	1,500 units
#P0742L	7,500 units



X= (H or oligosaccharide)

- *Removal of paucimonnose N-linked glycans from glycoproteins and glycopeptides*
- *Useful for determining N-glycosylation sites*

Description: Endo D, also known as Endoglycosidase D, is a recombinant glycosidase, which cleaves within the chitobiose core of paucimannose *N*-linked glycans, with or without extensions in the antennae.

Endo D is tagged with a chitin binding domain (CBD) for easy removal from a reaction, and is supplied glycerol-free for optimal performance in HPLC and MS intensive methods.

Source: A truncated Endo D gene cloned from *Streptococcus pneumoniae* and expressed in *E. coli* as a fusion to chitin binding domain

Reaction Conditions: GlycoBuffer 2, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- DTT
- GlycoBuffer 2

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of glycosidase-trimmed (trimannosyl core) Fetuin in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 140,000 daltons.

Concentration: 50,000 units/ml

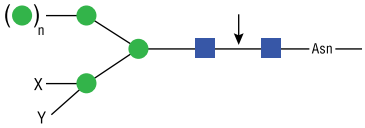
Endo H

Endo H

#P0702S	10,000 units
#P0702L	50,000 units

Endo H_f

#P0703S	100,000 units
#P0703L	500,000 units



Endo H and Endo Hf cleave only high mannose structures (n = 2–150, x = (Man)1–2, y = H) and hybrid structures (n = 2, x and/or y = AcNeu-Gal-GlcNAc).

- *Removal of high mannose N-glycans from glycoproteins*

Description: Endoglycosidase H is a recombinant glycosidase which cleaves within the chitobiose core of high mannose and some hybrid oligosaccharides from *N*-linked glycoproteins.

Endo Hf is a recombinant protein fusion of Endoglycosidase H and maltose binding protein. It has identical activity to Endo H.

Source: Endo H and Endo Hf have been cloned from *Streptomyces plicatus* and overexpressed in *E. coli*.

Reaction Conditions: Denature glycoprotein in 1X Glycoprotein Denaturing Buffer at 100°C for 10 minutes. Incubate in 1X GlycoBuffer 3 at 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- 10X Glycoprotein Denaturing Buffer
- 10X GlycoBuffer 3

Molecular Weight:

- Endo H: 29,000 daltons
- Endo Hf: 70,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: Endo H concentration: 500,000 units/ml, Endo H_f concentration: 1,000,000 units/ml

Note: Enzymatic activity is not affected by SDS. To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

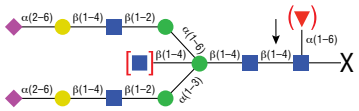
Max and Emily are new members of NEB's Small Scale Production Purification Department. Max began his career at NEB in 2022 as Production Scientist II and Emily joined NEB in 2023 as a Production Scientist I.

Gal Glc Man GalNAc GlcNAc Fuc NeuAc **R** = any sugar

Endo S

NEBU 37° 65°

#P0741S 6,000 units
#P0741L 30,000 units



- *Removal of N-glycans from native IgG*
- *Useful for determining N-glycosylation sites*

Description: Endo S is an endoglycosidase with a uniquely high specificity for removing *N*-linked glycans from the chitobiose core of the heavy chain of native IgG Endo S is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol-free for optimal performance in HPLC- and MS-intensive methods.

Source: Endo S is cloned from *Streptococcus pyogenes* and overexpressed as a fusion to the chitin binding domain in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Heat inactivation: 55°C for 10 minutes.

Reagents Supplied:

• GlycoBuffer 1

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 5 µg of native mouse monoclonal IgG in 1 hour at 37°C in a total reaction volume of 10 µl.

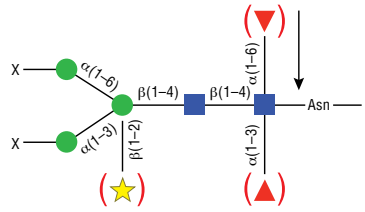
Molecular Weight: 136,000 daltons.

Concentration: 200,000 units/ml

PNGase A

NEBU 37° 65°

#P0707S 150 units
#P0707L 750 units



PNGase A hydrolyzes N-glycan chains from glycoproteins/peptides regardless of the presence of xylose or fucose. [x = H or Man or GlcNAc]

- *Removal of N-linked glycans from glycoproteins*

Description: PNGase A is a recombinant amidase, which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and short complex oligosaccharides such as those found in plant and insect cells from *N*-linked glycoproteins and glycopeptides. PNGase A differs from PNGase F in that it cleaves *N*-linked glycans with or without α(1,3)-linked core fucose residues.

Source: Cloned from *Oryza sativa* (rice) and expressed in *Pichia pastoris*.

Reaction Conditions: GlycoBuffer 3, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

• GlycoBuffer 3
• Glycoprotein Denaturing Buffer
• NP-40

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 1 µg of denatured recombinant Avidin produced in Maize in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 63 kDa.

Concentration: 5,000 units/ml

Note: PNGase A is active on both glycoproteins and glycopeptides. PNGase A cannot cleave larger *N*-glycans such as those from Fetuin, Fibrinogen, IgG, Lactoferrin and Transferrin. PNGase A is able to cleave high mannose *N*-glycan structures from Man 3 up to Man 9.

Tara has been with NEB for over 15 years, and began her career as Global Business Development Coordinator. Today, Tara is the Senior Manager of Project Management and Administration.

PNGase F & PNGase F, Recombinant

PNGase F
#P0704S 15,000 units
#P0704L 75,000 units

PNGase F (Glycerol-free)
#P0705S 15,000 units
#P0705L 75,000 units

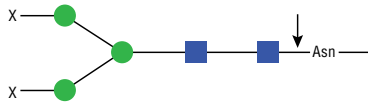
PNGase F, Recombinant
#P0708S 15,000 units
#P0708L 75,000 units

PNGase F (Glycerol-free), Recombinant
#P0709S 15,000 units
#P0709L 75,000 units

Companion Products:

RNase B
#P7817S 250 µg

Endoglycosidase Reaction Buffer Pack
#B0701S 4 ml



PNGase F hydrolyzes nearly all types of N-glycan chains from glycopeptides/proteins [x = H or oligosaccharide].

- *Removal of N-linked glycans from glycoproteins*

Description: Peptide-N-Glycosidase F, also known as PNGase F, is an amidase which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid and complex oligosaccharides from Nlinked glycoproteins. A glycerol-free version of PNGase F is also offered for HPLC methods.

Source: NEB #P0704 and #P0705 are purified from *Elizabethkingia miricola* (formerly *Flavobacterium meningosepticum*).

NEB #P0708 and #P0709 are purified from *Elizabethkingia miricola* (formerly *Flavobacterium meningosepticum*) and expressed in *E. coli*.

Reaction Conditions: Denature glycoprotein in 1X Glycoprotein Denaturing Buffer at 100°C for 10 minutes. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:

- 10X Glycoprotein Denaturing Buffer
- 10X GlycoBuffer 2
- 10% NP-40

Molecular Weight: 36,000 daltons

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl.

Concentration: 500,000 units/ml

Note: Since PNGase F activity is inhibited by SDS, it is essential to have NP-40 present in the reaction mixture.

To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.

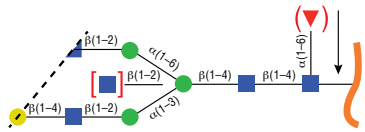
Rapid™ PNGase F & Rapid PNGase F (non-reducing format)

Rapid PNGase F
#P0710S 50 reactions

Rapid PNGase F (non-reducing format)
#P0711S 50 reactions

Companion Product:

Rapid PNGase F Antibody Standard
#P6043S 250 µg



- *Complete deglycosylation of antibodies and fusion proteins in minutes*
- *Release of all N-glycans rapidly and without bias*
- *Optimal activity is ensured for 12 months, if stored properly*
- *Purified to > 95% homogeneity, as determined by SDS-PAGE*

Description: Rapid PNGase F is an improved reagent that allows the complete and rapid deglycosylation of antibodies and fusion proteins in minutes. All *N*-glycans are released rapidly and without bias, and are ready to be prepared for downstream chromatography or mass spectrometry analysis. Rapid PNGase F creates an optimized workflow which reduces processing time without compromising sensitivity or reproducibility.

Developed for proteomic applications, Rapid PNGase F (non-reducing format) enables complete and rapid deglycosylation while preserving disulfide bonds. This facilitates high throughput proteomics applications and methods for antibody characterization by mass spectrometry such as intact mass analysis. Rapid PNGase F (non-reducing format) combines the advantages of Rapid PNGase F (fast processing time), with non-reducing conditions, preserving quaternary structure.

Heat inactivation: 75°C for 10 minutes.

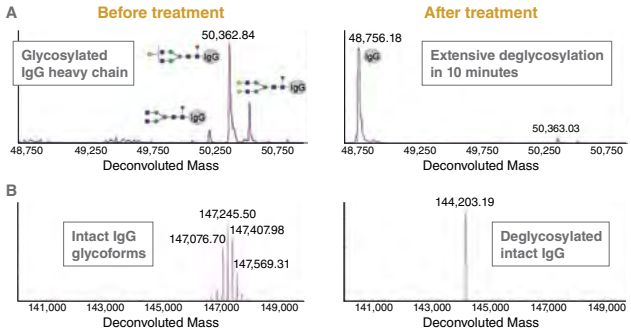
Reagents Supplied (NEB #P0710):

- Rapid PNGase F
- Rapid PNGase F Reaction Buffer (5X)

Reagents Supplied (NEB #P0711):

- Rapid PNGase F (non-reducing format)
- Rapid PNGase F (non-reducing format) Buffer (5X)

Specificity: Rapid PNGase F cleaves all complex, hybrid and high-mannose type glycans from antibodies and related proteins. Core α1-3 fucosylation (found in immunoglobulins expressed in plant or insect cells) is resistant to both PNGase F and Rapid PNGase F.



ESI-TOF analysis of an antibody before and after treatment with (A) Rapid PNGase F and (B) Rapid PNGase F (non-reducing format).

Gal Glc Man GalNAc GlcNAc Fuc NeuAc **R** = any sugar

Remove-iT® PNGase F

NEBU 37° 75°

#P0706S 6,750 units
#P0706L 33,750 units

Companion Products:

Chitin Magnetic Beads
#E8036S 5 ml

6-Tube Magnetic Separation Rack
#S1506S 6 tubes

12-Tube Magnetic Separation Rack
#S1509S 12 tubes

Description: Remove-iT PNGase F is an amidase which cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from *N*-linked glycoproteins. Remove-iT PNGase F is tagged with a chitin binding domain (CBD) for easy removal from a reaction and is supplied glycerol free for optimal performance in HPLC and MS intensive methods.

Source: Remove-iT PNGase F is purified from *Elizabethkingia miricola* (formerly *Flavobacterium meningosepticum*).

Reaction Conditions: GlycoBuffer 2, 37°C. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 2
- DTT

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 5 µg of DTT denatured RNase B in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 41,000 daltons.

Concentration: 225,000 units/ml

Note: Using typical RNase B denaturing conditions with NEB Glycoprotein Denaturing Buffer,containing SDS and DTT, Remove-iT PNGase F yields a higher concentration of 500,000 U/ml. If using Remove-iT PNGase F under typical PNGase F denaturing conditions, it is essential to have NP-40 in the reaction mixture as Remove-iT PNGase F is inhibited by SDS. It is not known why this non-ionic detergent counteracts the SDS inhibition. Removal of Remove-iT PNGase F from the deglycosylation reaction can be scaled up linearly with larger volumes of chitin magnetic beads.

O-Glycosidase

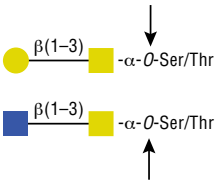
NEBU RR 37° 65°

#P0733S 2,000,000 units
#P0733L 10,000,000 units

Companion Products:

O-Glycosidase & Neuraminidase Bundle
#E0540S 1 set

α2-3,6,8 Neuraminidase
#P0720S 2,000 units
#P0720L 10,000 units



- *Removal of Core 1 and Core 3 O-linked disaccharide glycans from glycoproteins*

Description: *O*-Glycosidase, also known as Endo-a-*N*-Acetylgalactosaminidase, catalyzes the removal of Core 1 and Core 3 *O*-linked disaccharides from glycoproteins.

Source: Cloned from *Enterococcus faecalis* and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 2, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 2
- Glycoprotein Denaturing Buffer
- NP-40

Unit Definition: One unit is defined as the amount of enzyme required to remove 0.68 nmol of *O*-linked disaccharide from 5 mg of neuraminidase digested, non-denatured fetuin in 1 hour at 37°C in a total reaction volume of 100 µl (1 unit of both *O*-Glycosidase and PNGase F will remove equivalent molar amounts of *O*-linked disaccharides and *N*-linked oligosaccharides, respectively).

Molecular Weight: 147,000 daltons.

Concentration: 40,000,000 units/ml

Heat Inactivation: 65°C for 10 minutes

NEW

Boletopsis grisea Lectin (BGL)

RR 25° NG

#P0867S 1 ml

- *Binds to N-glycans having at least one terminal outer arm GlcNAc as well as O-glycans containing Gal-β1,3-GalNAc-α- within their structure.*
- *Enables enrichment of GlcNAc-capped N-glycans or mucin type O-glycopeptides from complex samples in glycomics and glycoproteomics analytical workflows.*

Boletopsis grisea Lectin (BGL) is a recombinant 15 kDa lectin from the Boletopsis grisea mushroom that has been expressed in *E. coli*. BGL has two separately functioning ligand binding sites. Site 1 binds to O-glycans bearing the Tn antigen (GalNAc-α-Ser/Thr) or Thomsen-Friedenreich antigen (TF-antigen; Gal-β1,3-GalNAc-α-) and Site 2 binds N-glycans with terminal GlcNAc residues.

Molecular Weight: 15 kDa.

Concentration: 1 mg/ml

This is an **Enzyme for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

Protein Deglycosylation Mix II

#P6044S 20 reactions

- *Fast reaction setup*
- *Enzyme mixture ensures effective deglycosylation of N-and O-linked glycans*
- *Can be used under native and reducing conditions*
- *Enzymatic deglycosylation leaves intact core structures suitable for mass spectrometry analysis*

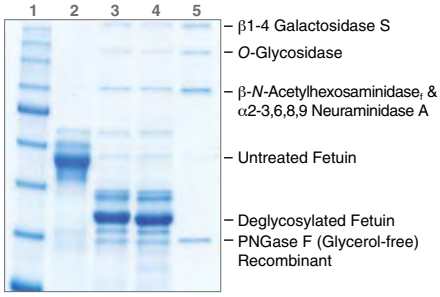
Description: The Protein Deglycosylation Mix II contains all of the enzymes, reagents and controls necessary to remove almost all *N*-linked and simple *O*-linked glycans, as well as some complex *O*-linked glycans. This mix contains enzymes sufficient for 20 reactions or the cleavage of as much as 2 mg of glycoprotein.

Deglycosylation Enzyme Mix II:

- PNGase F (Glycerol-free), Recombinant: 10,000 units/vial
- *O*-Glycosidase: 80,000 units/vial
- α2-3,6,8,9 Neuraminidase A: 400 units/vial
- β1-4 Galactosidase S: 960 units/vial
- β-*N*-Acetylhexosaminidase$_f$: 300 units/vial

Reagents Supplied:

- Deglycosylation Mix Buffer 1
- Deglycosylation Mix Buffer 2
- Fetuin



Enzymatic Deglycosylation of Bovine Fetuin under both native (10X Deglycosylation Mix Buffer 1) and reducing (10X Deglycosylation Mix Buffer 2) conditions. *20 µg reactions were loaded onto a 10-20% Tris-glycine SDS-PAGE gel. Lane 1: Color Prestained Protein Standard, Broad Range (11-245 kDa), Lane 2: 20 µg untreated Fetuin control, Lane 3: 20 µg Fetuin deglycosylated under native conditions with Deglycosylation Mix Buffer 1, Lane 4: 20 µg Fetuin deglycosylated under reducing conditions with Deglycosylation Mix Buffer 2, Lane 5: 5 µl Protein Deglycosylation Mix II.*

Fetuin

#P6042S 500 µg

Description: Fetuin is a glycoprotein containing sialylated *N*-linked and *O*-linked glycans that can be used as a positive control for endoglycosidase enzymes.

Source: Fetal Calf Serum

Molecular Weight: 48 kDa.

Concentration: 10 mg/ml

Note: 500 µg is enough for approximately 20 reactions. Due to heterogeneous glycosylation; Fetuin runs as a doublet on an SDS-PAGE gel.

Julie joined NEB in 2019 and is the Operations and Marketing Manager for NEB Australia. Find out what Julie likes best about NEB in her video reel.

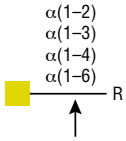
#NEBiographies

Gal Glc Man GalNAc GlcNAc Fuc NeuAc **R** = any sugar

α-*N*-Acetylgalactosaminidase

NEBU RR 37° 65° BSA

#P0734S 3,000 units



Description: α-*N*-Acetylgalactosaminidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α-linked d-*N*-Acetylgalactosamine residues from oligosaccharides and *N*-glycans attached to proteins.

Source: Cloned from *Chryseobacterium meningosepticum* and expressed in *E. coli* at NEB.

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 100 µg/ml Purified BSA. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1
- Purified BSA

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-D-*N*-acetylgalactosamine from 1 nmol (GalNAcα1-3)(Fucα1-2)Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

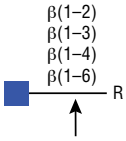
Molecular Weight: 47 kDa.

Concentration: 20,000 units/ml

β-*N*-Acetylglucosaminidase S

NEBU RR 37° No

#P0744S 100 units
#P0744L 500 units



- *Removal of bisecting β-GlcNAc residues*

Description: β-*N*-Acetylglucosaminidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of terminal, non-reducing β-*N*-Acetylglucosamine residues from oligosaccharides.

Source: Cloned from *Streptococcus pneumoniae* and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C.

Reagents Supplied:

- GlycoBuffer 1

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, non-reducing β-*N*-Acetylglucosamine from 1 nmol GlcNAcβ1-4GlcNAcβ1-4GlcNAc-7-amino-4-methylcoumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

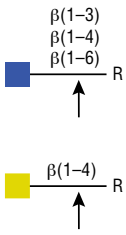
Molecular Weight: 125,000 daltons.

Concentration: 4,000 units/ml

β-*N*-Acetylhexosaminidase$_f$

NEBU RR 37° 75°

#P0721S 500 units



Description: β-*N*-Acetylhexosaminidase$_f$ is a recombinant protein fusion of β-*N*-Acetylhexosaminidase and maltose binding protein with identical activity to β-*N*-Acetylhexosaminidase. It catalyzes the hydrolysis of terminal β-*N*-Acetylgalactosamine and glucosamine residues from oligosaccharides.

Source: Cloned from *Streptomyces plicatus* and overexpressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Heat inactivation: 75°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1

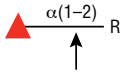
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal β-D-*N*-Acetylgalactosamine from 1 nmol of GalNAcβ1-4Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 100,000 daltons.

Concentration: 5,000 units/ml

α1-2 Fucosidase

#P0724S 1,000 units



- *Active only on linear substrates*

Description: α1-2 Fucosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of linear α1-2 linked fucose residues from oligosaccharides. In this case, a linear substrate is defined as having no branching on the adjacent residue.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 100 µg/ml Purified BSA. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1
- Purified BSA

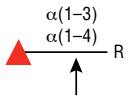
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the α-L-fucose from 1 nmol of Fucα1-2Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 70,000 daltons.

Concentration: 20,000 units/ml

α1-3,4 Fucosidase

#P0769S 200 units



Description: α1-3,4 Fucosidase, (also known as AMF) is a broad specificity exoglycosidase that catalyzes the hydrolysis of α1-3 and α1-4 linked fucose residues from oligosaccharides and glycoproteins.

Source: Cloned from the sweet almond tree (*Prunus dulcis*) and expressed in *Pichia pastoris*.

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 10X BSA. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1
- Purified BSA

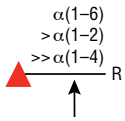
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the α-fucose from 1 nmol of Galβ1-4GlcNAcβ1-3(Fucα1-3)Galβ1- 4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl

Molecular Weight: 56,000 daltons.

Concentration: 4,000 units/ml

α1-2,4,6 Fucosidase O

#P0749S 80 units
#P0749L 400 units



Description: α1-2,4,6 Fucosidase O is a broad specificity exoglycosidase that catalyzes the hydrolysis of terminal α1-2, α1-4 and α1-6 linked fucose residues from oligosaccharides. α1-2,4,6 Fucosidase O cleaves α1-6 fucose residues more efficiently than other linkages.

Source: Cloned from *Omnitrophica* bacterium and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the fucose from 1 nmol of G0F from human IgG [GlcNAcβ1-2Manα1-6(GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4GlcNAc(Fucα1-6)-AMAC], in 1 hour at 37°C in a total reaction volume of 10 µl.

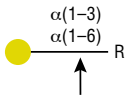
Molecular Weight: 49 kDa.

Concentration: 2,000 units/ml

α1-3,6 Galactosidase

NEBU BUTTERFLY RR 37° 65° BSA

#P0731S 100 units



Description: a1-3, 6 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of a1-3, 6 linked d-galactopyranosyl residues from oligosaccharides.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 100 µg/ml Purified BSA. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1
- Purified BSA

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, α-D-galactose from 1 nmol Galα1-3Galβ1-4Gal-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

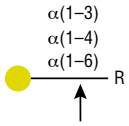
Molecular Weight: 70,000 daltons.

Concentration: 4,000 units/ml

α1-3,4,6 Galactosidase

NEBU BUTTERFLY RR 37° 65° BSA

#P0747S 200 units
#P0747L 1,000 units



Description: a1-3,4,6 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-3, α1-4 and α1-6 linked d-galactopyranosyl residues from oligosaccharides.

Source: Cloned from green coffee bean and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with Purified BSA. Heat inactivation: 65°C.

Reagents Supplied:

- GlycoBuffer 1
- Purified BSA

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, α-D-galactose from 1 nmol Galα1-3Galβ1-4Gal- 7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

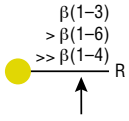
Molecular Weight: 39,700 daltons.

Concentration: 8,000 units/ml

β1-3 Galactosidase

NEBU BUTTERFLY RR 37° 65° BSA

#P0726S 500 units



Description: β1-3 Galactosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-3 and, at a much lower rate, β1-6 linked d-galactopyranosyl residues from oligosaccharides. The approximate kinetic data show > 100-fold preference for β1-3 over β1-6 linkages and > 500-fold preference from β1-3 over β1-4 linkages.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli*.

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 100 µg/ml Purified BSA. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1
- Purified BSA

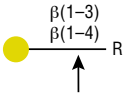
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal β-D-galactose from 1 nmol of Galβ1-3GlcNAcβ1-3Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 66 kDa.

Concentration: 10,000 units/ml

β1-3,4 Galactosidase

#P0746S 400 units



Description: β1-3,4 Galactosidase, cloned from bovine testis and also known as BTG, is a highly specific exoglycosidase that catalyzes the hydrolysis of terminal β1-3 and β1-4 linked galactose residues from oligosaccharides.

Source: Cloned from bovine testis and expressed in *Pichia pastoris.*

Reaction Conditions: GlycoBuffer 4, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

• GlycoBuffer 4

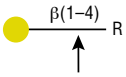
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, β-D-galactose from 1 nmol Galβ1-4GlcNAcβ1-3Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 71 kDa.

Concentration: 8,000 units/ml

β1-4 Galactosidase S

#P0745S 400 units
#P0745L 2,000 units



Description: β1-4 Galactosidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of β1-4 linked galactose residues from oligosaccharides.

Source: Cloned from *Streptococcus pneumoniae* and expressed in *E. coli.*

Reaction Conditions: GlycoBuffer 1, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

• GlycoBuffer 1

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal, β-D-galactose from 1 nmol Galβ1-4GlcNAcβ1- 3Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 231,000 daltons.

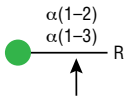
Concentration: 8,000 units/ml

Meet three members of our Marketing Team from our subsidiary office in Japan (pictured left to right). Naoki joined NEB in 2007 and is currently the Marketing & Technical Support Manager. Hiroshi joined NEB in 2022 as General Manager. Kumiko has been with NEB since 2018 and is currently a Marketing Specialist.

α1-2,3 Mannosidase

NEBU BUTTERFLY RR 37° 65° BSA

#P0729S 640 units



Description: α1-2,3 Mannosidase is a highly specific exoglycosidase that catalyzes the hydrolysis of α1-2 and α1-3 linked d-mannopyranosyl residues from oligosaccharides.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli.*

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 100 µg/ml Purified BSA. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1
- Purified BSA

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the non-reducing terminal α-D-mannose from 1 nmol Manα1-3Manβ1-4GlcNAc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

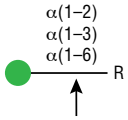
Molecular Weight: 90 kDa.

Concentration: 32,000 units/ml

α1-2,3,6 Mannosidase

NEBU BUTTERFLY RR 37° 95°

#P0768S 80 units



Description: α1-2,3,6 Mannosidase, cloned from Jack Bean, and also known as JBM, is a broad specificity exoglycosidase that catalyzes the hydrolysis of terminal α1-2, α1-3 and α1-6 linked mannose residues from oligosaccharides. α1-2,3,6 Mannosidase has a slight preference for α1-2 mannose residues over α1-3 and α1-6 mannose residues.

Source: Cloned from *Canavalia ensiformis* (Jack Bean) and expressed in *Pichia pastoris.*

Reaction Conditions: GlycoBuffer 4, 37°C. Supplement with 1X Zinc. Heat inactivation: 95°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 4
- Zinc

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal mannose from 1 nmol of Man(α1,3)-Man(β1,4)-GlcNAc-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

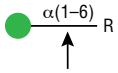
Molecular Weight: 110 kDa.

Concentration: 2,000 units/ml

α1-6 Mannosidase

NEBU BUTTERFLY RR 37° 65° BSA

#P0727S 800 units



Description: α1-6 Mannosidase is a highly specific exoglycosidase that removes unbranched α1-6 linked d-mannopyranosyl residues from oligosaccharides. When used in conjunction with α1-2,3 Mannosidase, the α1-6 Mannosidase will cleave α1-6 Mannose residues from branched carbohydrate substrates.

Source: Cloned from *Xanthomonas manihotis* and expressed in *E. coli.*

Reaction Conditions: GlycoBuffer 1, 37°C. Supplement with 100 µg/ml Purified BSA. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

- GlycoBuffer 1
- Purified BSA

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-D-mannose from 1 nmol of Manα1-6Manα1-6Man-7-amino-4-methyl-coumarin (AMC), in 1 hour at 37°C in a total reaction volume of 10 µl.

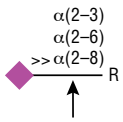
Molecular Weight: 51 kDa.

Concentration: 40,000 units/ml

Note: *p*-nitrophenyl-α-D-mannopyranoside is NOT a substrate for this enzyme.

α2-3,6,8 Neuraminidase

#P0720S 2,000 units
#P0720L 10,000 units



- *Active from pH 4.5 to 8.5*

Description: Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). This Neuraminidase catalyzes the hydrolysis of α2-3, α2-6 and α2-8 linked *N*-acetylneuraminic acid residues from glycoproteins and oligosaccharides.

Source: Cloned from *Clostridium perfringens* and overexpressed in *E. coli.*

Reaction Conditions: GlycoBuffer 1, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

• GlycoBuffer 1

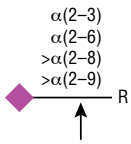
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-7-amino-4-methyl-coumarin (AMC), in 5 minutes at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 43 kDa.

Concentration: 50,000 units/ml

α2-3,6,8,9 Neuraminidase A

#P0722S 800 units
#P0722L 4,000 units



- *Removes branched sialic acid residues that are linked to an internal residue*

Description: Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). α2-3,6,8,9 Neuraminidase A catalyzes the hydrolysis of all linear and branched non-reducing terminal sialic acid residues from glycoproteins and oligosaccharides. The enzyme releases α2-3 and α2-6 linkages at a slightly higher rate than α2-8 and α2-9 linkages.

Source: Cloned from *Arthrobacter ureafaciens* and expressed in *E. coli.*

Reaction Conditions: GlycoBuffer 1, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

• GlycoBuffer 1

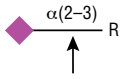
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 100,000 daltons.

Concentration: 20,000 units/ml

α2-3 Neuraminidase S

#P0743S 400 units
#P0743L 2,000 units



Description: Neuraminidase is the common name for Acetyl-neuraminyl hydrolase (Sialidase). α2-3 Neuraminidase S is a highly specific exoglycosidase that catalyzes the hydrolysis of α2-3 linked *N*-acetylneuraminic acid residues from glycoproteins and oligosaccharides.

Source: Cloned from *Streptococcus pneumoniae* and expressed in *E. coli.*

Reaction Conditions: GlycoBuffer 1, 37°C. Heat inactivation: 65°C for 10 minutes.

Reagents Supplied:

• GlycoBuffer 1

Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of the terminal α-Neu5Ac from 1 nmol Neu5Acα2-3Galβ1-3GlcNAcβ1-3Galβ1-4Glc-AMC, in 1 hour at 37°C in a total reaction volume of 10 µl.

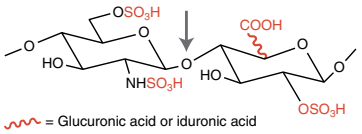
Molecular Weight: 74,000 daltons.

Concentration: 8,000 units/ml

Bacteroides Heparinase I

NEBU 30° 100°

#P0735S 240 units
#P0735L 600 units



= Glucuronic acid or iduronic acid
All structural determinants for enzyme specificity are displayed in red.

- *Degradation of heparin and heparan sulfate glycosaminoglycans*

Description: *Bacteroides* Heparinase I cloned from *Bacteroides eggerthii*, also called Heparin Lyase I, is active on heparin and the highly sulfated domains of heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.

Source: Cloned from *Bacteroides Eggerthii* and expressed in *E. coli*.

Reaction Conditions: Bacteroides Heparinase Reaction Buffer, 30°C. Heat inactivation: 100°C for 1 minute.

Reagents Supplied:

• Bacteroides Heparinase Reaction Buffer

Unit Definition: One unit is defined as the amount of enzyme that will liberate 1.0 µmol unsaturated oligosaccharides from porcine mucosal heparin per minute at 30°C and pH 7.0 in a total reaction volume of 100 µl.

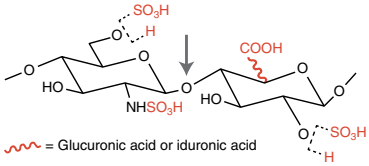
Molecular Weight: 42 kDa.

Concentration: 12,000 units/ml

Bacteroides Heparinase II

NEBU 30° 100°

#P0736S 80 units
#P0736L 200 units



= Glucuronic acid or iduronic acid
All structural determinants for enzyme specificity are displayed in red.

- *Degradation of heparin and heparan sulfate glycosaminoglycans*

Description: *Bacteroides* Heparinase II cloned from *Bacteroides eggerthii*, also called Heparin Lyase II, is active on heparin and heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.

Source: Cloned from *Bacteroides Eggerthii* and expressed in *E. coli*.

Reaction Conditions: Bacteroides Heparinase Reaction Buffer, 30°C. Heat inactivation: 100°C for 1 minute.

Reagents Supplied:

• Bacteroides Heparinase Reaction Buffer

Unit Definition: One unit is defined as the amount of enzyme that will liberate 1.0 µmol unsaturated oligosaccharides from porcine mucosal heparin per minute at 30°C and pH 7.0 in a total reaction volume of 100 µl.

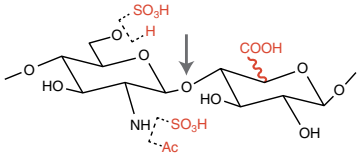
Molecular Weight: 86 kDa.

Concentration: 4,000 units/ml

Bacteroides Heparinase III

NEBU 30° 100°

#P0737S 14 units
#P0737L 35 units



= Glucuronic acid or iduronic acid
All structural determinants for enzyme specificity are displayed in red.

- *Degradation of heparan sulfate glycosaminoglycans*

Description: *Bacteroides* Heparinase III cloned from *Bacteroides eggerthii*, also called Heparin Lyase III, is active on heparan sulfate. The reaction yields oligosaccharide products containing unsaturated uronic acids which can be detected by UV spectroscopy at 232 nm.

Source: Cloned from *Bacteroides Eggerthii* and expressed in *E. coli*.

Reaction Conditions: Bacteroides Heparinase Reaction Buffer, 30°C. Heat inactivation: 100°C for 1 minute.

Reagents Supplied:

• Bacteroides Heparinase Reaction Buffer

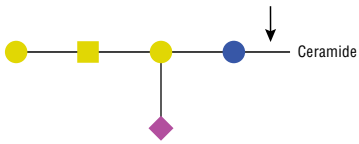
Unit Definition: One unit is defined as the amount of enzyme that will liberate 1.0 µmol unsaturated oligosaccharides from heparan sulfate per minute at 30°C and pH 7.0 in a total reaction volume of 100 µl.

Molecular Weight: 75 kDa.

Concentration: 700 units/ml

Endoglycoceramidase I (EGCase I)

#P0773S 150 milliunits



Description: Endoglycoceramidase I (ECGase I) catalyzes the hydrolysis of the β-glycosidic linkage between oligosaccharides and ceramides in various glycosphingolipids. One unit of *R. triatomea* EGCase I is defined as the amount of enzyme required to hydrolyze 1 µmol of ganglioside GM1a per minute at 37°C.

Source: EGCase I is isolated from a strain of *E. coli*, which contains the cloned EGCase I gene from *Rhodococcus triatomea.*

Reaction Conditions: EGCase I Reaction Buffer, 37°C. Heat inactivation: 65°C for 20 minutes.

Reagents Supplied:

- EGCase I Reaction Buffer

Unit Definition: One unit of *R. triatomea* EGCase I was defined as the amount of enzyme required to hydrolyze 1 µmol of ganglioside GM1a per minute at 37°C.

Molecular Weight: 50 kDa.

Concentration: 6 units/ml

This is an **Enzyme for Innovation (EFI)**. EFI is a project initiated by NEB to provide unique enzymes to the scientific community in the hopes of enabling the discovery of new and innovative applications. Visit **www.neb.com/EnzymesforInnovation** to view the full list.

IdeZ Protease (IgG-specific)

#P0770S 4,000 units

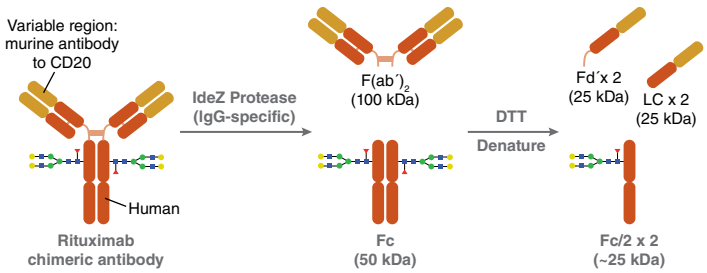
human IgG1, IgG3, IgG4: CPAPELLG▼GPSVF
human IgG2: CPAPPVA▼GPSVF
murine IgG2a: CPAPNLLG▼GPSVF
murine IgG3: CPPGNILG▼GPSVF

- *Complete fragmentation of antibodies and immunoglobulin fusion proteins in 30 minutes under native conditions*

Description: IdeZ Protease (IgG-specific) is a recombinant antibody specific protease that recognizes all human, sheep, monkey, and rabbit IgG subclasses, specifically cleaving at a single recognition site below the hinge region, yielding a homogenous pool of F(ab´)2 and Fc fragments. IdeZ Protease more effectively cleaves murine IgG2a than IdeS.

Source: Cloned from *Streptococcus equi* subspecies *zooepidemicus* and expressed in *E. coli.*

Reaction Conditions: GlycoBuffer 2, 37°C. Heat inactivation: 65°C for 10 minutes.



Reagents Supplied:

- GlycoBuffer 2

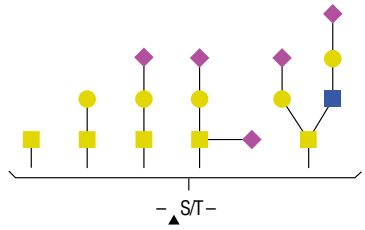
Unit Definition: One unit is defined as the amount of enzyme required to cleave > 95% of 1 µg of human IgG, in 15 minutes at 37°C in a total reaction volume of 10 µl.

Molecular Weight: 35,578 daltons.

Concentration: 80,000 units/ml

O-Glycoprotease (IMPa)

#P0761S 200 reactions



- *Efficiently cleaves glycoproteins with or without sialic acid; no neuraminidase treatment necessary*
- *200 reactions is sufficient for the cleavage of up to 2 mg of glycoprotein*

Description: O-Glycoprotease is a highly specific protease that cleaves the peptide bonds of a glycoprotein or glycopeptide immediately *N*-terminal to a serine or threonine residue containing a mucin-type *O*-linked glycan with or without sialylation.

Source: Cloned from *Pseudomonas aeruginosa* and expressed in *E. coli.*

Reaction Conditions: 20 mM Tris-HCl, pH 8.0, 37°C. Heat inactivation: 95°C for 10 minutes.

Unit Definition: One unit of O-Glycoprotease (IMPa) will cleave > 90% of 2 µM FAM-labelled O-glycopeptide in a total reaction volume of 20 µl in 2 hours at 37°C in 20 mM Tris-HCl, pH 8.0.

Molecular Weight: 97 kDa.

Concentration: 1,000 units/ml

Gal Glc Man GalNAc GlcNAc Fuc NeuAc **R** = any sugar

Proteases

Proteins found in nature vary greatly in size from 5 kDa to greater than 400 kDa. While it is possible to study intact proteins and the modifications present on these proteins by mass spectrometry, the most common proteomic approaches utilize digestion with site-specific proteases to generate smaller fragments, called peptides, as a first step in the analyses. Peptides are easier to characterize and can be separated using reverse phase supports, such as C18, combined with high performance liquid chromatography (HPLC). HPLC coupled with tandem Mass Spectrometry is used to obtain fragmentation data of individual peptides.

Trypsin-ultra, Mass Spectrometry Grade

NEBU 37°

#P8101S 100 µg

Lys/Arg▼XXX

- *Digestion of proteins for proteomic analysis by mass spectrometry*
- *Protein and peptide identification*
- *TPCK treatment eliminates chymotryptic activity*
- *Free of contaminating proteases*

Description: Trypsin-ultra, Mass Spectrometry Grade is a serine endopeptidase. It selectively cleaves peptide bonds C-terminal to lysine and arginine residues. Trypsin-ultra, Mass Spectrometry Grade is treated with L-(tosylamido-2-phenyl) ethyl chloromethyl ketone (TPCK) to inactivate any remaining chymotryptic activity. It is modified by acetylation of the ε-amino groups of lysine residues to prevent autolysis. Trypsin-ultra, Mass Spectrometry Grade cleaves at Lys-Pro and Arg-Pro bonds at a much slower rate than other amino acid residues.

Source: Isolated from bovine (*Bos taurus*) pancreas.

Reaction Conditions: Trypsin-ultra, Reaction Buffer, 37°C.

Reagents Supplied:

• Trypsin-ultra, Reaction Buffer

Molecular Weight: 23,675 daltons.

Reconstitution: Trypsin-ultra, Mass Spectrometry Grade should be reconstituted by the addition of 20–200 µl of high purity water. Rapid autolysis is a function of enzyme concentration.

Note: Can be stored frozen in solution at -20°C for up to 2 weeks. A decrease in activity will occur if stored in solution. Use only freshly reconstituted protease for best results.

α-Lytic Protease

37° 95°

#P8113S 20 µg
#P8113L 100 µg

XX-T/A/S/V▼XX

- *Analyze complex proteomes*
- *Suitable for both in-gel and solution digests*
- *Optimal activity and stability for up to 24 months*
- *Ideal for digestion of proteins for proteomic analysis by mass spectrometry*

Description: α-Lytic Protease (aLP) cleaves after Threonine (T), Alanine (A), Serine (S) and Valine (V) residues. Its specificity makes it an orthogonal and alternative protease to others commonly used in proteomics applications, including trypsin and chymotrypsin. Peptides generated by aLP are of similar average length to those of Trypsin.

Source: Purified from *Lysobacter enzymogenes*

Molecular Weight: 19 kDa.

Concentration: 0.4 mg/ml

Note: α-Lytic Protease is stable for at least 2 years at –20°C. No loss of activity is observed after 10 freeze-thaw cycles. To avoid autolysis, α-Lytic Protease should be stored long term in 10 mM Sodium Acetate pH 5.0.

Endoproteinase LysC

37°

#P8109S 20 µg

XX-Lys▼XXX

- *Ideal for proteomic analysis by mass spectrometry*
- *Free of contaminating proteases*
- *Best suited for peptide identification*

Description: LysC is a serine endoproteinase, isolated from *Lysobacter enzymogenes*, that cleaves peptide bonds C-terminal to lysine residues. LysC is a sequencing grade enzyme and is suitable for proteomics and glycobiology applications.

Source: Isolated from *Lysobacter enzymogenes*

Molecular Weight: 30,000 daltons.

Reconstitution: Endoproteinase LysC should be reconstituted in 200 µl double-distilled water to make a 100 ng/µl solution in 10 mM Tris-HCl, pH 8.0. Rapid autolysis is a function of enzyme concentration.

Note: Storage Conditions: Supplied in dry format from a Tris-HCl buffer. The solution can be stored at 4°C for several days or in single-use aliquots at –20°C for several months. Use only freshly reconstituted protease for best results.

Endoproteinase GluC

#P8100S 50 µg

XX-Glu▼XX

- *Ideal for proteomic analysis by mass spectrometry*
- *Protein & peptide identification*
- *Free of contaminating proteases. Produced from a protease-deficient Bacillus subtilis strain*

Description: Endoproteinase GluC (*Staphylococcus aureus* Protease V8) is a serine proteinase which selectively cleaves peptide bonds C-terminal to glutamic acid residues. Endoproteinase GluC also cleaves at aspartic acid residues at a rate 100–300 times slower than at glutamic acid residues.

Source: *Staphylococcus aureus* Protease V8 gene cloned and expressed with histidine-tag in *Bacillus subtilis*

Reaction Conditions: GluC Reaction Buffer, 37°C.

Reagents Supplied:

• GluC Reaction Buffer

Molecular Weight: 29,849 daltons.

Reconstitution: Endoproteinase GluC should be reconstituted by the addition of 50-500 µl of high purity water. Finger flick the volume of water in the tube to fully resuspend the enzyme. Rapid autolysis is a function of enzyme concentration; any sample reconstituted in a small volume should be used immediately. To get the most use out the enzyme, resuspend the enzyme in 500 µl H_2O and aliquot 50 ul each in 10 tubes. Freeze the tubes that are not being used immediately at -20°C for up to two weeks or less. Storage at -80°C will prolong enzyme stability approximately 2-4 additional weeks.

Endoproteinase AspN

#P8104S 50 µg

XX▼Asp-XXX

- *Ideal for proteomic analysis by mass spectrometry*
- *Free of contaminating proteases*
- *Best suited for peptide identification*

Description: Endoproteinase AspN (flavastacin) is a zinc metalloendopeptidase which selectively cleaves peptide bonds N-terminal to aspartic acid residues.

Source: Purified from *Flavobacterium menigosepticum.*

Reaction Conditions: Endoproteinase AspN Reaction Buffer, 37°C.

Reagents Supplied:

• Endoproteinase AspN Reaction Buffer

Molecular Weight: 40,089 daltons.

Reconstitution:
Endoproteinase AspN should be reconstituted by the addition of 50-500 µl of high purity water. Rapid autolysis is a function of enzyme concentration.

Note: Storage Conditions: Supplied in dry format. Can be stored frozen in solution at -20°C for up to 2 weeks. A decrease in activity will occur if stored in solution. Use only freshly reconstituted protease for best results.

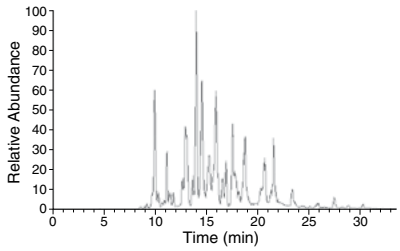
Trypsin-digested BSA MS Standard (CAM-modified)

#P8108S 500 pmol

Description: A complex mixture of peptides produced by Trypsin digestion of Bovine Serum Albumin (BSA) that was reduced and alkylated with Iodoacetimide (CAM modified). This peptide mixture can be used to test a Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) or Electrospray Ionization (ESI) mass spectrometer (TOF, Q-TOF or Ion Trap).

Source: BSA (GENBANK P02769) was digested using Trypsin (TPCK-treated).

Reconstitution: Suggested volume to resuspend: 500 µl. Avoid repeated freeze/thaw cycles once in solution.



One hundred fmol of resuspended peptide mix may be analyzed by reverse phase liquid chromatography with on-line MS/MS analysis, for example with a Proxeon EASY-nLC and by Orbitrap Mass Spectrometer. *Both analytical methods reveal a range of peptides in the standard. At least sixty percent sequence coverage is seen after database search, with greater than 15 unique peptides being identified.*

Proteinase K, Molecular Biology Grade

#P8107S 2 ml

- *Isolation of plasmid and genomic DNA*
- *Isolation of RNA*
- *Inactivation of RNases, DNases and enzymes in reactions*

Description: Proteinase K is a subtilisin-related serine protease that will hydrolyze a variety of peptide bonds.

Source: *Engyodontium album (Tritirachium album)*

Unit Definition: One unit will digest urea-denatured hemoglobin at 37°C (pH 7.5) per minute to produce equal absorbance as 1.0 µmol of L-tyrosine using Folin & Ciocalteu's phenol reagent.

Molecular Weight: 28 kDa.

Concentration: 800 units/ml

Note: Active in a wide range of buffers, including all NEB-specific restriction endonuclease buffers. It is highly active between pH 7.5 and 12 and temperatures 20-60°C. Proteinase K is also active in chelating agents such as EDTA and activity is stimulated in up to 2% SDS or 4M urea.

Thermolabile Proteinase K

#P8111S 30 units

- *Heat inactivated following incubation at 55°C for 10 minutes*
- *Isolation of plasmid and genomic DNA*
- *Inactivation of RNases, DNases and enzymes in reactions*
- *Removal of enzymes from DNA to improve cloning efficiency*
- *PCR purification*

Description: Thermolabile Proteinase K is an engineered, subtilisin-related serine protease that will hydrolyze a variety of peptide bonds.

Source: Cloned from *Engyodontium album* (formerly *Tritirachium album*), mutagenized to increase thermolability of the enzyme and expressed in *K. lactis*.

Unit Definition: One unit is defined as the amount of enzyme required to release 1.0 µmol of 4-nitroaniline per minute from N-Succinyl-Ala-Ala-Pro-Phe-p-nitroanilide at 25°C, in a total reaction volume of 105 µl.

Molecular Weight: 29 kDa.

Concentration: 120 units/ml

Note: Active in a wide range of buffers. It is highly active between pH 7.0 and 9.5 and temperatures 20-40°C. It is active in chelating agents such as EDTA up to 10 mM.

TEV Protease

#P8112S 1,000 units

ENLYFQ▾(G/S/M)

- *Removal of affinity purification tags such as MBP or poly-histidine from fusion proteins*
- *Contains a His-tag for easy removal from a reaction using NEBExpress Ni Resin (NEB #S1428), NEBExpress NiSpin Columns (NEB #S1427) or NEBExpress Ni-NTA Magnetic Beads (NEB #S1423)*

Description: TEV Protease, also known as Tobacco Etch Virus (TEV) Protease, is a highly specific cysteine protease that recognizes the amino-acid sequence Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser/Met) and cleaves between the Gln and Gly/Ser/Met residues. It is often used for the removal of affinity purification tags such as maltose-binding protein (MBP) or poly-histidine from fusion proteins. TEV Protease has a 7xHis-tag for easy removal from a reaction using nickel affinity resins and has been engineered to improve thermal stability and decrease autolysis.

Source: Cloned from Tobacco Etch Virus and expressed in *E. coli*.

Unit Definition: 1 unit of TEV Protease will cleave 2 µg of MBP-fusion protein, MBP5-TEV-paramyosin ΔSal, to 95% completion in a total reaction volume of 10 µl in 1 hour at 30°C in 50 mM Tris-HCl (pH 7.5 @ 25°C) with 0.5 mM EDTA and 1 mM DTT.

Molecular Weight: 28 kDa.

Concentration: 10,000 units/ml

Factor Xa Protease

#P8010S	50 µg
#P8010L	250 µg

Ile-Glu/Asp-Gly-Arg▼

Description: Factor Xa cleaves after the arginine residue in its preferred cleavage site Ile-(Glu or Asp)-Gly-Arg. It will sometimes cleave at other basic residues, depending on the substrate conformation. The most common secondary site, among those that have been sequenced, is Gly-Arg. There seems to be a correlation between proteins that are unstable in *E. coli* and those that are cleaved by Factor Xa at secondary sites; this may indicate that these proteins are in a partially unfolded state. Factor Xa will not cleave a site followed by proline or arginine.

Source: Factor Xa Protease is purified from bovine plasma and activated by treatment with the activating enzyme from Russell's viper venom.

Unit Definition: 1 µg of Factor Xa will cleave 50 µg of MBP fusion protein test substrate, MBP-ΔSal to 95% completion in a total reaction volume of 50 µl in 6 hours or less at 23°C in 20 mM Tris-HCl (pH 8.0 @ 25°C) with 100 mM NaCl and 2 mM CaCl2.

Molecular Weight: 43 kDa.

Concentration: 1 mg/ml

Removal: Factor Xa will bind specifically to benzamidine-agarose.

Enterokinase, light chain

#P8070S	480 units
#P8070L	2,560 units

Companion Product:

K. *lactis* Protein Expression Kit
#E1000S 1 set

Asp-Asp-Asp-Asp-Lys▼

Description: Enterokinase is a specific protease that cleaves after the lysine at its cleavage site, Asp-Asp-Asp-Asp-Lys. It will sometimes cleave at other basic residues, depending on the conformation of the protein substrate. Enterokinase will not cleave a site followed by proline.

Source: This preparation is purified from *Pichia pastoris* containing a clone of the light chain of the bovine enterokinase gene.

Unit Definition: 1 unit is defined as the amount of enzyme required to cleave 25 µg of a MBP-EK-paramyosin-ΔSal substrate to 95% completion in 16 hours at 25°C in a total reaction volume of 25 µl.

Molecular Weight: 26 kDa, Apparent Molecular Weight: 31 kDa.

Concentration: 16,000 units/ml

Removal: Enterokinase will bind specifically to trypsin inhibitor agarose (e.g., Sigma T-0637).

Furin

#P8077S	50 units
#P8077L	250 units

Arg-X-X-Arg▼

Description: Furin is an ubiquitous subtilisin-like proprotein convertase. It is the major processing enzyme of the secretory pathway and is localized in the trans-golgi network. Substrates of Furin include blood clotting factors, serum proteins and growth factor receptors such as the insulin-like growth factor receptor. The minimal cleavage site is Arg-X-X-Argt. However, the enzyme prefers the site Arg-X-(Lys/Arg)-Argt. An additional arginine at the P6 position appears to enhance cleavage. Furin is inhibited by EGTA, α1-Antitrypsin Portland and polyarginine compounds.

Note: The ability to cleave a particular substrate appears to depend on its tertiary structure as well as on the amino acids immediately surrounding the cleavage site.

Source: Isolated from *Spodoptera frugiperda* (Sf9) cells infected with recombinant baculovirus carrying truncated human furin.

Unit Definition: 1 unit is defined as the amount of enzyme required to cleave 25 µg of a MBP-FN-paramyosin-ΔSal substrate to 95% completion in 6 hours at 25°C in a total reaction volume of 25 µl.

Molecular Weight: 52 kDa.

Concentration: 2,000 units/ml

Lambda Protein Phosphatase (Lambda PP)

NEBU RR 30° 65°

#P0753S	20,000 units
#P0753L	100,000 units

Companion Products:

p-Nitrophenyl Phosphate (PNPP)

#P0757S	1 ml
#P0757L	5 ml

Sodium Orthovanadate (Vanadate)

#P0758S	1 ml
#P0758L	5 ml

Description: Lambda Protein Phosphatase (Lambda-PP) is a Mn^{2+}-dependent protein phosphatase with activity towards phosphorylated serine, threonine and tyrosine residues. Lambda-PP is active on phosphorylated histidine residues.

Source: Isolated from a strain of *E. coli* that carries the bacteriophage lambda ORF221 open reading frame under the control of a T7 expression system.

Reaction Conditions: NEBuffer Pack for Protein MetalloPhosphatases (PMP), 30°C. Supplement with 1 mM $MnCl_2$. Heat inactivation: 65°C for 60 minutes.

Reagents Supplied:

- NEBuffer Pack for Protein MetalloPhosphatases (PMP)
- $MnCl_2$

Unit Definition: One unit is defined as the amount of enzyme that hydrolyzes 1 nmol of *p*-Nitrophenyl Phosphate (50 mM) (NEB #P0757) in 1 minute at 30°C in a total reaction volume of 50 µl.

Molecular Weight: 25 kDa.

Concentration: 400,000 units/ml

Protein Kinases

The reversible addition of phosphate groups to proteins is important for the transmission of signals within eukaryotic cells and, as a result, protein phosphorylation and dephosphorylation regulate many diverse cellular processes. As the number of known protein kinases has increased at an ever-accelerating pace, it has become more challenging to determine which protein kinases interact with which substrates in the cell. The determination of consensus phosphorylation site motifs by amino acid sequence alignment of known substrates has proven useful in this pursuit. These motifs can be helpful for predicting phosphorylation sites for specific protein kinases within a potential protein substrate.

Since the determinants of protein kinase specificity involve complex 3-dimensional interactions, these motifs, short amino-acid sequences describing the primary structure around the phosphoacceptor residue, are a significant oversimplification of the issue. They do not take into account possible secondary and tertiary structural elements, or determinants from other polypeptide chains or from distant locations within the same chain. Furthermore, not all of the residues described in a particular specificity motif may carry the same weight in determining recognition and phosphorylation by the kinase. As a consequence, they should be used with some caution.

On the other hand, many of the residues within these consensus sequences have in fact proven to be crucial recognition elements, and the very simplicity of these motifs has made them useful in the study of protein kinases and their substrates. In addition to the prediction of phosphorylation sites, short synthetic oligopeptides based on consensus motifs are often excellent substrates for protein kinase activity assays.

The table below summarizes the specificity motifs for protein kinases that are available from NEB. Amino acids, which can function interchangeably at a particular residue, are separated by a slash (/), and residues which do not appear to contribute strongly to recognition are indicated by an "X".

Protein Kinase	NEB #	Recognition Determinant	Size
cAMP-dependent Protein Kinase (PKA), catalytic subunit	P6000S P6000L	R-R-X-S/T Y	100,000 units 500,000 units
Casein Kinase II (CK2)	P6010S P6010L	S-X-X-E/D	10,000 units 50,000 units

D = aspartic acid, E = glutamic acid, R = arginine, S = serine, T = threonine, Y = tyrosine/hydrophobic residue, X = any amino acid
Note: More specific information on recognition determinants for each kinase can be found on the corresponding product page at www.neb.com.

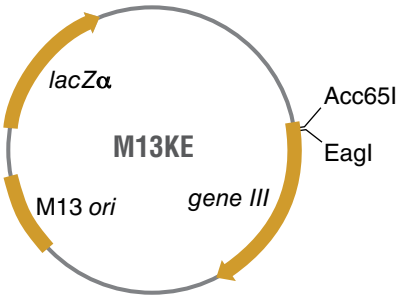
Ph.D.™ Peptide Display Cloning System

#E8101S	20 µg

Description: The Ph.D. Peptide Display Cloning System facilitates the display of custom peptide libraries on the surface of bacteriophage M13 as coat protein fusions, creating a physical linkage between each displayed peptide and its encoding DNA sequence. Peptide ligands for a variety of targets can then be selected by the straightforward method of panning. The supplied display vector M13KE is an M13 derivative with cloning sites engineered for N-terminal pIII fusion, resulting in a valency of 5 displayed peptides per virion. The use of a phage vector, rather than a phagemid, simplifies the intermediate amplification steps, since neither antibiotic selection nor helper phage superinfection are required. Since displayed proteins longer than 20–30 amino acids have a deleterious effect on the infectivity function of pIII in phage vectors, **this vector is suitable only for the display of short peptides.** Included with the cloning vector is an insert extension primer as well as a detailed protocol for cloning a peptide library into M13KE.

Kit Includes:

- M13KE gIII Cloning Vector
- M13 Extension Primer



NEBU Unique NEBuffer · Cloned at NEB · RR Recombinant Enzyme · BSA Requires BSA · 65° Heat Inactivation

Ph.D.™ Phage Display Peptide Library Kits

NEW
Ph.D.-7 Phage Display Peptide Library Kit v2
#E8211S 1 set

NEW
Ph.D.-12 Phage Display Peptide Library Kit v2
#E8210S 1 set

NEW
Ph.D.-C7C Phage Display Peptide Library Kit v2
#E8212S 1 set

Companion Products:

Ph.D. Peptide Display Cloning System
#E8101S 20 µg

Ph.D.-12 Phage Display Peptide Library
#E8111L 50 panning experiments

Protein G Magnetic Beads
#S1430S 1 ml

Description: Phage display describes a selection technique in which a library of peptide or protein variants is expressed on the outside of a phage virion, while the genetic material encoding each variant resides on the inside. This creates a physical linkage between each variant protein sequence and the DNA encoding it, which allows rapid partitioning based on binding affinity to a given target molecule (antibodies, enzymes, cell-surface receptors, etc.) by an *in vitro* selection process called panning. In its simplest form (Figure 1), panning is carried out by incubating a library of phage-displayed peptides with a plate (or bead) coated with the target, washing away the unbound phage, and eluting the specifically-bound phage. The eluted phage is then amplified and taken through additional binding/amplification cycles to enrich the pool in favor of binding sequences. After 3–4 rounds, individual clones are characterized by DNA sequencing and ELISA. The Ph.D. v2 kits have been updated with a new control panning target for an optional epitope mapping experiment.

NEB offers 3 pre-made random peptide libraries, as well as the cloning vector M13KE for construction of custom libraries. The pre-made libraries consist of linear heptapeptide (Ph.D.-7) and dodecapeptide (Ph.D.-12) libraries, as well as a disulfide-constrained heptapeptide (Ph.D.-C7C) library. All of the libraries have complexities in excess of 2 billion independent clones. The randomized peptide sequences in all three libraries are expressed at the N-terminus of the minor coat protein pIII, resulting in a valency of 5 copies of the displayed peptide per virion.

The Ph.D. libraries have been used for myriad applications, including epitope mapping (Figure 2), identification of protein-protein contacts and enzyme inhibitors and discovery of peptide ligands for GroEL, HIV, semiconductor surfaces and small-molecule fluorophores and drugs.

The Ph.D. Kits Include:

- Sufficient Phage Display Library for 10 separate panning experiments, complexity of 10^9 clones
- -96 gIII Sequencing Primer (500 pmol)
- Host *E. coli* K12 strain ER2738
- Monoclonal antibody (DYKDDDDK) and Protein G Magnetic Beads included for new control panning experiment
- Detailed Protocols

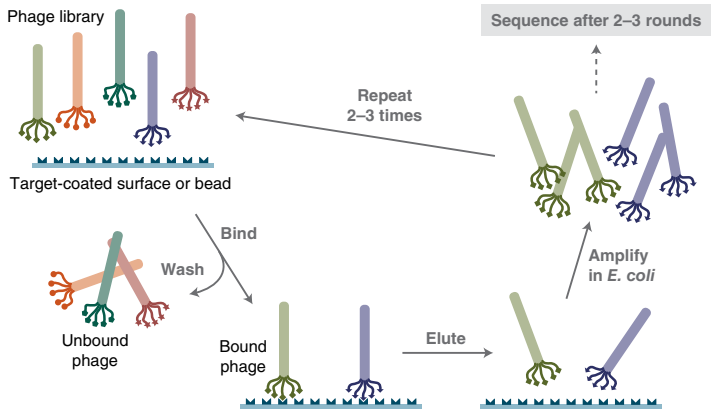


Figure 1: *Routine Phage Display Workflow. Round 1: Incubate 1011 pfu Ph.D. library + target incubation, wash away non-binders, elute bound phage, enrich selected phage with amplification in E. coli. Carry out 3-4 rounds of selection and then proceed with sequencing and/or phage-ELISA.*

Figure 2: *Epitope mapping of an anti-β-endorphin monoclonal antibody with the Ph.D.-12 library. The Ph.D.-12 library was panned against anti-β-endorphin antibody 3-E7 in solution (10 nM antibody), followed by affinity capture of the antibody-phage complexes onto Protein A-agarose (rounds 1 and 3) or Protein G-agarose (round 2). Bound phage were eluted with 0.2 M glycine-HCl, pH 2.2. Selected sequences from each round are shown aligned with the first 12 residues of β-endorphin; consensus elements are boxed. The results clearly show that the epitope for this antibody spans the first 7 residues of β-endorphin, and that the bulk of the antibody-antigen binding energy is contributed by the first 4 residues (YGGF), with some flexibility allowed in the third position. Additionally, the conserved position of the selected sequences within the 12 residue window indicates that the free ?-amino group of the N-terminal tyrosine is part of the epitope.*

Gal Glc Man GalNAc GlcNAc Fuc NeuAc **R** = any sugar

Tapping into clean and renewable geothermal energy

Thermal energy originates from deep within the Earth's core and has been used for bathing and cooking for thousands of years. Water seeps into underground reservoirs and breaks through the surface as steam or hot water in hot springs or geysers. Typically, this occurs where tectonic plates meet – examples include the intersection of the Eurasian and North American tectonic plates in Iceland or the Ring of Fire, which encircles the Pacific Ocean. Thermal heat can be harnessed to provide sustainable energy all day, every day, without relying on the intermittency of favorable weather conditions, as wind turbines and solar panels do. It is clean energy that generates very low emissions, requires only a small physical footprint, and can even recycle wastewater.

Geothermal energy production is not limited to locations where tectonic plates meet. Wells can be drilled in other regions of the world to between 600 meters (2000 ft) and over 3 km (2 miles) deep into the ground, where the temperature is approximately 316˚C (600˚F). There are various methods of producing energy from these wells – steam can be directly piped from the well (dry steam), water from the well can be pumped at high pressure into a tank where it rapidly cools and turns to steam (flash steam), or water can be pumped in closed-loop pipes adjacent to an intermediate liquid that has a much lower boiling point, transferring its heat and traveling back into the ground, while the intermediate liquid cools and releases steam (binary power). In all of these cases, the steam generated spins a turbine, producing electricity.

While Iceland is well-known for heating its homes, businesses and greenhouses using geothermal power, the top geothermal energy-producing countries are the U.S., Indonesia and the Philippines. The U.S. has the largest geothermal power plant in the world – The Geysers, located in California, draws steam from 350 wells.

Nevertheless, geothermal technologies are not experiencing the same level of investment as wind and solar power. Globally it only provides approximately 1% of electricity. This is because, like all forms of energy production, there are advantages and disadvantages. The main drawback of this technology is that temperatures high enough to produce steam are not within drillable depths everywhere.

Enhanced Geothermal Systems (EGS) is a promising solution that doesn't rely on underground hydrothermal reservoirs. EGS involves drilling two deep wells into dry, hot rock. Cold water is injected between the wells at high pressure, and the temperature differential within the hot rock re-opens pre-existing fractures, creating a reservoir. Water is pumped down one well into the cracks and then returned hot to the surface. This technique allows geothermal energy to be produced anywhere and, in theory, could produce 10% of global energy needs.

One note of caution is the potential for this 'fracking' technique to cause seismic activity. This occurred in South Korea in 2017 when the water injected into an EGS well activated an unknown fault, which then caused a 5.5-magnitude earthquake. Another drawback is that this method is costly, and there is no guarantee that a drill will result in a suitable well for harnessing geothermal energy.

Geothermal power is undoubtedly a great alternative to fossil fuels, but it still requires more research and development to overcome technical challenges. Still, with regulations to protect sensitive geysers, hot springs and pre-existing fault lines, it is a viable part of the solution to becoming carbon neutral.

View a geyser eruption in action.

Geyser eruption in Iceland.
Credit: logoboom, Adobe Stock

Epigenetics

Simplify your epigenetics research.

Epigenetics is the study of heritable changes in the phenotype of a cell or organism that are not encoded in the DNA of the genome. The molecular basis of an epigenetic profile arises from covalent modifications of the protein and DNA components of chromatin. The epigenetic profile of a cell often dictates cell memory and cell fate and, thus influences mammalian development.

The epigenetic code is hypothesized to be the combined effects of histone modifications and DNA methylation on gene expression. While the genetic code for an individual is the same in every cell, the epigenetic code is tissue- and cell-specific, and may change over time as a result of aging, disease or environmental stimuli (e.g., nutrition, life style, toxin exposure). Cross-talk between histone modifications, DNA methylation or RNAi pathways are being studied in such areas as cancer, X chromosome inactivation, and imprinting.

For almost 50 years, New England Biolabs has been committed to understanding the mechanisms of restriction and methylation of DNA. This expertise in enzymology has led to the development of a suite of validated products for epigenetics research. These unique solutions to study DNA and histone modifications are designed to address some of the challenges of the current methods. All NEB products pass stringent quality control assays to ensure the highest level of functionality and purity.

Featured Products

283 **Methylation-dependent Restriction Enzymes**

285 **NEBNext Enzymatic Methyl-seq Products**

Featured Tools & Resources

Videos of NEB Scientists Discussing Epigenetics

Feature Articles

Epigenetics-related FAQs

Visit **www.EpiMark.com** to view an interactive tutorial explaining the phenomenon of epigenetics at the molecular level.

Find an interactive tutorial on epigenetics.

Epigenetics

RR T4 Phage β-glucosyltransferase (T4-BGT) 282
EpiMark N6-Methyladenosine Enrichment Kit 282
RR EpiMark Hot Start *Taq* DNA Polymerase 282

Control DNA

5-methyl-dCTP 282

Methylation-Dependent Restriction Enzymes

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NEBNext Reagents for Enzymatic Methyl-seq (EM-seq)

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NEBNext Enzymatic Methyl-seq Conversion Module 285
NEBNext Enzymatic 5hmC-seq Kit 285
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NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs) 285
NEBNext Multiplex Oligos for Enzymatic 5hmC-seq 285

RR Recombinant Enzyme

T4 Phage β-glucosyltransferase (T4-BGT)

#M0357S 500 units
#M0357L 2,500 units

- *Glucosylation of 5-hydroxymethylcytosine in DNA*
- *Immunodetection of 5-hydroxymethylcytosine in DNA*
- *Labeling of 5-hydroxymethylcytosine residues by incorporation of [^{3}H]- or [^{14}C]-glucose into 5-hmC-containing DNA acceptor after incubation with [^{3}H]- or [^{14}C]-UDP-Glc*
- *Detection of 5-hydroxymethylcytosine in DNA by protection from endonuclease cleavage*

Description: T4 Phage β-glucosyltransferase specifically transfers the glucose moiety of uridine diphosphoglucose (UDP-Glc) to the 5-hydroxymethylcytosine (5-hmC) residues in double-stranded DNA, making beta-glucosyl-5-hydroxymethylcytosine.

Reaction Conditions: NEBuffer 4, 37°C. Supplement with 40 µM Uridine Diphosphate Glucose. Heat inactivation: 65°C for 10 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to protect 0.5 µg T4gt-DNA against cleavage by MfeI restriction endonuclease.

Concentration: 10,000 units/ml

Reagents Supplied:

- NEBuffer 4
- Uridine Diphosphate Glucose

EpiMark® N6-Methyladenosine Enrichment Kit

#E1610S 20 reactions

- *Enrichment for m6A modified RNA in immunoprecipitation protocols*
- *Enriched RNA can be used directly for next gen sequencing or RT-qPCR*

Description: The EpiMark N6-Methyladenosine Enrichment Kit contains a rabbit monoclonal antibody specific for N6-Methyladenosine (m6A). The kit also contains two control RNAs, one with m6A modification (*Gaussia* luciferase) and one without (*Cypridina* luciferase) to monitor enrichment and depletion. The GLuc RNA control was transcribed in the presence of 20% m6ATP and 80% ATP.

This kit can be used to enrich m6A modified RNA in immunoprecipitation protocols for downstream analysis by next-generation RNA sequencing or RT-qPCR. Modified RNA is isolated from a fragmented RNA sample by binding to the N6-Methyladenosine antibody attached to Protein G Magnetic Beads. After multiple wash and clean-up steps, the enriched RNA is eluted in nuclease-free water and is ready for further analysis.

EpiMark® Hot Start *Taq* DNA Polymerase

#M0490S 100 reactions
#M0490L 500 reactions

- *Ideal for use on bisulfite-converted DNA and AT-rich templates*
- *Specially-formulated reaction buffer system*

Description: EpiMark Hot Start *Taq* DNA Polymerase is an excellent choice for use on bisulfite-converted DNA. It is a mixture of *Taq* DNA Polymerase and a temperature-sensitive, aptamer-based inhibitor. The inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 45°C, but releases the enzyme during normal PCR cycling conditions. This permits room temperature reaction assembly with no separate high-temperature incubation step to activate the enzyme.

Molecular Weight: 94,000 daltons.

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 15 nmol dNTP into acid insoluble material in 30 minutes at 75°C.

Concentration: 5,000 units/ml

EPIGENETICS

5-methyl-dCTP

#N0356S 1 µmol

Description: Cytosine modification at carbon 5 (C5) represents an important epigenetic modification. It is also believed to be the starting substrate for the TenEleven Translocation (TET) family of enzymes and their associated oxidation pathways. 5-methyl-dCTP offers the ability to enzymatically make defined fully methylated cytosine-substituted DNA, which can be used for a variety of biochemical and cellular applications.

5-methyl-dCTP (2´-deoxy-5-methylcytidine 5´-triphosphate) is supplied as a 10 mM solution at pH 7. Nucleotide concentration is determined by measurements of absorbance at 260 nm.

Formula: $C_{10}H_{15}N_3O_{13}P_3$ (free acid)

Diluent Compatibility: Can be diluted using sterile distilled water, preferably Milli-Q® water, or can be diluted using sterile TE [10 mM Tris-HCl, 1 mM EDTA (pH 7.5)]

Concentration: 10 mM

Methylation-Dependent Restriction Enzymes

- *Specificity to epigenetically-relevant DNA modifications (5-mC and 5-hmC)*
- *Easy-to-follow protocols*
- *Less harsh than bisulfite conversion*
- *Simplified data analysis*

The EpiMark Suite of products has been validated for use in epigenetics applications. Visit **EpiMark.com** for more information.

Many restriction enzymes are sensitive to DNA methylation states. Cleavage can be blocked or impaired when a particular base in the recognition site is modified. The MspJI family of restriction enzymes are dependent on methylation and hydroxymethylation for cleavage to occur (1). These enzymes excise 32-base pair DNA fragments containing a centrally located 5-hmC or 5-mC modified residue that can be extracted and sequenced. Due to the known position of this epigenetic modification, bisulfite conversion is not required prior to downstream analysis. These EpiMark validated, methylation-dependent restriction enzymes expand the potential for mapping epigenetic modifications and simplify the study of DNA methylation. Additionally, they provide an opportunity to better understand the role of 5-hydroxymethylcytosine in the genome.

(1) Cohen-Karni, D. et al. (2011) *PNAS*, 108, 11040–11045.

AbaSI

rCutSmart RR dilC 25° 65° Epi

#R0665S 1,000 units

5´...^{ghm}C $N_{11\text{-}13}$▼$N_{9\text{-}10}$ G...3´
3´..... G $N_{9\text{-}10}$ ▲$N_{11\text{-}13}$ ^{x}C...5´

^{x}C = ^{ghm}C, ^{hm}C, ^{m}C or C

Description: AbaSI is a DNA modification-dependent endonuclease that recognizes 5-glucosylhydroxymethylcytosine (^{ghm}C) in double-stranded DNA and cleaves 11–13 bases 3´ from the modified C leaving a 2–3 base 3´ overhang. The enzyme only cleaves if there is a G residue 20–23 nucleotides 3´ from the modified C. AbaSI also recognizes 5-hydroxymethylcytosine (^{hm}C) at a much lower efficiency. It does not recognize DNA with 5-methylcytosine (^{m}c) or unmodified cytosine.

Reaction Conditions: rCutSmart Buffer, 25°C. Supplement with 1 mM DTT. Heat inactivation: 65°C for 20 minutes.

Concentration: 10,000 units/ml

Reagents Supplied:
- rCutSmart Buffer
- DTT

FspEI

rCutSmart RR dilB 37° 80° Epi

#R0662S 200 units

5´...C^{m}C $(N)_{12}$▼...3´
3´...G G $(N)_{16}$▲...5´

Description: FspEI is a modification-dependent endonuclease which recognizes C^{m}C sites and generates a double-stranded DNA break on the 3´ side of the modified cytosine at N_{12}/N_{16}. Recognized cytosine modifications include C5-methylation (5-mC) and C5-hydroxymethylation (5-hmC).

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with 1X Enzyme Activator Solution. Heat inactivation: 80°C for 20 minutes.

Concentration: 5,000 units/ml

Reagents Supplied:
- rCutSmart Buffer
- Enzyme Activator Solution

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

LpnPI

rCutSmart RR dilB 37° 65° Epi

#R0663S 200 units

5´...C^{m}C D G $(N)_{10}$▼...3´
3´...G GHC $(N)_{14}$▲...5´

Description: LpnPI is a modification-dependent endonuclease which recognizes C^{m}CDG sites and generates a double-stranded DNA break on the 3´ side of the modified cytosine at N_{10}/N_{14}. Recognized cytosine modifications include C5-methylation (5-mC) and C5-hydroxymethylation (5-hmC).

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with 1X Enzyme Activator Solution. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Reagents Supplied:
- rCutSmart Buffer
- Enzyme Activator Solution

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

MspJI

rCutSmart RR dilB 37° 65° Epi

#R0661S 200 units
#R0661L 1,000 units

5´...^{m}C N N R $(N)_{9}$▼...3´
3´... G N N Y $(N)_{13}$▲...5´

Description: MspJI is a modification-dependent endonuclease that recognizes ^{m}CNNR sites and generates a double-stranded DNA break on the 3´ side of the modified cytosine at N_9/N_{13}. The recognized cytosine modifications include C5-methylation (5-mC) and C5-hydroxymethylation (5-hmC).

Reaction Conditions: rCutSmart Buffer, 37°C. Supplement with 1X Enzyme Activator Solution. Heat inactivation: 65°C for 20 minutes.

Concentration: 5,000 units/ml

Reagents Supplied:
- rCutSmart Buffer
- Enzyme Activator Solution

Note: Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of >5%.

Single Letter Code:
R = A or G **Y** = C or T **M** = A or C **K** = G or T **S** = C or G **W** = A or T **H** = A or C or T (not G) **B** = C or G or T (not A) **V** = A or C or G (not T) **D** = A or G or T (not C) **N** = A or C or G or T

McrBC

NEB2 RR dilB 37° 65° rAlbumin Epi

#M0272S 500 units
#M0272L 2,500 units

5′...Pu^{m}C ($N_{40–3000}$) Pu^{m}C... 3′

- *Determination of the methylation state of CpG dinucleotides*
- *Detection of cytosine methylated DNA*

Description: McrBC is an endonuclease that cleaves DNA containing methylcytosine* on one or both strands. McrBC will not act upon unmethylated DNA. Sites on the DNA recognized by McrBC consist of two half-sites of the form (G/A)^{m}C. These half-sites can be separated by up to 3 kb, but the optimal separation is 55–103 base pairs. McrBC requires GTP for cleavage, but in the presence of a non-hydrolyzable analog of GTP, the enzyme will bind to methylated DNA specifically, without cleavage. McrBC will act upon a pair of Pu^{m}CG sequence elements, thereby detecting a high proportion of methylated CpGs, but will not recognize HpaII/MspI sites (CCGG) in which the internal cytosine is methylated.

*5-methylcytosine, 5-hydroxymethylcytosine or N4-methylcytosine

Reaction Conditions: NEBuffer 2, 37°C. Supplement with 1 mM GTP and 200 µg/ml Recombinant Albumin, Molecular Biology Grade. Heat inactivation: 65°C for 20 minutes.

Unit Definition: One unit is defined as the amount of enzyme required to cleave 0.5 µg of a plasmid containing multiple McrBC sites in 1 hour at 37°C in a total reaction volume of 50 µl. A pilot titration of enzyme is recommended for cleavage of genomic DNA.

Concentration: 10,000 units/ml

Reagents Supplied:

- NEBuffer 2
- Recombinant Albumin, Molecular Biology Grade
- Plasmid DNA for McrBC Linearized methylated (20 µl)
- GTP

Note: McrBC makes one cut between each pair of half-sites, cutting close to one half-site or the other, but cleavage positions are distributed over several base pairs approximately 30 base pairs from the methylated base (2). Therefore, the enzyme does not produce defined DNA ends upon cleavage. Also, when multiple McrBC half-sites are present in DNA (as is the case with cytosine-methylated genomic DNA) the flexible nature of the recognition sequence results in an overlap of sites, and so a smeared rather than a sharp banding pattern is produced.

Additional Restriction Enzymes for Epigenetic Analysis

DpnI
#R0176S 1,000 units
#R0176L 5,000 units

DpnII
#R0543S 1,000 units
#R0543L 5,000 units

for high (5X) concentration
#R0543T 1,000 units
#R0543M 5,000 units

HpaII
#R0171S 2,000 units
#R0171L 10,000 units

for high (5X) concentration
#R0171M 10,000 units

MspI
#R0106S 5,000 units
#R0106L 25,000 units

for high (5X) concentration
#R0106T 5,000 units
#R0106M 25,000 units

Methylation sensitive restriction enzymes can be used to generate fragments for further analysis. When used in conjunction with an isoschizomer that has the same recognition site, but is methylation insensitive, information about methylation status can be obtained.

See the Technical Reference section for more information about Dam, Dcm and CpG methylation.

DNA Methyltransferases

CpG Methyltransferase (M.SssI)
#M0226S 100 units
#M0226L 500 units

for high (5X) concentration
#M0226M 500 units

GpC Methyltransferase (M.CviPI)
#M0227S 200 units
#M0227L 1,000 units

AluI Methyltransferase
#M0220S 100 units

BamHI Methyltransferase
#M0223S 100 units

dam Methyltransferase
#M0222S 500 units
#M0222L 2,500 units

EcoGII Methyltransferase
#M0603S 200 units

EcoRI Methyltransferase
#M0211S 10,000 units

HaeIII Methyltransferase
#M0224S 500 units

HhaI Methyltransferase
#M0217S 1,000 units

HpaII Methyltransferase
#M0214S 100 units

MspI Methyltransferase
#M0215S 100 units

*Taq*I Methyltransferase
#M0219S 1,000 units

NEB offers a selection of DNA methyltransferases that can be used in epigenetics research. More information on these products can be found in the DNA Modifying Enzymes & Cloning Technologies chapter or at www.neb.com.

NEBNext® Reagents for ChIP-Seq Library Preparation Epi

NEBNext reagents are a series of highly pure reagents that facilitate library preparation of DNA or RNA for downstream applications, such as next generation sequencing and expression library construction. These reagents undergo stringent quality controls and functional validation, ensuring maximum yield, convenience and value.

For sample preparation of a ChIP-Seq DNA library, NEB offers kits, oligos and modules that support fast workflows. To decide which products to choose, use the selection chart below. For more information, see our NEBNext Reagents for Library Preparation.

ChIP

- **Kits**
  - **NEBNext Ultra II DNA Library Prep Kit +/– Sample Purification Beads** (#E7645/#E7103)
- **Modules**
  - **NEBNext Ultra II End Repair/dA-Tailing Module** (#E7546)
  - **NEBNext Ultra II Ligation Module** (#E7595)
  - **NEBNext Ultra II Q5 Master Mix** (#M0544)

Multiplex Oligos

Single Index	Dual Index	Unique Dual Index	Unique Dual Index UMIs
Set 1 (#E7335), Set 2 (#E7500), Set 3 (#E7710), Set 4 (#E7730), 96 Index Primers (#E6609)	Set 1(#E7600), Set 2 (#E7780)	Set 1 (#E6440), Set 2 (#E6442), Set 3 (#E6444), Set 4 (#E6446), Set 5 (#E6448), Primer Pairs for EM-seq (#E7140)	Set 1(#E7395), Set 2 (#E7874), Set 3 (#E7876), Set 4 (#E7878)

NEBNext® Enzymatic Methyl-seq (EM-seq™)

NEBNext Enzymatic Methyl-seq Kit
#E7120S 24 reactions
#E7120L 96 reactions

NEBNext Enzymatic Methyl-seq Conversion Module
#E7125S 24 reactions
#E7125L 96 reactions

NEW
NEBNext Enzymatic 5hmC-seq Kit
#E3350S 24 reactions
#E3350L 96 reactions

NEW
NEBNext Enzymatic 5hmC-seq Conversion Module
#E3365S 24 reactions
#E3365L 96 reactions

NEBNext Q5U® Master Mix
#M0597S 50 reactions
#M0597L 250 reactions

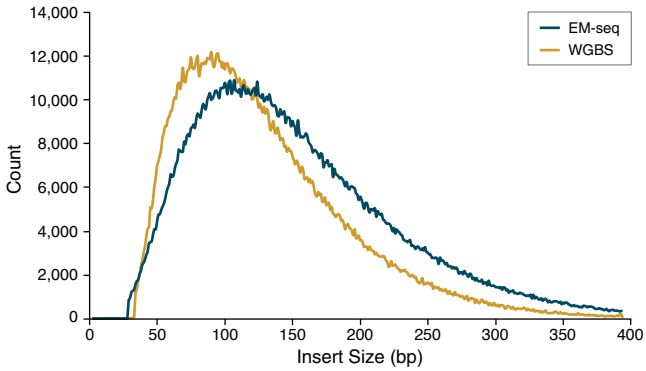
NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)
#E7140S 24 reactions
#E7140L 96 reactions

NEW
NEBNext Multiplex Oligos for Enzymatic 5hmC-seq
#E3360S 24 reactions
#E3360L 96 reactions

- *Superior sensitivity of detection of 5mC and 5hmC*
- *Larger library insert sizes*
- *More uniform GC coverage*
- *Greater mapping efficiency*
- *High-efficiency library preparation*

Description: While bisulfite sequencing has been the gold standard for the study of DNA methylation, this conversion treatment is damaging to DNA, resulting in DNA fragmentation, loss and GC bias. The NEBNext Enzymatic Methyl-seq Kit (EM-seq) provides an enzymatic alternative to whole genome bisulfite sequencing (WGBS), combined with high-efficiency streamlined library preparation suitable for Illumina sequencing.

The highly effective EM-seq enzymatic conversion minimizes damage to DNA and, in combination with the supplied NEBNext Ultra II library preparation workflow reagents, results in high quality libraries that enable superior detection of 5mC and 5hmC from fewer sequencing reads. Products specific for 5hmC detection (E5hmc-seq™) are also available.



NEBNext Enzymatic Methyl-seq libraries have larger insert sizes. *50 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris® S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold kit for bisulfite conversion. Libraries were sequenced on an Illumina MiSeq (2 x 76 bases) and insert sizes were determined using Picard 2.18.14. The normalized frequency of each insert size was plotted, illustrating that library insert sizes are larger for EM-seq than for WGBS, and indicating that EM-seq does not damage DNA as bisulfite treatment does in WGBS.*

COVARIS® is a registered trademark of Covaris, Inc.

NEBNext Enzymatic Methyl-seq is an enzymatic alternative to bisulfite conversion with superior performance. For more information, including extensive performance data, visit **NEBNext.com.**

The cost of fast fashion

Many of us consider ourselves conscientious consumers of food. We read the ingredient list and know how it was produced, but how many of us think about what goes into manufacturing the clothes we wear? Fast fashion, or the mass production of cheap clothing made from artificial fibers produced using fossil fuels, is responsible for 10% of the world's greenhouse gas emissions. Eighty billion items of clothes are made each year globally, 10% of which are never sold and go straight to landfills. We buy more, wear each item less often, and readily dispose of clothing because it is cheap and of poor quality.

Much of the clothing made in the past thirty years has had a carbon-intensive journey before it is sold – raw materials are grown or produced in one country, shipped to another for processing, another to be sewn into garments, and eventually to the final selling destination. Every step generates carbon emissions, and the fast fashion industry is on track to increase emissions by 60% in 2030.

While individuals can feel powerless to impact climate change when large-scale challenges call for technical innovations or policy changes, giving up fast fashion is in the hands of the consumer. Scaling back our wardrobes and becoming informed about the environmental impacts throughout the product lifecycle can prevent the millions of tons of clothes from ending up in a landfill.

Some of the first eco-friendlier options that come to mind are renting, donating and recycling – but these all have drawbacks. Recent analyses have determined that renting clothes, once considered an environmentally friendly option, creates more greenhouse gases due to extensive shipping and dry cleaning of garments. Donating may seem like a charitable, eco-conscious option, but 90% of clothes donated to thrift stores are rejected and sent to a landfill or a textile waste mill, creating massive graveyards of clothing in countries such as Ghana and Chile. Here, the local communities are exposed to toxicity caused by incinerating synthetic fabrics and dyes that leech into groundwater supplies. Recycling, at this time, also has drawbacks. Over 92 million tons of textile waste are created each year globally, and only 1% of these garments are recycled. Fast fashion garments are typically made from a combination of natural fibers like cotton or wool and non-degradable synthetic fibers such as polyester derived from petroleum. Recycling is not economically feasible because separating these fibers is a complex, labor-intensive process. However, the European Union is taking action with recycling resolutions requiring more clothes made from single fibers to increase their recyclability and lifespan.

High-quality, long-lasting, single-fiber clothing is not universally affordable. Sometimes fast fashion purchases cannot be avoided, but thoughtful purchases lead to less waste. Consignment or thrift stores have unique bargains, and mending clothes rather than discarding them is also an option.

Some of the biggest fast fashion brands have committed to significant changes and sustainable fabrics as soon as 2030. This is a big step forward; however, an item of clothing is made of many different materials and dyes. The manufacturer needs to make each piece of the garment and process less harmful to the environment. Look to brands that have transparency throughout the whole supply and production chain.

The core issue is overconsumption and over-production. Buy less overall – resist the urge to give in to trends dictated by fast fashion brands. Purchase items that can withstand the test of time. Consumers can influence and pressure big brands to make a difference. So, become informed but mostly, buy less.

Find out how your fashion choices can make a difference.

Industrial textile factory.
Coils with threads in production.
Credit: andreysha74, Adobe Stock

Cellular Analysis

Novel tools to study expression & function of proteins.

Cell imaging analysis can use fluorescent dyes, fluorophore-labeled molecules or recombinant protein plasmid systems. Recombinant protein labeling systems and bioluminescent reporter systems are among the most sensitive fluorescence methods for imaging expression, transport, co-localization and degradation in either fixed or living cells. Protein labeling systems offer many advantages. For example, color changes can be easily implemented by using different substrates. Protein labeling systems can involve the use of tag-specific antibodies or antibodies to separate epitopes engineered into a plasmid tag system for detection. Protein labeling systems can be used with non-cell permeable substrates to enable the specific imaging of cell surface targets. This strategy is not possible with bioluminescent recombinant systems. In living cells, protein labeling substrates can be introduced and followed in cells over time. Two separate cellular targets can also be imaged simultaneously, using protein labeling systems with mutually exclusive, tag-specific fluorescent substrates.

Studies of protein expression, interactions and structure, often use reporter systems to introduce and select for gene targets in cells. Reporter genes confer drug resistance, bioluminescence or fluorescence properties in the cells into which they are introduced. Typical reporter studies link reporter genes directly to a promoter region of interest, the function of which can be monitored by the reporter activity. Protein fusion tagging is used to detect subcellular localization, degradation, protein-protein interactions, etc. Typical fusion tags are fluorescent proteins (e.g., eGFP) or small protein epitopes (e.g., FLAG, Myc HA) which can be detected by fluorescence FACS or western blots. New generations of reporter gene systems expand the range of applications and enhance experimental possibilities.

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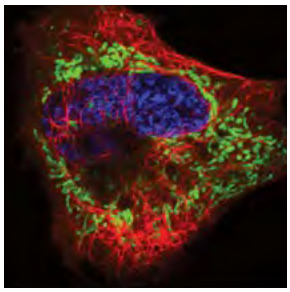
Cellular Imaging & Analysis



Live HeLa cell transfected with pSNAP$_f$-tubulin and pCLIP$_f$-Cox8A (mitochondrial cytochrome oxidase 8A). *Cells were labeled with 3 µM SNAP-Cell TMR-Star (red) and 5 µM CLIP-Cell 505 (green) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.*

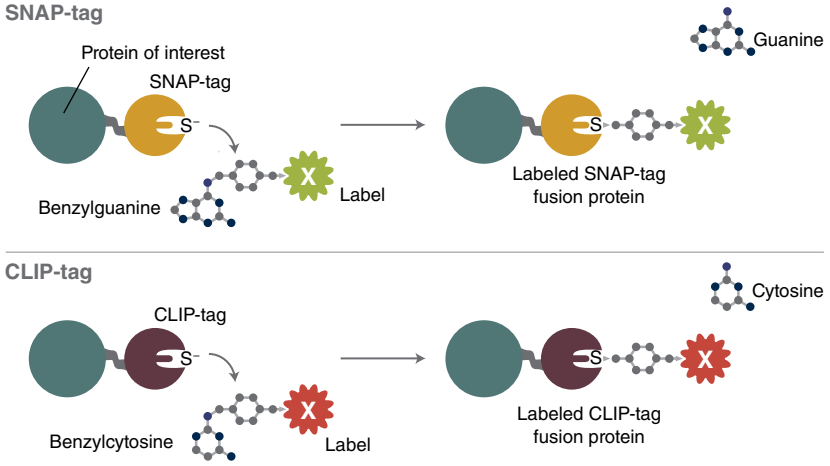
Features of SNAP-tag and CLIP-tag:

- *Clone and express once, then use with a variety of substrates*
- *Non-toxic to living cells*
- *Wide selection of fluorescent substrates*
- *Highly specific covalent labeling*
- *Simultaneous dual labeling*

Applications of SNAP-tag and CLIP-tag:

- *Simultaneous dual protein labeling inside or on the surface of live cells*
- *Protein localization and translocation*
- *Pulse-chase experiments*
- *Receptor internalization studies*
- *Selective cell surface labeling*
- *Protein pull down assays*
- *Protein detection in SDS-PAGE*
- *Flow cytometry*
- *High throughput binding assays in microtiter plates*
- *Biosensor interaction experiments*
- *FRET-based binding assays*
- *Single molecule labeling*
- *Super-resolution microscopy*

New England Biolabs offers an innovative technology for studying the function and localization of proteins in live and fixed cells. Covalent protein labeling brings simplicity and versatility to the imaging of mammalian proteins in live cells, as well as the ability to capture proteins *in vitro*. The creation of a single genetic construct generates a fusion protein which, when covalently attached to a variety of fluorophores, biotin or beads, provides a powerful tool for studying proteins. For added flexibility, NEB offers two systems in which the protein is labeled by a self-labeling fusion protein (SNAP-tag® and CLIP-tag™).



Protein labeling with SNAP-tag and CLIP-tag. *The SNAP- or CLIP-tag is fused to the protein of interest. Labeling occurs through covalent attachment to the tag, releasing either a guanine or a cytosine moiety.*

SNAP-tag and CLIP-tag – Self-Labeling Tag Technology

The SNAP- and CLIP-tag protein labeling systems enable the specific, covalent attachment of virtually any molecule to a protein of interest. There are two steps to using this system: cloning and expression of the protein of interest as a SNAP-tag fusion, and labeling of the fusion with the SNAP-tag substrate of choice. The SNAP-tag is based on the human O6-alkylguanine-DNA-alkyltransferase (hAGT), a DNA repair protein. SNAP-tag substrates are fluorophores, biotin or beads conjugated to guanine or chloropyrimidine leaving groups via a benzyl linker. In the labeling reaction, the substituted benzyl group of the substrate is covalently attached to the SNAP-tag. CLIP-tag is a modified version of SNAP-tag, engineered to react with benzylcytosine rather than benzylguanine derivatives. When used in conjunction with SNAP-tag, CLIP-tag enables the orthogonal and complementary labeling of two proteins simultaneously in the same cells.

SNAP-Cell®: SNAP-Cell labels are cell-permeant and uniquely suited for the labeling of SNAP-tag fusion proteins inside living or fixed cells, on cell surfaces or *in vitro*. These labels are spread across the visible spectrum, ranging from blue to red. Non-fluorescent cell-permeable blocking agent is also available.

SNAP-Surface®: SNAP-Surface labels are non-cell-permeant and routinely used to label SNAP-tag fusion proteins on the surface of living cells, in fixed cells or *in vitro*. These labels are spread across the visible spectrum and include the photostable AlexaFluor® dyes and a variety of other commonly used fluorophores.

CLIP-Cell™: CLIP-Cell labels are cell-permeant and uniquely suited for the labeling of CLIP-tag fusion proteins inside living or fixed cells, on cell surfaces or *in vitro*. The CLIP-tag is a derivative of the SNAP-tag that reacts with orthogonal substrates, allowing simultaneous labeling of two expressed proteins with different fluorophores.

CLIP-Surface™: CLIP-Surface labels are non-cell permeant and routinely used to label CLIP-tag fusion proteins on the surface of living cells, in fixed cells or *in vitro*. The CLIP-tag is a derivative of the SNAP-tag that reacts with orthogonal substrates, allowing simultaneous labeling of two expressed proteins with different fluorophores. The labels include fluorophores at commonly used areas of the visible spectrum, such as 488, 547 and 647 nm.

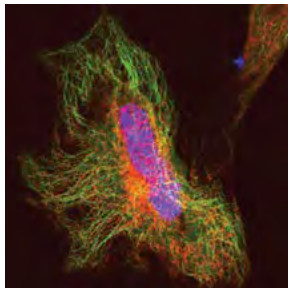
Find an overview of SNAP-tag labeling.

Comparison of SNAP-tag/CLIP-tag Technologies to GFP

While SNAP/CLIP-tag technologies are complementary to GFP, there are several applications for which SNAP- and CLIP-tag self-labeling technologies are advantageous.

Application	SNAP-tag/CLIP-tag	GFP and Other Fluorescent Proteins
Time-resolved fluorescence	Fluorescence can be initiated upon addition of label	Color is genetically encoded and always expressed. Photoactivatable fluorescent proteins require high intensity laser light, which may activate undesired cellular pathways (e.g., apoptosis)
Pulse-chase analysis	Labeling of newly synthesized proteins can be turned off using available blocking reagents (e.g., SNAP-Cell® Block)	Fluorescence of newly synthesized proteins cannot be specifically quenched to investigate dynamic processes
Ability to change colors	A single construct can be used with different fluorophore substrates to label with multiple colors	Requires separate cloning and expression for each color
Surface specific labeling	Can specifically label subpopulation of target protein expressed on cell surface using non-cell permeant substrates	Surface subpopulation cannot be specifically visualized
Single molecule detection	Conjugation with high quantum yield and photostable fluorophores	Fluorescent proteins are generally less bright and photobleach quicker than most organic fluorophores
Visualizing fixed cells	Resistant to fixation; strong labeling	Labile to fixation; weak labeling
Pull-down studies	"Bait" proteins can be covalently captured on BG beads	Requires anti-GFP antibody to non-covalently capture "bait" protein, complicating downstream analysis
Live animal imaging	Cell permeable far-red dye available, permitting deep tissue visualization	Signal is easily quenched by fixation (whole-mount specimens or thick sections); limited spectral flexibility and weaker fluorescence

Fluorescent Substrates for Protein Labeling



Live HeLa cell transfected with pSNAP$_f$-ER (endoplasmic reticulum) and pCLIP$_f$-tubulin.
Cells were labeled with 3 µM SNAP-Cell TMR-Star (red) and 5 µM CLIP-Cell 505 (green) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.

- *Fluorescently label SNAP-tag or CLIP-tag fusions for cellular imaging*
- *Labels span fluorescent imaging spectrum from aqua (430 nm) to far-red (647+ nm) wavelengths*
- *Cell-permeable and non-cell-permeable labels available*

NEB offers a large selection of fluorescent labels (substrates) for SNAP-tag and CLIP-tag fusion proteins. SNAP-tag substrates consist of a fluorophore conjugated to guanine or chloropyrimidine leaving groups via a benzyl linker, while CLIP-tag substrates consist of a fluorophore conjugated to a cytosine leaving group via a benzyl linker. These substrates will label their respective tags without the need for additional enzymes. Cell-permanent substrates (SNAP- and CLIP-Cell) are suitable for both intracellular and cell-surface labeling, whereas non-cell-permeant substrates (SNAP- and CLIP-Surface) are specific for fusion proteins expressed on the cell surface only.

Fluorescent substrates for SNAP-tag and CLIP-tag

Self-Labeling Tag	Applications	NEB #	Excitation*	Emission*[1]	Size
SNAP-tag	**Cell-permeable**				
	SNAP-Cell 430	S9109S	421	444,484	50 nmol
	SNAP-Cell 505-Star	S9103S	504	532	50 nmol
	SNAP-Cell Oregon Green®	S9104S	490	514	50 nmol
	SNAP-Cell TMR-Star	S9105S	554	580	30 nmol
	SNAP-Cell 647-SiR	S9102S	645	661	30 nmol
	Non-cell-permeable				
	SNAP-Surface Alexa Fluor 488	S9129S	496	520	50 nmol
	SNAP-Surface 488	S9124S	506	526	50 nmol
	SNAP-Surface Alexa Fluor 546	S9132S	558	574	50 nmol
	SNAP-Surface 549	S9112S	560	575	50 nmol
	SNAP-Surface 594	S9134S	606	626	50 nmol
	SNAP-Surface Alexa Fluor 647	S9136S	652	670	50 nmol
	SNAP-Surface 649	S9159S	655	676	50 nmol
CLIP-tag	**Cell-permeable**				
	CLIP-Cell 505	S9217S	504	532	50 nmol
	CLIP-Cell TMR-Star	S9219S	554	580	30 nmol
	Non-cell-permeable				
	CLIP-Surface 488	S9232S	506	526	50 nmol
	CLIP-Surface 547	S9233S	554	568	50 nmol
	CLIP-Surface 647	S9234S	660	673	50 nmol

* Excitation and emission values determined experimentally for labeled protein tag.
[1] Colors are based on the electromagnetic spectrum. Actual color visualization may vary.

Blocking Agents

- *Irreversible blocking*
- *Ideal for pulse-chase applications*

Blocking agents are non-fluorescent substrates that block the reactivity of the SNAP-tag intracellularly (SNAP-Cell Block) or on the surface of live and fixed cells (SNAP-Surface Block). They can be used to generate inactive controls in live and fixed cells, as well as in *in vitro* labeling experiments performed with SNAP-tag fusion proteins.

SNAP-Cell Block is highly membrane permeant and once inside the cell reacts with the SNAP-tag, irreversibly inactivating it for subsequent labeling steps.

SNAP-Surface Block also reacts with the SNAP-tag irreversibly, inactivating it for subsequent labeling steps. This blocker is largely membrane impermeant essentially limiting blocking to cell surface-exposed SNAP-tags.

Product	NEB #	Application	Size
SNAP-Cell Block	S9106S	Block SNAP-tag inside live cells, fixed cells and *in vitro*	100 nmol
SNAP-Surface Block	S9143S	Block SNAP-tag on the surface of live cells, fixed cells and *in vitro*	200 nmol

Anti-SNAP-tag® Antibody (Polyclonal)

#P9310S 100 µl

Description: The Anti-SNAP-tag Antibody (Polyclonal) can be used in Western blots with SNAP-tag and CLIP-tag proteins. Polyclonal antibodies are produced from the immunization of rabbit with purified recombinant SNAP-tag protein and affinity purified using SNAP-BG resin.

Sensitivity: 5 ng of SNAP-tag per load in Western blotting.

Recommended Dilution: 1:1000

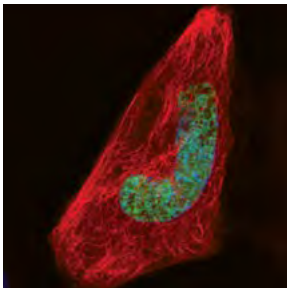
SNAP-tag® Purified Protein

#P9312S 50 µg

Description: SNAP-tag Purified Protein can be used as a positive control for *in vitro* labeling with various SNAP-tag fluorescent substrates. The coding sequence of SNAP-tag was cloned into a pTXB1 derived *E. coli* T7 expression vector. SNAP-tag protein was expressed and purified according to the instructions in the IMPACT™ kit manual (NEB #E6901). The purified SNAP-tag protein was dialyzed into 1X phosphate buffered saline (PBS) solution containing 1 mM DTT at 1 mg/ml (50 µM) and stored at -80°C.

Molecular Weight: 19,694 Da

Cloning Vectors



Live HeLa cell transfected with pSNAP$_f$-tubulin and pCLIPf-H2B constructs generated using pSNAP$_f$ and pCLIP$_f$ vectors. *Cells were labeled with 3 µM SNAP-Cell TMR-Star (red) and 5 µM CLIP-Cell 505 (green) for 25 minutes and counterstained with Hoechst 33342 (blue) for nuclei.*

- *Vectors for mammalian and bacterial expression available*

Vectors are available for SNAP-tag and CLIP-tag fusion protein expression and labeling in mammalian and bacterial systems. The mammalian SNAP$_f$ and CLIP$_f$ vectors express faster-reacting variants of the SNAP- and CLIP-tags than previously available vectors. Improved polylinker sequences both upstream and downstream from the tag allow expression of the tag on either end of the protein of interest, under control of the CMV promoter. SNAP$_f$-tag and CLIP$_f$-tag expression vectors contain a neomycin resistance (NeoR) gene for selection of stable transfectants, together with an IRES element for efficient expression of both the fusion protein and NeoR. Codon usage has been optimized for mammalian expression. Control plasmids encoding fusion proteins that are localized to the nucleus (H2B), mitochondria (Cox8A) and cell surface (ADRβ2, NK1R) are also available through Addgene.

The bacterial expression vector pSNAP-tag(T7)-2 includes cloning sites both upstream and downstream from the SNAP-tag, which is under the control of the T7 promoter. Codon usage in the SNAP-tag gene has been optimized for *E. coli* expression.

Source: Isolated from an *E. coli* strain by a standard plasmid purification procedure. Plasmids have been purified free of endotoxins for efficient transfection.

Concentration: 500 µg/ml

Restriction Map: The restriction map for pSNAP$_f$ Vector can be found in the Technical Reference section. Additional sequence and map files for expression and control plasmids can be found at www.neb.com.

Product	NEB #	Features	Size
pSNAP$_f$ Vector	N9183S	Stable and transient mammalian expression	20 µg
pSNAP-tag (T7)-2 Vector	N9181S	Bacterial expression under T7 control	20 µg
pCLIP$_f$ Vector	N9215S	Stable and transient mammalian expression	20 µg

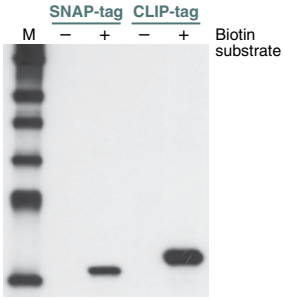
Biotin Labels

SNAP-Biotin
#S9110S 50 nmol

CLIP-Biotin
#S9221S 50 nmol

- *Label SNAP-tag and CLIP-tag fusions with biotin*
- *Compatible with a variety of streptavidin conjugates*
- *Attach to streptavidin surfaces on microtiter plates*

For optimal flexibility with existing technologies, biotinylated labels are available for studies using streptavidin platforms. Cell-permeant (SNAP-Biotin and CLIP-Biotin) substrates are based on biotin with an amidocaproyl linker. Biotin labels are suitable for applications such as biotinylation of fusion proteins for detection with streptavidin fluorophore conjugates or labeling in solution for analysis by SDS-PAGE/ Western blot. Biotin labels are also used for capture with streptavidin surfaces for binding and interaction studies.



Western blot analysis of biotin labeling reactions using anti-Biotin Antibody (CST #7075). *SNAP-tag and CLIP-tag (5 µM) labeled with a biotin-containing substrate (10 µM). Marker M is Biotinylated Protein Ladder (CST #7727).*

SNAP-Capture Magnetic Beads

#S9145S 2 ml

- *Selectively capture SNAP-tag fusion proteins from solution*
- *Ideal for protein pull-down experiments or proteomic analysis*

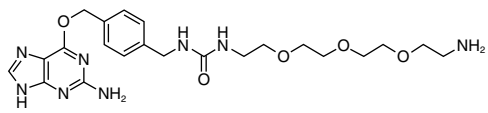
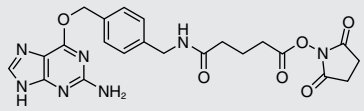
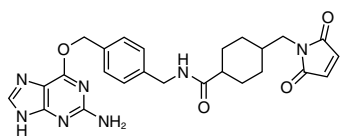
SNAP-Capture Magnetic Beads are magnetic agarose beads coupled to a benzylguanine substrate, used to selectively capture and immobilize SNAP-tag fusion proteins from solution. These beads have a high loading capacity for SNAP-tag fusion proteins and show very low non-specific adsorption of proteins from a complex lysate, making them especially suitable for pull-down applications.

Building Blocks

- *Synthesize new SNAP-tag and CLIP-tag substrates*
- *Make surfaces for protein immobilization*
- *Attach novel molecules or ligands to proteins*
- *Create custom substrates for protein labeling*

For advanced users with novel probes interested in working with SNAP-tag labeling technologies, building blocks are available for linkage of the core benzylguanine (BG) moiety to activated esters, primary amines and thiol groups. A variety of functional groups allows the choice of chemical coupling approaches to suit the molecule or surface to be coupled. Couple onto surfaces such as Biacore® chips or microarrays for specific protein immobilization. Couple onto peptides, proteins and DNA oligomers. Couple onto new fluorophores or affinity reagents for specific protein labeling. Labeling is gentle, precise, and versatile: one label is covalently bound under biological conditions in a defined position.

BIACORE® is a registered trademark of GE Healthcare Life Sciences

Product	NEB #	Structure	Application	Size
BG-PEG-NH$_2$	S9150S		SNAP-tag substrate. PEG-linker gives superior flexibility. Particularly suited for immobilization on solid surfaces.	2 mg
BG-GLA-NHS	S9151S		SNAP-tag substrate. Activated as NHS ester. Reacts with primary amines.	2 mg
BG-Maleimide	S9153S		SNAP-tag substrate. Activated as maleimide. Reacts with thiols.	2 mg

How can you reduce your carbon footprint in the lab?

Scientists can take low- or no-cost steps to reduce the carbon footprint of laboratory work to de-escalate climate change. Resources, such as My Green Lab® and Laboratory Efficiency Assessment Framework (LEAF) provide implementation programs. Labconscious® helps to drive awareness of the latest lab sustainability challenges and what solutions biologists are using to overcome them.

The scientific consensus is that the accelerating rate of climate change is a threat tied to atmospheric greenhouse gases (GHG) originating from human activities. Over the past two million years life has evolved in tandem with approximately 1°C of global warming every thousand years, based on paleoclimate data. However, global warming has risen at 0.18°C per decade since 1981. This rate shift from millennium to century timeframes presents a stark challenge. Climate models predict that another 2°C warming will greatly intensify heat and precipitation patterns. Unfortunately, species adaptation is often slower than the climate changes that provoke it. Already, more than one million species are on the brink of extinction. The atmosphere has been flooded with heat absorbing GHG, primarily carbon dioxide (CO_2), from burning fossil fuels. Natural carbon sinks like plants, soil and oceans cannot keep up.

Climatologists predict that cutting carbon emissions by approximately 50% by 2030 will help avoid the worst threats. Highly industrialized nations are responsible for sixty eight percent of global GHG emissions, giving individuals in these societies an outsized impact. Into the bargain, laboratories represent resource intensive spaces primed for reductions in GHG emissions.

Carbon footprint is a useful measure because it can attach outcomes to separate conditions, to some degree. It is reported in metric tonnes of carbon dioxide equivalent (CO_2e) to represent global warming potential generated by an activity or entity. Carbon footprint calculators incorporate different parameters and methodologies. In recent years, carbon footprint calculators have been applied to lab work. These can be open source, commercial, or accessible through public sustainability frameworks. Estimating the carbon footprint of a laboratory is not necessary for improvements, but it can support decision making.

Biologists have many low-cost options to reduce laboratory energy and waste. Sustainability objectives should be communicated in lab meetings, training and procedures. Signage is an especially effective tool to set clear expectations for lab users. “Shut the sash” stickers on variable air volume (VAV) fume hoods dramatically reduce energy consumption. As does strategically raising ultra-low temperature freezers to -70 °C and consolidating inventory. “Turn off” signs save energy with small lab equipment with monitors, chilling or heating components. Autoclaves should be run at full capacity. Waste diversion goals should prioritize avoidance of non-contaminated materials deposited in hazardous waste streams associated with higher emissions. Polypropylene pipette tip boxes can be refilled with bulk bagged tips and autoclaved. Recycling services with verified downstream resin buyers divert emissions from virgin materials. Replacing consumables with reusable labware whenever possible reduces CO_2e. Techniques that require fewer single-use plastic flasks, plates, tubes, tips and multi-well plates reduce waste. Consolidating lab orders reduces packaging and transport. Virtual meetings and conferences eliminate travel resulting in a 1000–3000-fold lower carbon footprint. Each of these strategies can empower individual scientists or life science organizations to reduce carbon footprints.

Science background pipette tips.
Credit: eplisterra, Adobe Stock

Test your sustainability IQ with this Virtual Lab Scavenger Hunt.

Reference Appendix

Technical Support – for scientists, by scientists

As a partner to the scientific community, New England Biolabs is committed to providing top quality tools and scientific expertise. This philosophy has led to long-standing relationships with many of our fellow scientists. NEB's commitment to scientists is the same regardless of whether or not they purchase products from NEB; their ongoing research is supported by our catalog, website and technical staff.

NEB's technical support model is unique as it utilizes most of our scientists. Several of our product lines have designated technical support scientists assigned to serving customers in those application areas. Questions regarding a product could be dealt with by one of the technical support scientists, the product manager who manufactures it, the product development scientist who optimizes it, or a researcher who uses the product in their daily research. As such, customers are supported by scientists and experts in the product or its application.

To access technical support:

- Call 1-800-632-7799 (Monday – Friday: 9:00 am - 6:00 pm EST)
- Submit an online form at www.neb.com/techsupport
- Email info@neb.com
- International customers can contact a local NEB subsidiary or distributor. For more information see inside back cover.

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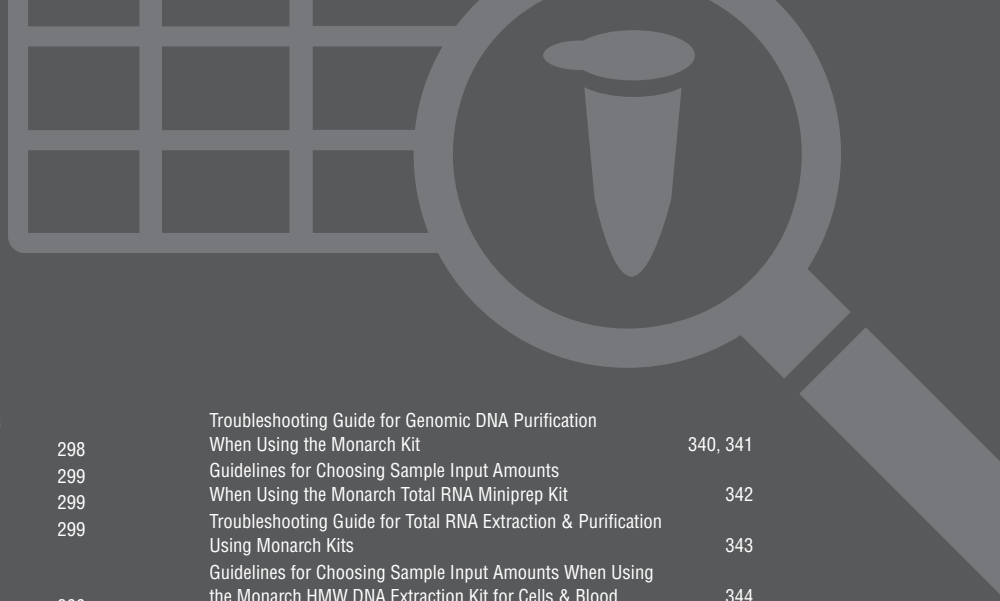
Visit the **Tools & Resources tab** at **www.neb.com** to find additional online tools, video tech tips and tutorials to help you in your research.

Learn more about NEB's tech support program.



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Online Interactive Tools, Databases & Mobile Apps

Use the Tools & Resources tab at www.neb.com to access our growing selection of interactive technical tools. These tools can also be accessed directly by visiting **www.neb.com/nebtools**.

NEB scientists are often involved in the development of online tools that will aid in their research. We are now making these tools and in some circumstances, the source code, available for you to evaluate. To learn more, visit **www.neb.com/NEBetaTools**.

Online Tools

Competitor Cross-Reference Tool

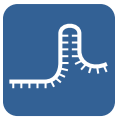
Use this tool to select another company's product and find out which NEB product is compatible. Choose either the product name or catalog number from the available selections, and this tool will identify the recommended NEB product.

DNA Sequences and Maps Tool

With the DNA Sequences and Maps Tool, find the nucleotide sequence files for commonly used molecular biology tools, including plasmid, viral and bacteriophage vectors.

Double Digest Finder

Use this tool to guide your reaction buffer selection when setting up double digests, a common timesaving procedure. Choosing the right buffers will help you to avoid star activity and loss of product.



EnGen sgRNA Template Oligo Designer

EnGen sgRNA Template Oligo Designer can be used to design target-specific DNA oligos for use with the EnGen sgRNA Synthesis Kit, *S. pyogenes* (NEB #E3322).

Enzyme Finder

Use this tool to select restriction enzymes by name, sequence, overhang or type. Enter your sequence using single letter code nomenclature, and Enzyme Finder will identify the right enzyme for the job.

Exo Selector

Use this tool to simplify the process of selecting the appropriate exonucleases for use in your nucleic acid digestion workflows. The tool guides you to product recommendations based on your answers to a few simple questions.

Glycan Analyzer

Use this tool to interpret ultra or high pressure liquid chromatography (UPLC/HPLC) N-glycan profiles following exoglycosidase digestions.

NEBridge™ Ligase Fidelity Tools

These tools can be used to design your Golden Gate Assemblies – visualize overhang ligation preferences with the Ligase Fidelity Viewer™, Predict high-fidelity junction sets with GetSet™, and split DNA sequences for scarless high-fidelity assembly with SplitSet™.

NEBridge® Golden Gate Assembly Tool

Use this tool to assist with in silico DNA construct design for Golden Gate DNA assembly. It enables the accurate design of primers with appropriate Type IIS restriction sites and overlaps, quick import of sequences in many formats and export of the final assembly, primers and settings.

NEB LAMP Primer Design Tool

NEB LAMP Primer Design Tool can be used to design primers for your Loop-mediated Isothermal Amplification. Fixed primers can be specified for the design of LAMP primers, and subsequent Loop primers are then designed based on LAMP primer selection.

NEBaseChanger®

NEBaseChanger can be used to design primers specific to the mutagenesis experiment you are performing using the Q5® Site-Directed Mutagenesis Kit. This tool will also calculate a recommended custom annealing temperature based on the sequence of the primers by taking into account any mismatches.

NEBcloner®

Use this tool to find the right products and protocols for each step (digestion, end modification, ligation, transformation and mutagenesis) of your next traditional cloning experiment. Also, find other relevant tools and resources to enable protocol optimization.

NEBcutter® V3.0

Identify the restriction sites within your DNA sequence using NEBcutter. Choose between Type II and commercially available Type III restriction enzymes to digest your DNA. NEBcutter will indicates cut frequency and methylation-state sensitivity.

NEBioCalculator®

Use this tool for your scientific calculations and conversions for DNA and RNA. Options include conversion of mass to moles, ligation amounts, conversion of OD to concentration, dilution and molarity. Additional features include sgRNA template oligo design and qPCR library quantification.

NEBNext Custom RNA Depletion Design Tool

This tool designs probes to be used with the NEBNext RNA Depletion Core Reagent Set or to supplement an existing NEBNext Depletion Kit for the depletion of unwanted RNA species.

NEBNext Selector

Use this tool to guide you through the selection of NEBNext reagents for next generation sequencing sample preparation.

Online Interactive Tools, Databases & Mobile Apps (continued)

NEBuilder® Assembly Tool

Use this tool to design primers for your DNA assembly reaction, based on the entered fragment sequences and the polymerase being used for amplification.

PCR Fidelity Estimator

Estimate the percentage of correct DNA copies (those without base substitution errors) per cycle of PCR for selected DNA polymerases.

PCR Selector

Use this tool to help select the right DNA polymerase for your PCR setup. Whether your amplicon is long, complex, GC-rich or present in a single copy, the PCR selection tool will identify the perfect DNA polymerase for your reaction.

Read Coverage Calculator

This tool allows for easy calculation of values associated with read coverage in NGS protocols.

Thermostable Ligase Reaction Temperature Calculator

This tool will help you estimate an optimal reaction temperature to minimize mismatch for thermostable ligation of two adjacent ssDNA probes annealed to a template.

Tm Calculator

Determine the optimal annealing temperature for your amplicon with our Tm Calculator. Simply input your DNA polymerase, primer concentration and your primer sequence, and the Tm Calculator will guide you to successful reaction conditions.

Additional Databases

Polbase®

Polbase is a repository of biochemical, genetic and structural information about DNA Polymerases.

REBASE®

Use this tool as a guide to the ever-changing landscape of restriction enzymes. REBASE, the Restriction Enzyme DataBASE, is a dynamic, curated database of restriction enzymes and related proteins.

Freezer Locator

NEBnow Locator

NEBnow Freezer Programs are ideally suited for researchers in academics and industry looking for on-site access to the world's finest restriction enzymes and related products. NEB freezers offer you convenience, flexibility and value.

Mobile Apps

NEB Tools for iPhone®, iPad® or Android®

NEB Tools brings New England Biolabs' most popular web tools to your iPhone, iPad or Android devices.

- Use Enzyme Finder to select a restriction enzyme by category or recognition sequence, or search by name to find information on any NEB enzyme. Sort your results so they make sense to you, then email them to your inbox or connect directly to www.neb.com.
- Use Double Digest Finder to determine buffer and reaction conditions for experiments requiring two restriction enzymes.
- Use Tm Calculator to calculate annealing temperatures for your PCR reaction.
- Also included are several popular calculators from the NEBioCalculator web app.

NEB Tool for Augmented Reality

Download the NEB Augmented Reality (AR) app for iPhone or iPad at the Apple® App Store or for Android on Google Play™.

Optimizing Restriction Enzyme Reactions

While standard recommended reaction conditions are a good place to start, in some cases, optimization may be necessary to achieve the best results. Depending on the enzyme(s) being used, variables such as incubation time and number of enzyme units used can be tested to find the optimal reaction conditions for your substrate DNA and enzyme(s) of choice.

Protocol Restriction Enzyme Reactions

	Standard Protocol	Time-Saver Protocol
DNA	up to 1 µg	up to 1 µg
10X Buffer	5 µl (1X)	5 µl (1X)
Restriction Enzymes	10 units*	1 µl
Total Volume	50 µl	50 µl
Incubation Temperature	Enzyme-dependent	Enzyme-dependent
Incubation Time	60 minutes	5–15 minutes**

* Sufficient to digest all types of DNAs.
** Time-Saver qualified enzymes can also be incubated overnight with no star activity.

Tools & Resources

Visit NEBRestrictionEnzymes.com to find:

- Online tutorials for setting up restriction enzyme digests
- Tips to avoid Star activity
- Restriction Enzyme Performance Chart
- Troubleshooting Guide
- Access to NEB's online tools, including: **Enzyme Finder, DoubleDigest Finder** and **NEBcloner**

Tips for Optimization

Enzyme

- Keep on ice when not in the freezer
- Should be the last component added to reaction
- Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube. Follow with a quick ("touch") spin-down in a microcentrifuge. Do not vortex the reaction.
- In general, we recommend 5–10 units of enzyme per µg DNA, and 10–20 units per µg of genomic DNA in a 1 hour digest
- Some restriction enzymes require more than one recognition site to cleave efficiently. These are designated with the "multi-site" icon 2-site. Please review recommendations on working with these enzymes at www.neb.com.

Star Activity

- Unwanted cleavage that can occur when enzyme is used under sub-optimal conditions, such as:
  - Too much enzyme present
  - Too long of an incubation time
  - Using a non-recommended buffer
  - Glycerol concentrations above 5%
- Star activity can be reduced by using a High-Fidelity (HF®) enzyme, reducing incubation time, using a Time-Saver™ enzyme or increasing reaction volume

DNA

- Should be free of contaminants such as phenol, chloroform, alcohol, EDTA, detergents and salts. Extra wash steps during purification are recommended.
- Methylation of DNA can affect digestion with certain enzymes. For more information about methylation visit www.neb.com/methylation

Buffer

- Use at a 1X concentration
- Recombinant Albumin is included in NEBuffer r1.1, r2.1, r3.1 and rCutSmart™ Buffer.

Reaction Volume

- A 50 µl reaction volume is recommended for digestion of up to 1 µg of substrate. This helps maintain salt levels introduced by miniprepped DNA low enough that they don't affect enzyme activity.
- Enzyme volume should not exceed 10% of the total reaction volume to prevent star activity due to excess glycerol
- Additives in the restriction enzyme storage buffer (e.g., glycerol, salt), as well as contaminants found in the substrate solution (e.g., salt, EDTA or alcohol), can be problematic in smaller reaction volumes

Incubation Time

- Incubation time for the Standard Protocol is 1 hour. Incubation for the Time-Saver Protocol is 5–15 minutes.

	Restriction Enzyme*	DNA	10X NEBuffer
10 µl rxn**	1 unit	0.1 µg	1 µl
25 µl rxn	5 units	0.5 µg	2.5 µl
50 µl rxn	10 units	1 µg	5 µl

* Restriction enzymes can be diluted using the recommended diluent buffer when smaller amounts are needed

** 10 µl rxns should not be incubated for longer than 1 hour to avoid evaporation.

- Visit www.neb.com/timesaver for list of Time-Saver qualified enzymes
- It is possible, with many enzymes, to use fewer units and digest for up to 16 hours. For more information, visit www.neb.com

Storage

- Storage at –20°C is recommended for most restriction enzymes. For a few enzymes, storage at–80°C is recommended. Visit www.neb.com for storage information.
- 10X NEBuffers should be stored at –20°C

Stability

- The expiration date is found on the label
- Long term exposure to temperatures above –20°C should be minimized whenever possible

Double Digestion

Digesting a DNA substrate with two restriction enzymes simultaneously (double digestion) is a common timesaving procedure. Over 210 restriction enzymes are 100% active in rCutSmart Buffer, making double digestion simple. If you are using an enzyme that is not supplied with rCutSmart Buffer, the Performance Chart for Restriction Enzymes rates the percentage activity of each restriction endonuclease in the four standard NEBuffers.

Setting up a Double Digestion

- Double digests with CutSmart restriction enzymes can be set up in rCutSmart Buffer. Otherwise, choose an NEBuffer that results in the most activity for both enzymes. If star activity is a concern, consider using one of our High-Fidelity (HF) enzymes.
- Set up reaction according to recommended protocol. The final concentration of glycerol in any reaction should be less than 5% to minimize the possibility of star activity. For example, in a 50 µl reaction, the total amount of enzyme added should not exceed 5 µl. NEBcloner can also be used to determine recommended double-digest conditions.
- If two different incubation temperatures are necessary, choose the optimal reaction buffer and set up reaction accordingly. Add the first enzyme and incubate at the desired temperature. Then, heat inactivate the first enzyme, if it can be heat inactivated, add the second enzyme and incubate at the recommended temperature.
- Depending on an enzyme's activity rating in a non-optimal NEBuffer, the number of units or incubation time may be adjusted to compensate for the slower rate of cleavage. The Performance Chart for Restriction Enzymes indicates if star activity is an issue with sub-optimal buffers.

Setting up a Double Digestion with a Unique Buffer (designated "U")

- NEB currently supplies three enzymes with unique buffers: EcoRI, SspI and DpnII. In most cases, DpnII requires a sequential digest. Note that EcoRI and SspI have HF versions (NEB #R3101 and NEB #R3132, respectively) which is supplied with rCutSmart Buffer.

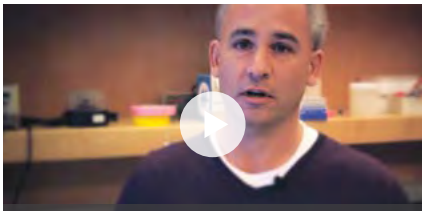
Setting up a Sequential Digestion

- If there is no buffer in which the two enzymes exhibit > 50% activity, a sequential digest can be performed.
- Set up a reaction using the restriction endonuclease that has the lowest salt concentration in its recommended buffer and incubate to completion.
- Adjust the salt concentration of the reaction (using a small volume of a concentrated salt solution) to approximate the reaction conditions of the second restriction endonuclease.
- Add the second enzyme and incubate to complete the second reaction.
- Alternatively, a spin column can be used to isolate the DNA prior to the second reaction. NEB recommend's using our Monarch Nucleic Acid Purification Kits (see the Nucleic Acid Purification chapter or visit **NEBmonarch.com**).

Tools & Resources

Visit www.neb.com/nebtools for:

- Help choosing double digest conditions using NEB's **DoubleDigest Finder** and **NEBcloner®**



TIPS FOR SETTING UP DOUBLE DIGESTS

Types of Restriction Enzymes

Restriction enzymes are traditionally classified into four types on the basis of subunit composition, cleavage position, sequence specificity and cofactor requirements. However, amino acid sequencing has uncovered extraordinary variety among restriction enzymes and revealed that at the molecular level there are many more than four different types.

Type I Enzymes are complex, multisubunit, combination restriction-and-modification enzymes that cut DNA at random far from their recognition sequences. Type I enzymes are of considerable biochemical interest, but they have little practical value since they do not produce discrete restriction fragments or distinct gel-banding patterns.

Type II Enzymes cut DNA at defined positions close to or within their recognition sequences. They produce discrete restriction fragments and distinct gel banding patterns, and they are the predominant class used in the laboratory for DNA analysis and gene cloning. Type IIS restriction enzymes recognize asymmetric DNA sequences and cleave outside of their recognition sequences. They are useful for many applications, including Golden Gate Assembly.

Rather than forming a single family of related proteins, Type II enzymes are a collection of unrelated proteins of many different sorts. Type II enzymes frequently differ greatly in amino acid sequence from one another, and indeed from every other known protein, that they exemplify the class of rapidly evolving proteins that are often indicative of involvement in host-parasite interactions.

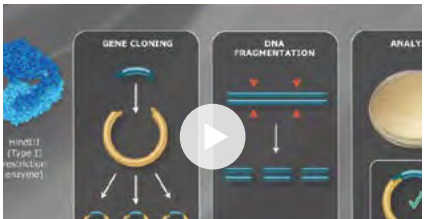
Type III Enzymes are also large combination restriction-and-modification enzymes. They cleave outside of their recognition sequences and require two such sequences in opposite orientations within the same DNA molecule to accomplish cleavage;they rarely yield complete digests.

Type IV Enzymes recognize modified, typically methylated DNA and are exemplified by the McrBC and Mrr systems of *E. coli*.

Tools & Resources

Visit the video library at www.neb.com to find:

- Tutorials on Type I, II and III restriction enzymes



TYPE I, II AND III RESTRICTION ENZYMES

View double digest protocol.

Restriction Enzyme Troubleshooting Guide

Problem	Cause	Solution
Few or no transformants	Restriction enzyme(s) didn't cleave completely	• Check the methylation sensitivity of the enzyme(s) to determine if the enzyme is blocked by methylation of the recognition sequence • Use the recommended buffer supplied with the restriction enzyme • Clean up the DNA to remove any contaminants that may inhibit the enzyme • When digesting a PCR fragment, make sure to have at least 6 nucleotides between the recognition site and the end of the DNA molecule
The digested DNA ran as a smear on an agarose gel	The restriction enzyme(s) is bound to the substrate DNA	• Lower the number of units • Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the DNA or use Gel Loading Dye, Purple (6X) (NEB #B7024)
	Nuclease contamination	• Use fresh, clean running buffer and a fresh agarose gel • Clean up the DNA. We recommend the Monarch PCR & DNA Cleanup Kit (NEB #T1030).
Incomplete restriction enzyme digestion	Cleavage is blocked by methylation	• Check the methylation sensitivity of the enzyme(s) to determine if the enzyme is blocked by methylation of the recognition sequence • DNA isolated from a bacterial source may be blocked by Dam and Dcm methylation • If the enzyme is inhibited by Dam or Dcm methylation, grow the plasmid in a dam-/dcm- strain (NEB #C2925) • DNA isolated from eukaryotic source may be blocked by CpG methylation
	Salt inhibition	• Enzymes that have low activity in salt-containing buffers (NEBuffer r3.1) may be salt sensitive, so clean up the DNA (NEB #T1030) prior to digestion • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. To prevent this, DNA solution should be no more than 25% of total reaction volume.
	Inhibition by PCR components	• Clean up the PCR fragment prior to restriction digest
	Using the wrong buffer	• Use the recommended buffer supplied with the restriction enzyme
	Too few units of enzyme used	• Use at least 3–5 units of enzyme per µg of DNA (NEB #T1030)
	Incubation time was too short	• Increase the incubation time
	Digesting supercoiled DNA	• Some enzymes have a lower activity on supercoiled DNA. Increase the number of enzyme units in the reaction.
	Presence of slow sites	• Some enzymes can exhibit slower cleavage towards specific sites. Increase the incubation time, 1–2 hours is typically sufficient.
	Two sites required	• Some enzymes require the presence of two recognition sites to cut efficiently
	DNA is contaminated with an inhibitor	• Assay substrate DNA in the presence of a control DNA. Control DNA will not cleave if there is an inhibitor present. Miniprep DNA is particularly susceptible to contaminants. • Clean DNA with a spin column or increase volume to dilute contaminant. We recommend the Monarch PCR & DNA Cleanup Kit (NEB #T1030)
Extra bands in the gel	If larger bands than expected are seen in the gel, this may indicate binding of the enzyme(s) to the substrate	• Lower the number of units in the reaction • Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the substrate, or use Gel Loading Dye, Purple (6X) (NEB #B7024)
	Star activity	• Use the recommended buffer supplied with the restriction enzyme • Decrease the number of enzyme units in the reaction • Make sure the amount of enzyme added does not exceed 10% of the total reaction volume. This ensures that the total glycerol concentration does not exceed 5% v/v. • Decrease the incubation time. Using the minimum reaction time required for complete digestion will help prevent star activity. • Try using a High-Fidelity (HF) restriction enzyme. HF enzymes have been engineered for reduced star activity.
	Partial restriction enzyme digest	• Enzymes that have low activity in salt-containing buffers (e.g., NEBuffer r3.1) may be salt sensitive. Make sure to clean up the DNA prior to digestion. • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. To prevent this, DNA solution should be no more than 25% of total reaction volume. • Clean-up the PCR fragment prior to restriction digest • Use the recommended buffer supplied with the restriction enzyme • Use at least 5–10 units of enzyme per µg of DNA and digest the DNA for 1–2 hours

Performance Chart for Restriction Enzymes

New England Biolabs supplies > 210 restriction enzymes that are 100% active in rCutSmart Buffer. This results in increased efficiency, flexibility and ease-of-use, especially when performing double digests.

This performance chart summarizes the activity information of NEB restriction enzymes. To help select the best conditions for double digests, this chart shows the optimal (supplied) NEBuffer and approximate activity in the four standard NEBuffers for each enzyme. In addition, this performance chart shows recommended reaction temperature, heat-inactivation temperature, recommended diluent buffer, methylation sensitivity and whether the enzyme is Time-Saver qualified (e.g., cleaves substrate in 5–15 minutes under recommended conditions, and can be used overnight without degradation of DNA), and whether the enzyme works better in a substrate with multiple sites.

Chart Legend

- **U** — Supplied with a unique reaction buffer that is different from the four standard NEBuffers. The compatibility with the four standard NEBuffers is indicated in the chart.
- RR — Recombinant
- e — Engineered enzyme for maximum performance
- Time-Saver qualified
- 2*-site — Indicates that the restriction enzyme requires two or more sites for cleavage
- dcm — dcm methylation sensitivity
- dam — dam methylation sensitivity
- CpG — CpG methylation sensitivity

Activity Notes (see last column)

FOR STAR ACTIVITY

1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.
2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.

* May exhibit star activity in this buffer.

\+ For added flexibility, NEB offers an isoschizomer or HF enzyme, supplied with rCutSmart Buffer.

FOR LIGATION AND RECUTTING

a. Ligation is less than 10%

b. Ligation is 25% – 75%

c. Recutting after ligation is < 5%

d. Recutting after ligation is 50% – 75%

e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or the recognition sequence contains variable sequences.

			Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1	rCutSmart	Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity		Notes
RR	⏱		AatII	rCutSmart	<10	50*	50	100	37°	80°	B	λ DNA		CpG	
RR			AbaSI	rCutSmart + DTT	25	50	50	100	25°	65°	C	T4 wild-type phage DNA (fully ghmC-modified)			e
RR	⏱		Acc65I	r3.1	10	75*	100	25	37°	65°	A	pBC4 DNA	dcm	CpG	
RR	⏱		AccI	rCutSmart	50	50	10	100	37°	80°	A	λ DNA		CpG	
RR	⏱		AciI	rCutSmart	<10	25	100	100	37°	65°	A	λ DNA		CpG	
RR	⏱		AclI	rCutSmart	<10	<10	<10	100	37°	No	B	λ DNA		CpG	
RR	⏱		AcuI	rCutSmart	50	100	50	100	37°	65°	B	λ DNA			1, b, d
RR			AfeI	rCutSmart	25	100	25	100	37°	65°	B	pXba DNA		CpG	
RR	⏱		AflII	rCutSmart	50	100	10	100	37°	65°	A	ΦX174 RF I DNA			
RR	⏱		AflIII	r3.1	10	50	100	50	37°	80°	B	λ DNA			
RR	⏱	e	AgeI-HF	rCutSmart	100	50	10	100	37°	65°	A	λ DNA		CpG	
RR	⏱		AhdI	rCutSmart	25	25	10	100	37°	65°	A	λ DNA		CpG	a
RR		e	AleI-v2	rCutSmart	<10	<10	<10	100	37°	65°	B	λ DNA		CpG	
RR	⏱		AluI	rCutSmart	25	100	50	100	37°	80°	B	λ DNA			b
RR			AlwI	rCutSmart	50	50	10	100	37°	No	A	λ DNA (dam-)	dam		1, b, d
RR	⏱		AlwNI	rCutSmart	10	100	50	100	37°	80°	A	λ DNA	dcm		
RR	⏱		ApaI	rCutSmart	25	25	<10	100	37°	65°	A	pXba DNA	dcm	CpG	
RR	⏱		ApaLI	rCutSmart	100	100	10	100	37°	No	A	λ DNA (HindIII digest)		CpG	
RR	⏱		ApeKI	r3.1	25	50	100	10	75°	No	B	λ DNA		CpG	
RR	⏱	e	ApoI-HF	rCutSmart	10	100	10	100	37°	80°	B	λ DNA			
RR	⏱		AscI	rCutSmart	<10	10	10	100	37°	80°	A	λ DNA		CpG	
RR	⏱		AseI	r3.1	<10	50*	100	10	37°	65°	B	λ DNA			3
RR			AsiSI	rCutSmart	100	100	25	100	37°	80°	B	XhoI digested pXba		CpG	2, b
RR	⏱		AvaI	rCutSmart	<10	100	25	100	37°	80°	A	λ DNA		CpG	
RR	⏱		AvaII	rCutSmart	50	75	10	100	37°	80°	A	λ DNA	dcm	CpG	
RR	⏱		AvrII	rCutSmart	100	50	50	100	37°	No	B	λ DNA (HindIII digest)			
RR	⏱		BaeGI	r3.1	75	75	100	25	37°	80°	A	λ DNA			

Performance Chart for Restriction Enzymes (Continued)

Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1	rCutSmart	Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity	Notes
BaeI	rCutSmart	50	100	50	100	37°	65°	A	λ DNA	CpG	e
BamHI	r3.1	75*	100*	100	100*	37°	No	A	λ DNA		3
BamHI-HF	rCutSmart	100	50	10	100	37°	No	A	λ DNA		
BanI	rCutSmart	10	25	<10	100	37°	65°	A	λ DNA	dcm CpG	1
BanII	rCutSmart	100	100	50	100	37°	80°	A	λ DNA		2
BbsI	r2.1	100	100	25	75	37°	65°	B	λ DNA		
BbsI-HF	rCutSmart	10	10	10	100	37°	65°	B	λ DNA		
BbvCI	rCutSmart	10	100	50	100	37°	No	B	λ DNA	CpG	1, a
BbvI	rCutSmart	100	100	25	100	37°	65°	B	pBR322 DNA		3
BccI	rCutSmart	100	50	10	100	37°	65°	A	pXba DNA		3, b
BceAI	r3.1	100*	100*	100	100*	37°	65°	A	pBR322 DNA	CpG	1
BcgI	r3.1	10	75*	100	50*	37°	65°	A	λ DNA	dam CpG	e
BciVI	rCutSmart	100	25	<10	100	37°	80°	C	λ DNA		b
BclI	r3.1	50	100	100	75	50°	No	A	λ DNA (dam-)	dam	
BclI-HF	rCutSmart	100	100	10	100	37°	65°	B	λ DNA (dam-)	dam	
BcoDI	rCutSmart	50	75	75	100	37°	No	B	λ DNA	CpG	
BfaI	rCutSmart	<10	10	<10	100	37°	80°	B	λ DNA		2, b
BfuAI	r3.1	<10	25	100	10	50°	65°	B	λ DNA	CpG	3
BglI	r3.1	10	25	100	10	37°	65°	B	λ DNA	CpG	
BglII	r3.1	10	10	100	<10	37°	No	A	λ DNA		
BlpI	rCutSmart	50	100	10	100	37°	No	A	λ DNA		d
BmgBI	r3.1	<10	10	100	10	37°	65°	B	λ DNA	CpG	3, b, d
BmrI	r2.1	75	100	75	100*	37°	65°	B	λ DNA (HindIII digest)		b
BmtI-HF	rCutSmart	50	100	10	100	37°	65°	B	pXba DNA		
BpmI	r3.1	75	100	100	100*	37°	65°	B	λ DNA		2
Bpu10I	r3.1	10	25	100	25	37°	80°	B	λ DNA		3, b, d
BpuEI	rCutSmart	50*	100	50*	100	37°	65°	B	λ DNA		d
BsaAI	rCutSmart	100	100	100	100	37°	No	C	λ DNA	CpG	
BsaBI	rCutSmart	50	100	75	100	60°	80°	B	λ DNA (dam-)	dam CpG	2
BsaHI	rCutSmart	50	100	100	100	37°	80°	C	λ DNA	dcm CpG	
BsaI-HFv2	rCutSmart	100	100	100	100	37°	80°	B	pXba DNA	dcm CpG	
BsaJI	rCutSmart	50	100	100	100	60°	80°	A	λ DNA		
BsaWI	rCutSmart	10	100	50	100	60°	80°	A	λ DNA		
BsaXI	rCutSmart	50*	100*	10	100	37°	No	C	λ DNA		e
BseRI	rCutSmart	100	100	75	100	37°	80°	A	λ DNA		d
BseYI	r3.1	10	50	100	50	37°	80°	B	λ DNA	CpG	d
BsgI	rCutSmart	25	50	25	100	37°	65°	B	λ DNA		d
BsiEI	rCutSmart	25	50	<10	100	60°	No	A	λ DNA	CpG	
BsiHKAI	rCutSmart	25	100	100	100	65°	No	A	λ DNA		
BsiWI	r3.1	25	50*	100	25	55°	65°	B	ΦX174 DNA	CpG	
BsiWI-HF	rCutSmart	50	100	10	100	37°	No	B	ΦX174 DNA	CpG	
BsII	rCutSmart	50	75	100	100	55°	No	A	λ DNA	dcm CpG	b
BsmAI	rCutSmart	50	100	100	100	55°	No	B	λ DNA	CpG	

a. Ligation is less than 10%
b. Ligation is 25% – 75%
c. Recutting after ligation is < 5%
d. Recutting after ligation is 50% – 75%
e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or the recognition sequence contains variable sequences.

Performance Chart for Restriction Enzymes (Continued)

Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1	rCutSmart	Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity		Notes
BsmBI-v2	r3.1	<10	50	100	25	55°	80°	B	λ DNA		CpG	
BsmFI	rCutSmart	25	50	50	100	65°	80°	A	pBR322 DNA	dcm	CpG	1
BsmI	rCutSmart	25	100	<10	100	65°	80°	A	λ DNA			
BsoBI	rCutSmart	25	100	100	100	37°	80°	A	λ DNA			
Bsp1286I	rCutSmart	25	25	25	100	37°	65°	A	λ DNA			3
BspCNI	rCutSmart	100	75	10	100	37°	80°	A	λ DNA			b
BspDI	rCutSmart	25	75	50	100	37°	80°	A	λ DNA	dam	CpG	
BspEI	r3.1	<10	10	100	<10	37°	80°	B	λ DNA (dam-)	dam	CpG	
BspHI	rCutSmart	10	50	25	100	37°	80°	A	λ DNA	dam		
BspMI	r3.1	10	50*	100	10	37°	65°	B	λ DNA			
BspQI	r3.1	100*	100*	100	100*	50°	80°	B	λ DNA			3
BsrBI	rCutSmart	50	100	100	100	37°	80°	A	λ DNA		CpG	d
BsrDI	r2.1	10	100	75	25	37°	80°	A	λ DNA			3, d
BsrFI-v2	rCutSmart	25	25	0	100	37°	No	C	pBR322 DNA		CpG	
BsrGI-HF	rCutSmart	10	100	100	100	37°	80°	A	λ DNA			
BsrI	r3.1	<10	50	100	10	65°	80°	B	ΦX174 DNA			b
BssHII	rCutSmart	100	100	100	100	50°	65°	B	λ DNA		CpG	
BssSI-v2	rCutSmart	10	25	<10	100	37°	No	B	λ DNA			
BstAPI	rCutSmart	50	100	25	100	60°	80°	A	λ DNA		CpG	b
BstBI	rCutSmart	75	100	10	100	65°	No	A	λ DNA		CpG	
BstEII-HF	rCutSmart	<10	10	<10	100	37°	No	A	λ DNA			
BstNI	r3.1	10	100	100	75	60°	No	A	λ DNA			a
BstUI	rCutSmart	50	100	25	100	60°	No	A	λ DNA		CpG	b
BstXI	r3.1	<10	50	100	25	37°	80°	B	λ DNA	dcm		3
BstYI	rCutSmart	25	100	75	100	60°	No	A	λ DNA			
BstZ17I-HF	rCutSmart	100	100	10	100	37°	No	A	λ DNA		CpG	
Bsu36I	rCutSmart	25	100	100	100	37°	80°	C	λ DNA (HindIII digest)			b
BtgI	rCutSmart	50	100	100	100	37°	80°	B	pBR322 DNA			
BtgZI	rCutSmart	10	25	<10	100	60°	80°	A	λ DNA		CpG	3, b, d
BtsCI	rCutSmart	10	100	25	100	50°	80°	B	λ DNA			
BtsI-v2	rCutSmart	100	100	25	100	37°	No	A	λ DNA			1
BtsIMutI	rCutSmart	100	50	10	100	55°	80°	A	pUC19 DNA			b
Cac8I	rCutSmart	50	75	100	100	37°	65°	B	λ DNA		CpG	b
ClaI	rCutSmart	10	50	50	100	37°	65°	A	λ DNA (dam-)	dam	CpG	
CspCI	rCutSmart	10	100	10	100	37°	65°	A	λ DNA			e
CviAII	rCutSmart	50	50	10	100	25°	65°	C	λ DNA			
CviKI-1	rCutSmart	25	100	100	100	37°	No	A	pBR322 DNA			1, b
CviQI	r3.1	75	100*	100	75*	25°	No	C	λ DNA			b
DdeI	rCutSmart	75	100	100	100	37°	65°	B	λ DNA			
DpnI	rCutSmart	100	100	75	100	37°	80°	B	pBR322 DNA (dam methylated)		CpG	b
DpnII	U	25	25	100*	25	37°	65°	B	λ DNA (dam-)	dam		
DraI	rCutSmart	75	75	50	100	37°	65°	A	λ DNA			

1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.
2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.

* May exhibit star activity in this buffer.
+ NEB isoschizomer or HF enzyme supplied with rCutSmart Buffer.

Performance Chart for Restriction Enzymes (Continued)

Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1	rCutSmart	Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity		Notes
DraIII-HF	rCutSmart	<10	50	10	100	37°	No	B	λ DNA		CpG	b
DrdI	rCutSmart	25	50	10	100	37°	65°	A	pUC19 DNA		CpG	3
EaeI	rCutSmart	10	50	<10	100	37°	65°	A	λ DNA	dcm	CpG	b
EagI-HF	rCutSmart	25	100	100	100	37°	65°	B	pXba DNA		CpG	
EarI	rCutSmart	50	10	<10	100	37°	65°	B	λ DNA		CpG	b, d
EciI	rCutSmart	100	50	50	100	37°	65°	A	λ DNA		CpG	2
Eco53kI	rCutSmart	100	100	<10	100	37°	65°	A	pXba DNA		CpG	3, b
EcoNI	rCutSmart	50	100	75	100	37°	65°	A	λ DNA			b
EcoO109I	rCutSmart	50	100	50	100	37°	65°	A	λ DNA (HindIII digest)	dcm		3
EcoP15I	r3.1 + ATP	75	100	100	100	37°	65°	A	pUC19 DNA			e
EcoRI	U	25	100*	50	50*	37°	65°	C	λ DNA		CpG	
EcoRI-HF	rCutSmart	10	100	<10	100	37°	65°	C	λ DNA		CpG	
EcoRV	r3.1	10	50	100	10	37°	80°	A	λ DNA		CpG	
EcoRV-HF	rCutSmart	25	100	100	100	37°	65°	B	λ DNA		CpG	
Esp3I	rCutSmart	100	100	<10	100	37°	65°	B	λ DNA		CpG	
FatI	r2.1	10	100	50	50	55°	80°	A	pUC19 DNA			
FauI	rCutSmart	100	50	10	100	55°	65°	A	λ DNA		CpG	3, b, d
Fnu4HI	rCutSmart	<10	<10	<10	100	37°	No	A	λ DNA		CpG	a
FokI	rCutSmart	100	100	75	100	37°	65°	A	λ DNA	dcm	CpG	3, b, d
FseI	rCutSmart	100	75	<10	100	37°	65°	B	pBC4 DNA	dcm	CpG	
FspEI	rCutSmart + Enz. Activ.	<10	<10	<10	100	37°	80°	B	pBR322 (dcm+) DNA			1, e
FspI	rCutSmart	10	100	10	100	37°	No	C	λ DNA		CpG	b
HaeII	rCutSmart	25	100	10	100	37°	80°	A	λ DNA		CpG	
HaeIII	rCutSmart	50	100	25	100	37°	80°	A	λ DNA			
HgaI	r1.1	100	100	25	100*	37°	65°	A	ΦX174 DNA		CpG	1
HhaI	rCutSmart	25	100	100	100	37°	65°	A	λ DNA		CpG	
HinP1I	rCutSmart	100	100	100	100	37°	65°	A	λ DNA		CpG	
HincII	rCutSmart	25	100	100	100	37°	65°	B	λ DNA		CpG	
HindIII	r2.1	25	100	50	50	37°	80°	B	λ DNA			2
HindIII-HF	rCutSmart	10	100	10	100	37°	80°	B	λ DNA			
HinfI	rCutSmart	50	100	100	100	37°	80°	A	λ DNA		CpG	
HpaI	rCutSmart	<10	75*	25	100	37°	No	A	λ DNA		CpG	1
HpaII	rCutSmart	100	50	<10	100	37°	80°	A	λ DNA		CpG	
HphI	rCutSmart	50	50	<10	100	37°	65°	B	λ DNA	dcm		1, b, d
Hpy166II	rCutSmart	100	100	50	100	37°	65°	C	pBR322 DNA		CpG	
Hpy188I	rCutSmart	25	100	50	100	37°	65°	A	pBR322 DNA	dam		1, b
Hpy188III	rCutSmart	100	100	10	100	37°	65°	B	pUC19 DNA	dam	CpG	3, b
Hpy99I	rCutSmart	50	10	<10	100	37°	65°	A	λ DNA		CpG	
HpyAV	rCutSmart	100	100	25	100	37°	65°		λ DNA		CpG	3, b, d
HpyCH4III	rCutSmart	100	25	<10	100	37°	65°	A	λ DNA			b
HpyCH4IV	rCutSmart	100	50	25	100	37°	65°	A	pUC19 DNA		CpG	
HpyCH4V	rCutSmart	50	50	25	100	37°	65°	A	λ DNA			

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b. Ligation is 25% – 75%
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d. Recutting after ligation is 50% – 75%
e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or the recognition sequence contains variable sequences.

Performance Chart for Restriction Enzymes (Continued)

Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1	rCutSmart	Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity		Notes
I-CeuI	rCutSmart	10	10	10	100	37°	65°	B	pBHS ScaI-linearized Control Plasmid			
I-SceI	rCutSmart	10	50	25	100	37°	65°	B	pGPS2 NotI-linearized Control Plasmid			
KasI	rCutSmart	50	100	50	100	37°	65°	B	pBR322 DNA		CpG	3
KpnI-HF	rCutSmart	100	25	<10	100	37°	No	A	pXba DNA			
LpnPI	rCutSmart + Enz. Activ.	<10	<10	<10	100	37°	65°	B	pBR322 (dcm+) DNA			1, e
MboI	rCutSmart	75	100	100	100	37°	65°	A	λ DNA (dam-)	dam	CpG	
MboII	rCutSmart	100*	100	50	100	37°	65°	C	λ DNA (dam-)	dam		b
MfeI-HF	rCutSmart	75	25	<10	100	37°	No	A	λ DNA			
MluCI	rCutSmart	100	10	10	100	37°	No	A	λ DNA			
MluI-HF	rCutSmart	25	100	100	100	37°	No	A	λ DNA		CpG	
MlyI	rCutSmart	50	50	10	100	37°	65°	A	λ DNA			b, d
MmeI	rCutSmart	50	100	50	100	37°	65°	B	ΦX174 RF I DNA		CpG	b, c
MnlI	rCutSmart	75	100	50	100	37°	65°	B	λ DNA			b
MscI	rCutSmart	25	100	100	100	37°	80°	C	λ DNA	dcm		
MseI	rCutSmart	75	100	75	100	37°	65°	A	λ DNA			
MslI	rCutSmart	50	50	<10	100	37°	80°	A	λ DNA			
MspA1I	rCutSmart	10	50	10	100	37°	65°	B	λ DNA		CpG	
MspI	rCutSmart	75	100	50	100	37°	No	A	λ DNA			
MspJI	rCutSmart + Enz. Activ.	<10	<10	<10	100	37°	65°	B	pBR322 (dcm+) DNA			1, e
MwoI	rCutSmart	<10	100	100	100	60°	No	B	λ DNA		CpG	
NaeI	rCutSmart	25	25	<10	100	37°	No	A	pXba DNA		CpG	b
NarI	rCutSmart	100	100	10	100	37°	65°	A	pXba DNA		CpG	
Nb.BbvCI	rCutSmart	25	100	100	100	37°	80°	A	supercoiled plasmid DNA			e
Nb.BsmI	r3.1	<10	50	100	10	65°	80°	A	supercoiled plasmid pBR322 DNA			e
Nb.BsrDI	rCutSmart	25	100	100	100	65°	80°	A	supercoiled pUC19 DNA			e
Nb.BssSI	r3.1	10	100	100	25	37°	No	B	supercoiled pUC19 DNA			e
Nb.BtsI	rCutSmart	75	100	75	100	37°	80°	A	supercoiled pUC101 DNA (dam-/dcm-)			e
NciI	rCutSmart	100	25	10	100	37°	No	A	λ DNA		CpG	b
NcoI	r3.1	100	100	100	100	37°	80°	A	λ DNA			
NcoI-HF	rCutSmart	50	100	10	100	37°	80°	B	λ DNA			
NdeI	rCutSmart	75	100	100	100	37°	65°	A	λ DNA			
NgoMIV	rCutSmart	100	50	10	100	37°	No	A	pXba DNA		CpG	1
NheI-HF	rCutSmart	100	25	10	100	37°	80°	C	λ DNA (HindIII digest)		CpG	
NlaIII	rCutSmart	<10	<10	<10	100	37°	65°	B	ΦX174 RF I DNA			
NlaIV	rCutSmart	10	10	10	100	37°	65°	B	pBR322 DNA	dcm	CpG	
NmeAIII	rCutSmart	10	10	<10	100	37°	65°	B	ΦX174 RF I DNA			c
NotI	r3.1	<10	50	100	25	37°	65°	C	pBC4 DNA		CpG	
NotI-HF	rCutSmart	25	100	25	100	37°	65°	A	pBC4 DNA		CpG	
NruI-HF	rCutSmart	0	25	50	100	37°	No	A	λ DNA	dam	CpG	
NsiI	r3.1	10	75	100	25	37°	65°	B	λ DNA			

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2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.

* May exhibit star activity in this buffer.
+ NEB isoschizomer or HF enzyme supplied with rCutSmart Buffer.

Performance Chart for Restriction Enzymes (Continued)

Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1	rCutSmart	Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity	Notes
NsiI-HF	rCutSmart	<10	20	<10	100	37°	80°	B	λ DNA		
NspI	rCutSmart	100	100	<10	100	37°	65°	A	λ DNA		
Nt.AlwI	rCutSmart	10	100	100	100	37°	80°	A	pUC101 DNA (dam-/dcm-)	dam	e
Nt.BbvCI	rCutSmart	50	100	10	100	37°	80°	A	supercoiled plasmid DNA	CpG	e
Nt.BsmAI	rCutSmart	100	50	10	100	37°	65°	A	supercoiled plasmid DNA	CpG	e
Nt.BspQI	r3.1	<10	25	100	10	50°	80°	B	supercoiled pUC19 DNA		e
Nt.BstNBI	r3.1	0	10	100	10	55°	80°	A	T7 DNA		e
Nt.CviPII	rCutSmart	10	100	25	100	37°	65°	A	pUC19 DNA	CpG	e
PI-PspI	U + rAlbumin	10	10	10	10	65°	No	B	pAKR7 XmnI-linearized Control Plasmid		
PI-SceI	U + rAlbumin	10	10	10	10	37°	65°	B	pBSvdeX XmnI-linearized Control Plasmid		
PacI	rCutSmart	100	75	10	100	37°	65°	A	pNEB193 DNA		
PaeR7I	rCutSmart	25	100	10	100	37°	No	A	λ DNA (HindIII digest)	CpG	
PaqCI	rCutSmart PaqCI Activator	10	100	10	100	37°	65°	B	λ DNA	CpG	1
PciI	r3.1	50	75	100	50*	37°	80°	B	pXba DNA		
PflFI	rCutSmart	25	100	25	100	37°	65°	A	pBC4 DNA		b
PflMI	r3.1	0	100	100	50	37°	65°	A	λ DNA	dcm	3, b, d
PleI	rCutSmart	25	50	25	100	37°	65°	A	λ DNA	CpG	b, d
PluTI	rCutSmart	100	25	<10	100	37°	65°	A	pXba DNA	CpG	b
PmeI	rCutSmart	<10	50	10	100	37°	65°	A	λ DNA	CpG	
PmlI	rCutSmart	100	50	<10	100	37°	65°	A	λ DNA (HindIII digest) DNA	CpG	
PpuMI	rCutSmart	<10	<10	<10	100	37°	No	B	λ DNA (HindIII digest)	dcm	
PshAI	rCutSmart	25	50	10	100	37°	65°	A	λ DNA	CpG	
PsiI-v2	rCutSmart	25	50	10	100	37°	65°	B	λ DNA		3
PspGI	rCutSmart	25	100	50	100	75°	No	A	T7 DNA	dcm	3
PspOMI	rCutSmart	10	10	<10	100	37°	65°	B	pXba DNA	dcm CpG	
PspXI	rCutSmart	<10	100	25	100	37°	No	B	λ DNA (HindIII digest)	CpG	
PstI	r3.1	75	75	100	50*	37°	80°	C	λ DNA		
PstI-HF	rCutSmart	10	75	50	100	37°	No	C	λ DNA		
PvuI-HF	rCutSmart	25	100	100	100	37°	No	B	pXba DNA	CpG	
PvuII	r3.1	50	100	100	100*	37°	No	B	λ DNA		
PvuII-HF	rCutSmart	<10	<10	<10	100	37°	No	B	λ DNA		
RsaI	rCutSmart	25	50	<10	100	37°	No	A	λ DNA	CpG	
RsrII	rCutSmart	25	75	10	100	37°	65°	C	λ DNA	CpG	
SacI-HF	rCutSmart	10	50	<10	100	37°	65°	A	λ DNA (HindIII digest)	CpG	
SacII	rCutSmart	10	100	10	100	37°	65°	A	pXba DNA	CpG	
SalI	r3.1	<10	<10	100	<10	37°	65°	A	λ DNA (HindIII digest)	CpG	
SalI-HF	rCutSmart	10	100	100	100	37°	65°	A	λ DNA (HindIII digest)	CpG	
SapI	rCutSmart	75	50	<10	100	37°	65°	B	λ DNA		
Sau3AI	r1.1	100	50	10	100	37°	65°	A	λ DNA	CpG	b
Sau96I	rCutSmart	50	100	100	100	37°	65°	A	λ DNA	dcm CpG	
SbfI-HF	rCutSmart	50	25	<10	100	37°	80°	B	λ DNA		

a. Ligation is less than 10%
b. Ligation is 25% – 75%
c. Recutting after ligation is < 5%
d. Recutting after ligation is 50% – 75%
e. Ligation and recutting after ligation is not applicable since the enzyme is either a nicking enzyme, is affected by methylation, or the recognition sequence contains variable sequences.

Performance Chart for Restriction Enzymes (Continued)

Enzyme	Supplied NEBuffer	% Activity in NEBuffers r1.1	r2.1	r3.1	rCutSmart	Incub. Temp. (°C)	Inactiv. Temp. (°C)	Dil.	Unit Substrate	Methylation Sensitivity		Notes
ScaI-HF	rCutSmart	100	100	10	100	37°	80°	B	λ DNA			
ScrFI	rCutSmart	100	100	100	100	37°	65°	C	λ DNA	dcm	CpG	2, a
SexAI	rCutSmart	100	75	50	100	37°	65°	A	pBC4 DNA (dcm-)	dcm		3, b, d
SfaNI	r3.1	<10	75	100	25	37°	65°	B	ΦX174 RF I DNA		CpG	3, b
SfcI	rCutSmart	75	50	25	100	37°	65°	B	λ DNA			3
SfiI	rCutSmart	25	100	50	100	50°	No	C	pXba DNA	dcm	CpG	
SfoI	rCutSmart	50	100	100	100	37°	No	B	λ DNA (HindIII digest)	dcm	CpG	
SgrAI	rCutSmart	100	100	10	100	37°	65°	A	λ DNA		CpG	1
SmaI	rCutSmart	<10	<10	<10	100	37°	65°	B	λ DNA (HindIII digest)		CpG	b
SmlI	rCutSmart	25	75	25	100	55°	No	A	λ DNA			b
SnaBI	rCutSmart	50*	50	10	100	37°	80°	A	T7 DNA		CpG	1
SpeI-HF	rCutSmart	25	50	10	100	37°	80°	C	pXba-XbaI DNA			
SphI	r2.1	100	100	50	100	37°	65°	B	λ DNA			2
SphI-HF	rCutSmart	50	25	10	100	37°	65°	B	λ DNA			
SrfI	rCutSmart	10	50	0	100	37°	65°	B	pNEB193-SrfI DNA		CpG	
SspI-HF	rCutSmart	25	100	<10	100	37°	65°	B	λ DNA			
StuI	rCutSmart	50	100	50	100	37°	No	A	λ DNA	dcm		
StyD4I	rCutSmart	10	100	100	100	37°	65°	B	λ DNA	dcm	CpG	
StyI-HF	rCutSmart	25	100	25	100	37°	65°	A	λ DNA			
SwaI	r3.1	10	10	100	10	25°	65°	B	pXba DNA			b, d
TaqI-v2	rCutSmart	50	100	50	100	65°	No	B	λ DNA	dam		
TfiI	rCutSmart	50	100	100	100	65°	No	C	λ DNA		CpG	
TseI	rCutSmart	75	100	100	100	65°	No	B	λ DNA		CpG	3
Tsp45I	rCutSmart	100	50	<10	100	65°	No	A	λ DNA			
TspMI	rCutSmart	50*	75*	50*	100	75°	No	B	pBC4 DNA		CpG	d
TspRI	rCutSmart	25	50	25	100	65°	No	B	λ DNA			
Tth111I	rCutSmart	25	100	25	100	65°	No	B	pBC4 DNA			b
WarmStart Nt.BstNBI	r3.1	0	10	100	25	55°	80°	A	T7 DNA			
XbaI	rCutSmart	<10	100	75	100	37°	65°	A	λ DNA (dam-/Hind III digest)	dam		
XcmI	r2.1	10	100	25	100*	37°	65°	C	λ DNA			2
XhoI	rCutSmart	75	100	100	100	37°	65°	A	λ DNA (HindIII digest)		CpG	b
XmaI	rCutSmart	25	50	<10	100	37°	65°	A	pXba DNA		CpG	3
XmnI	rCutSmart	50	75	<10	100	37°	65°	A	λ DNA			b
ZraI	rCutSmart	100	25	10	100	37°	80°	B	λ DNA		CpG	

1. Star activity may result from extended digestion, high enzyme concentration or a glycerol concentration of > 5%.
2. Star activity may result from extended digestion.
3. Star activity may result from a glycerol concentration of > 5%.

* May exhibit star activity in this buffer.
+ NEB isoschizomer or HF enzyme supplied with rCutSmart Buffer.

Activity at 37°C for Restriction Enzymes with Alternate Incubation Temperatures

Listed below is the percentage of activity exhibited at 37°C for enzymes that have an optimal incubation temperature higher (thermophiles) or lower (25°C) than 37°C.

Enzyme	Optimal Temp. (°C)	% Activity at 37°C
AbaSI	25°	0
ApeKI	75°	10
BclI	50°	50
BfuAI	50°	25
BsaBI	60°	25
BsaJI	60°	25
BsaWI	60°	50
BsiEI	60°	10
BsiHKAI	65°	10
BsiWI	55°	25
BslI	55°	50
BsmAI	55°	50
BsmBI-v2	55°	10
BsmFI	65°	100
BsmI	65°	10
BspQI	50°	50
BsrI	65°	10

Enzyme	Optimal Temp. (°C)	% Activity at 37°C
BssHII	50°	100
BstAPI	60°	25
BstBI	65°	10
BstNI	60°	25
BstUI	60°	10
BstYI	60°	10
BtgZI	60°	50
BtsCI	50°	25
BtsIMutI	55°	50
CviAII	25°	10
CviQI	25°	25
FatI	55°	100
FauI	55°	50
MwoI	60°	25
Nb.BsmI	65°	100
Nb.BsrDI	65°	50

Enzyme	Optimal Temp. (°C)	% Activity at 37°C
Nt.BspQI	50°	50
Nt.BstNBI	55°	50
PI-PspI	65°	10
PspGI	75°	25
SfiI	50°	10
SmlI	55°	10
SwaI	25°	25
TaqI-v2	65°	10
TfiI	65°	10
TseI	65°	10
Tsp45I	65°	10
TspMI	75°	10
TspRI	65°	10
Tth111I	65°	10
WarmStart® Nt. BstNBI	55°	0

Activity of DNA Modifying Enzymes in rCutSmart Buffer

A selection of DNA modifying enzymes were assayed in rCutSmart Buffer, in lieu of their supplied buffers. Functional activity was compared to the activity in its supplied buffer, plus required supplements. Reactions were set up according to the recommended reaction conditions, with rCutSmart Buffer replacing the supplied buffer.

Enzyme	Activity in rCutSmart	Required Supplements
Antarctic Phosphatase	+++	Requires Zn^{2+}
Bst DNA Polymerase	+++	
CpG Methyltransferase (M.SssI)	+++	
DNA Polymerase I	+++	
DNA Polymerase I, Large (Klenow) Fragment	+++	
DNA Polymerase Klenow Exo⁻	+++	
DNase I (RNase-free)	+++	Requires Ca^{2+}
DNase I-XT	+++	Requires Ca^{2+}
E. coli DNA Ligase	+++	Requires NAD
Endonuclease III (Nth), recombinant	+++	
Endonuclease VIII	+++	
Exonuclease I	+++	
Exonuclease III	+++	
Exonuclease VII	+++	
Exonuclease V (Rec BCD)	+++	Requires ATP
Fpg	+++	
GpC Methyltransferase (M.CviPI)	+	Requires DTT
Hi-T4 DNA Ligase	+++	Requires ATP
Lambda Exonuclease	++	
McrBC	+++	
Micrococcal Nuclease	+++	Requires Ca^{2+}

Enzyme	Activity in rCutSmart	Required Supplements
phi29 DNA Polymerase	+++	Requires DTT
Quick CIP	+++	
$RecJ_f$	+++	
Salt-T4 DNA Ligase	+	Requires ATP
Shrimp Alkaline Phosphatase (rSAP)	+++	
T3 DNA Ligase	+++	Requires ATP + PEG
T4 DNA Ligase	+++	Requires ATP
T4 DNA Polymerase	+++	
T4 Phage β-glucosyltransferase (T4-BGT)	+++	
T4 Polynucleotide Kinase	+++	Requires ATP + DTT
T4 PNK (3´ phosphatase minus)	+++	Requires ATP + DTT
T5 Exonuclease	+++	
T7 DNA Ligase	+++	Requires ATP + PEG
T7 DNA Polymerase (unmodified)	+++	
T7 Exonuclease	+++	
Thermolabile Exonuclease I	+++	
Thermolabile USER II Enzyme	+++	
Thermolabile USER III Enzyme	++	
Thermostable OGG	+++	
USER Enzyme, recombinant	+++	

+++ full functional activity ++ 50–100% functional activity + 0–50% functional activity

Tips for Avoiding Star Activity

Under non-standard reaction conditions, some restriction enzymes are capable of cleaving sequences which are similar but not identical to their defined recognition sequence. This altered specificity has been termed "star activity". Although the propensity for star activity varies, the vast majority of enzymes from New England Biolabs will not exhibit star activity when used under recommended conditions in their supplied NEBuffers. If an enzyme has been reported to exhibit star activity, it will be indicated in the product entry found in the catalog, on the supplied card and on our website.

Conditions That Contribute to Star Activity	Steps That Can Be Taken to Inhibit Star Activity
High glycerol concentration (> 5% v/v)	• Restriction enzymes are stored in 50% glycerol, therefore the amount of enzyme added should not exceed 10% of the total reaction volume. • Use the standard 50 µl reaction volume to reduce evaporation during incubation.
High concentration of enzyme/µg of DNA ratio (varies with each enzyme, usually 100 units/µg)	• Use the fewest units possible to achieve digestion. This avoids overdigestion and reduces the final glycerol concentration in the reaction.
Non-optimal buffer	• Whenever possible, set up reactions in the recommended buffer. Buffers with differing ionic strengths and pHs may contribute to star activity.
Prolonged reaction time	• Use the minimum reaction time required for complete digestion. Prolonged incubation may result in increased star activity, as well as evaporation.
Presence of organic solvents [DMSO, ethanol (1), ethylene glycol, dimethylacetamide, dimethylformamide, sulphalane (2)]	• Make sure the reaction is free of any organic solvents, such as alcohols, that might be present in the DNA preparation.
Substitution of Mg^{2+} with other divalent cations (Mn^{2+}, Cu^{2+}, Co^{2+}, Zn^{2+})	• Use Mg^{2+} as the divalent cation. Other divalent cations may not fit correctly into the active site of the restriction enzyme, possibly interfering with proper recognition.

Note: The relative significance of each of these altered conditions will vary from enzyme to enzyme.

New England Biolabs recommends setting up restriction enzyme digests in a 50 µl reaction volume. However, different methods may require smaller reaction volumes. When performing restriction enzyme digests in smaller reaction volumes, extra care must be taken to follow the steps listed above to avoid star activity. Alternatively, using our line of **High Fidelity (HF) restriction enzymes** will allow greater flexibility in reaction setup.

Reference:
(1) Nasri, M. and Thomas, D. (1986) *Nucleic Acids Res.* 14, 811.
(2) Tikchinenko, T.I. et al (1978) *Gene* 4, 195–212.

Tools & Resources

Visit NEBRestrictionEnzymes.com to find:

- Online tutorials for setting up restriction enzyme digests
- Access to troubleshooting guides & usage guidelines

High-Fidelity (HF) Restriction Enzymes

As part of our ongoing commitment to the study and improvement of restriction enzymes, NEB offers a line of High-Fidelity (HF) restriction enzymes. These engineered enzymes have the same specificity as the native enzymes, are all active in rCutSmart or CutSmart Buffer and have reduced star activity. Star activity, or off-target cleavage, is an intrinsic property of restriction enzymes. Most restriction enzymes will not exhibit star activity when used under recommended reaction conditions. However, for enzymes that have reported star activity, extra caution must be taken to set up reactions according to the recommended conditions to avoid unwanted cleavage.

Many techniques such as cloning, genotyping, mutational analysis, mapping, probe preparation, sequencing and methylation detection employ a wide range of reaction conditions and require the use of enzymes under suboptimal conditions. HF enzymes with reduced star activity offer increased flexibility to reaction setup and help maximize results under a wide range of conditions.

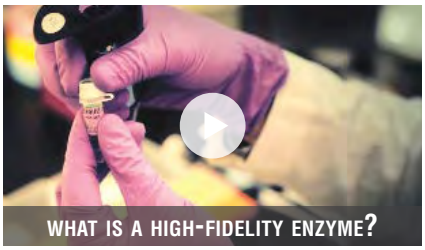
In addition to reduced star activity, all of these engineered enzymes work optimally in rCutSmart or CutSmart Buffer, which has the highest level of enzyme compatibility and will simplify double digest reactions. They are all Time-Saver qualified and digest substrate DNA in 5–15 minutes, and can also be incubated overnight without degradation of DNA. HF enzymes are available at the same price as the native enzymes and are supplied with purple loading dye.

Visit **www.neb.com/HF** to learn more about HF enzymes.

Tools & Resources

Visit www.neb.com/HF

- The full list of HF enzymes available
- Online tutorials on how to avoid star activity and setting up digests using the Time-Saver protocol



Learn how star activity is reduced with HF enzymes.

Isoschizomers

Restriction enzymes that recognize the same sequence are isoschizomers. The first example discovered is called a prototype, and all subsequent enzymes that recognize the same sequence are isoschizomers of the prototype.

NEB provides a list of isoschizomers for commercially-available restriction endonucleases at **www.neb.com/isoschizomers**. This table also specifies which isoschizomer is available from New England Biolabs.

All recognition sequences are written 5´ to 3´ using the single letter code nomenclature with the point of cleavage indicated by a "/".

Numbers in parentheses indicate point of cleavage for non-palindromic enzymes.
For example, GGTCTC(1/5) indicates cleavage at:

5´ ...GGTCTCN/...3´
3´ ...CCAGAGNNNNN/...5´

Isoschizomers with alternative cleavage sites (neoschizomers) are indicated with a "^". Enzymes that are not currently commercially available are indicated with a "⊗".

For more information on isoschizomers, visit **REBASE.neb.com**

Survival in a Reaction

Restriction enzymes vary with respect to their ability to maintain activity in a reaction over an extended period of time.

+ + +	Enzyme is active > 8 hours	N/A	Not Available
+ +	Enzyme is active 4–8 hours		
+	Enzyme is active 2–4 hours		
–	No benefit from digesting over 1 hour		

While most routine restriction digests are incubated for one hour or less at 37°C, there are certain applications that require the addition of less than 1 unit/µg of DNA and increasing the reaction time beyond one hour. The table below can be used as a guide when low levels of enzyme and extended reaction times are needed.

For example, 1 unit of AatII can be used to digest 8 µg of DNA in a 16 hour digest (+ + +).

Extended activity was determined by performing the restriction endonuclease unit assay, using a 16 hour incubation in place of the standard 1 hour digestion. After the 16 hour digestion, extended activity enzymes (+ + +) required only 0.13 units to completely digest 1 µg of DNA. Intermediate activity enzymes required either 0.25 (+ +) or 0.50 (+) units for complete digestion over this extended incubation time. Finally, enzymes marked (–) required 1.0 unit for complete digestion, the same amount of enzyme required for a 1 hour digestion.

Note: Reaction temperature is 37°C, unless otherwise noted.

Enzyme	Survival
AatII	+ + +
AbaSI @25°C	N/A
AccI	+ + +
Acc65I	+
AciI	–
AclI	+
AcuI	–
AfeI	+ +
AflII	+ + +
AflIII	+ + +
AgeI-HF	+ +
AhdI	+ + +
AleI-v2	+ + +
AluI	+ +
AlwI	+
AlwNI	+ + +
ApaI @25°C	+ + +
ApaLI	+ + +
ApeKI @75°C	+ + +
ApoI-HF	+ + +
AscI	+ + +
AseI	+ + +
AsiSI	+ + +
AvaI	+ +
AvaII	+ +
AvrII	+ + +
BaeI @25°C	+
BaeGI	+
BamHI	+
BamHI-HF	+
BanI	+ + +
BanII	+
BbsI	+ +
BbsI-HF	–
BbvI	–
BbvCI	+ + +

Enzyme	Survival
BccI	+
BceAI	+ + +
BcgI	+
BciVI	–
BclI @50°C	+
BclI-HF	N/A
BcoDI	+ + +
BfaI	+
BfuAI @50°C	+ +
BglI	+ + +
BglII	+ +
BlpI	+
BmgBI	–
BmrI	–
BmtI-HF	+ + +
BpmI	–
Bpu10I	+
BpuEI	–
BsaI-HFv2	+ + +
BsaAI	+ +
BsaBI @60°C	+
BsaHI	+ + +
BsaJI @60°C	+ + +
BsaWI @60°C	+ + +
BsaXI	+ +
BseRI	+
BseYI	+ +
BsgI	+
BsiEI @60°C	+ +
BsiHKAI @65°C	–
BsiWI @55°C	+ + +
BsiWI-HF	+ + +
BsmI @65°C	+ + +
BsmI @65°C	+
BsmAI @55°C	+ +
BsmBI-v2 @55°C	+

Enzyme	Survival
BsmFI @65°C	+ + +
BsoBI	+ + +
Bsp1286I	+
BspCNI @25°C	–
BspDI	+ +
BspEI	+ + +
BspHI	+ + +
BspMI	+ +
BspQI @50°C	–
BsrI @65°C	+ +
BsrBI	+
BsrDI @65°C	+
BsrFI-v2	+ + +
BsrGI-HF	+ + +
BssHII @50°C	+
BssSI-v2	+ + +
BstAPI @60°C	+ +
BstBI @65°C	+ + +
BstEII-HF	–
BstNI @60°C	–
BstUI @60°C	+ + +
BstXI @55°C	+ + +
BstYI @60°C	+ + +
BstZ17I-HF	+ +
Bsu36I	+ + +
BtgI	+
BtgZI @60°C	–
BtsI-v2 @55°C	+ + +
BtsMutI @55°C	+
BtsCI @50°C	+
Cac8I	+ +
ClaI	+
CspCI	+
CviAII @25°C	–
CviKI-1	–
CviQI @25°C	+ +

Enzyme	Survival
DdeI	+ + +
DpnI	+ + +
DpnII	+ + +
DraI	+ +
DraIII-HF	+ + +
DrdI	+ + +
EaeI	+ + +
EagI-HF	+ + +
EarI	+ + +
EciI	–
Eco53kI	+ +
EcoNI	+ + +
Eco0109I	+ + +
EcoP15I	–
EcoRI	+ + +
EcoRI-HF	+ + +
EcoRV	+
EcoRV-HF	+
Esp3I	+ + +
FatI @55°C	+
FauI @55°C	–
Fnu4HI	+ +
FokI	–
FseI	–
FspI	+ + +
FspEI	+ + +
HaeII	–
HaeIII	+ +
HgaI	–
HhaI	+ +
HinclI	+ + +
HindIII	+ + +
HindIII-HF	+ + +
HinfI	+ +
HinP1I	+ +
HpaI	+ +

Survival in a Reaction (continued)

Enzyme	Survival
HpaII	++
HphI	++
Hpy99I	–
Hpy166II	+++
Hpy188I	–
Hpy188III	++
HpyAV	–
HpyCH4III	+++
HpyCH4IV	++
HpyCH4V	++
I-CeuI	++
I-SceI	++
KasI	–
KpnI-HF	+
LpnPI	–
MboI	+
MboII	–
MfeI-HF	++
MluI-HF	+++
MluCI	–
MlyI	–
MmeI	–
MnlI	++
MscI	+
MseI	+++
MslI	–
MspI	–
MspA1I	++
MspJI	+++
MwoI @60°C	+++
NaeI	+

Enzyme	Survival
NarI	–
Nb.BbvCI	+++
Nb.BsmI @65°C	++
Nb.BsrDI @65°C	++
Nb.BssSI	+++
Nb.BtsI	++
NciI	+
NcoI	++
NcoI-HF	++
NdeI	+++
NgoMIV	+++
NheI-HF	+++
NlaIII	+
NlaIV	+
NmeAIII	–
NotI	++
NotI-HF	+++
NruI-HF	+++
NsiI	++
NsiI-HF	N/A
NspI	++
Nt.AlwI	+++
Nt.BbvCI	+++
Nt.BsmAI	+++
Nt.BspQI @50°C	++
Nt.BstNBI @55°C	+
Nt.CviPII	–
PacI	+++
PaeR7I	+++
PaqCI	++
PciI	++

Enzyme	Survival
PflFI	+++
PflMI	+
PI-PspI @65°C	+++
PI-SceI	+++
PleI	+
PluTI	+
PmeI	–
PmlI	+
PpuMI	+++
PshAI	–
PsiI-v2	+++
PspGI @75°C	++
PspOMI	+++
PspXI	+++
PstI	+
PstI-HF	+
PvuI-HF	+++
PvuII	+++
PvuII-HF	–
RsaI	++
RsrII	++
SacI-HF	+++
SacII	+++
SalI	+++
SalI-HF	++
SapI	–
Sau3AI	+
Sau96I	++
SbfI-HF	–
ScaI-HF	++
ScrFI	++

Enzyme	Survival
SexAI	++
SfaNI	+
SfcI	–
SfiI @50°C	++
SfoI	–
SgrAI	–
SmaI @25°C	+++
SmlI @55°C	++
SnaBI	+
SpeI-HF	+
SphI	+++
SphI-HF	–
SrfI	+++
SspI-HF	+
StuI	++
StyI-HF	++
StyD4I	+++
SwaI @25°C	++
TaqI-v2 @65°C	+
TfiI @65°C	++
TseI @65°C	+
Tsp45I @65°C	+
TspMI @75°C	+++
TspRI @65°C	+++
Tth111I @65°C	++
WarmStart Nt. BstNBI	+
XbaI	+++
XcmI	+++
XhoI	+++
XmaI	+
XmnI	++
ZraI	+

Kyle joined NEB in 2021 as a Development Scientist I in the Applications & Product Development Group. In his free time, Kyle enjoys hiking, fishing, camping, and playing board games.

Compatible Cohesive Ends and Generation of New Restriction Sites

Restriction enzymes that produce compatible cohesive ends often produce recleavable ligation products. The combinations listed were identified by computeranalysis, and havenot necessarily been confirmed by experimentation.

Where isoschizomers exist, only one member of each set is listed. A selection of enzymes available from New England Biolabs has been listed. For a more complete listing visit our website, **www.neb.com**

Enzymes that have degenerate recognition sequences (e.g., recognize more than one sequence) are followed by a specific sequence in parentheses and are only listed if a non-degenerate equivalent does not exist. Be aware that these degenerate enzymes will cleave sequences in addition to the one listed.
A "–" denotes a ligation product that cannot be recleaved.

Enzyme	Ligated To	Recleaved By
Acc65I (G/GTACC)	BanI (G/GTACC) BsiWI, BsrGI	Acc65I, BanI, KpnI, NlaIV, RsaI RsaI
AccI (GT/CGAC) (GT/CGAC)	AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI ClaI, BstBI, TaqI-v2	 — TaqI-v2
AciI (C/CGC)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI BsaHI (GR/CGCC), HinP1I, NarI HpaII	— AciI HpaII
AclI (AA/CGTT)	AccI (GT/CGAC), AciI, ClaI, BstBI, HinP1I, HpaII, NarI, TaqI	 —
AgeI* (A/CCGGT)	AvaI (C/CCGGG), XmaI BsaWI, BspEI BsrFI (A/CCGGT), SgrAI (CA/CCGGTG) NgoMIV	HpaII, NciI, ScrFI BsaWI, HpaII AgeI, BsaWI, BsrFI, HpaII BsrFI, HpaII
ApaI (GGGCC/C)	BanII (GGGCC/C), Bsp1286I (GGGCC/C)	ApaI, BanII, Bsp120I, Bsp1286I, HaeIII, NlaIV, Sau96I
ApaLI (G/TGCAC)	SfcI (C/TGCAG)	BsgI
ApoI* (A/AATTY) (G/AATTY) (R/AATTY)	 EcoRI EcoRI MfeI, Tsp509I	 ApoI, Tsp509I ApoI, EcoRI, Tsp509I Tsp509I
AscI (GG/CGCGCC)	AflIII (A/CGCGT), MluI BssHII	BstUI, HhaI BssHII, BstUI, Cac8I, HhaI
AseI (AT/TAAT)	BfaI, Csp6I, NdeI MseI	— MseI
AsiSI (GCGAT/CGC)	BsiEI (CGAT/CG) PacI PvuI	DpnII, PvuI MseI DpnII, PvuI
AvaI (C/CCGGG) (C/TCGAG) (C/TCGAG) (C/CCGGG)	 AgeI, BsaWI, BspEI, BsrFI (R/CCGGY), NgoMIV, SgrAI (CR/CCGGYG) XhoI SalI XmaI	 HpaII, NciI, ScrFI AvaI, TaqI-v2, XhoI TaqI-v2 AvaI, BsaJI, HpaII, NciI, ScrFI, SmaI
AvaII (G/GWCC)	PpuMI (RG/GACCY) RsrII PpuMI (RG/GTCCY)	AvaII, NlaIV, Sau96I AvaII, Sau96I AvaII, BsmFI, NlaIV, Sau96I
AvrII (C/CTAGG)	NheI, SpeI, XbaI StyI (C/CTAGG)	BfaI AvrII, BfaI, BsaJI, StyI
BamHI* (G/GATCC)	BclI, DpnII BglII, BstYI (R/GATCY) BstYI (G/GATCC)	AlwI, DpnII AlwI, BstYI, DpnII AlwI, BamHI, BstYI, DpnII, NlaIV
BanI (G/GTACC) (G/GCGCC) (G/GTACC)	 Acc65I KasI BsiWI, BsrGI	 Acc65I, BanI, KpnI, NlaIV, RsaI BanI, BsaHI, HaeII, HhaI, KasI, NarI, NlaIV RsaI
BanII (GGGCC/C) (GAGCT/C)	 ApaI, Bsp1286I (GGGCC/C) Bsp1286I (GAGCT/C), SacI	 ApaI, BanII, Bsp1286I, HaeIII, NlaIV, Sau96I AluI, BanII, BsiHKAI, Bsp1286I, SacI
BclI* (T/GATCA)	BamHI, BstYI (R/GATCY) BglII, MboI	AlwI, DpnII DpnII
BfaI (C/TAG)	AseI, Csp6I, MseI, NdeI	—

Enzyme	Ligated To	Recleaved By
BglII (A/GATCT)	BamHI, BstYI (R/GATCY) BclI, DpnII	AlwI, BstYI, DpnII DpnII
BsaHI (GR/CGYC) (GA/CGYC) (GG/CGYC) (GG/CGYC) (GA/CGYC) (GG/CGYC)	 AccI (GT/CGAC), ClaI, BstBI, TaqI-v2 AciI, HinP1I AciI, HinP1I HpaII NarI NarI	 — HgaI HhaI AciI BsaHI, HgaI BanI, BsaHI, HaeII, HhaI, NarI, NlaIV
BsaWI (W/CCGGW)	AgeI, BsrFI (R/CCGGY), SgrAI (CR/ CCGGYG) AvaI (C/CCGGG), XmaI BspEI BsrFI (R/CCGGY), NgoMIV NgoMIV	AgeI, BsaWI, BsrFI, HpaII HpaII, NciI, ScrFI BsaWI, BspEI, HpaII BsrFI, HpaII HpaII
BsiEI (CGAT/CG) (CGAT/CG) (CGGC/CG)	 PacI PvuI SacII	 MseI BsiEI, DpnII, PvuI AciI
BsiHKAI (GTGCA/C)	Bsp1286I (GTGCA/C) Bsp1286I (GAGCA/C) Bsp1286I (GAGCT/C), SacI NsiI PstI, SbfI	BsiHKAI, Bsp1286I BsiHKAI, Bsp1286I AluI, BanII, BsiHKAI, Bsp1286I, SacI — BsgI
BsiWI* (C/GTACG)	Acc65I, BanI (G/GTACC), BsrGI	RsaI
Bsp1286I (GGGCC/C) (GTGCA/C) (GGGCC/C) (GAGCT/C) (GWGCW/C) (GTGCA/C) (GTGCA/C)	 ApaI, BanII (GGGCC/C) BsiHKAI BanII (GGGCC/C) BanII (GAGCT/C), BsiHKAI, SacI BsiHKAI NsiI PstI, SbfI	 ApaI, BanII, Bsp1286I, HaeIII, NlaIV, Sau96I ApaLI, BsiHKAI, Bsp1286I BanII, Bsp1286I AluI, BanII, BsiHKAI, Bsp1286I, SacI BsiHKAI, Bsp1286I — BsgI
BspEI (T/CCGGA)	AgeI, BsaWI, BsrFI (R/CCGGY), SgrAI (CR/CCGGYG) AvaI (C/CCGGG), XmaI BsaWI BsrFI (R/CCGGY), NgoMIV	BsaWI, HpaII HpaII, NciI, ScrFI BsaWI, BspEI, HpaII HpaII
BspHI (T/CATGA)	FatI, NcoI, PciI	FatI, NlaIII
BsrFI (A/CCGGY) (G/CCGGY) (R/CCGGY) (A/CCGGY) (R/CCGGY) (G/CCGGY) (CR/CCGGYG)	 AgeI, BsaWI AgeI, BsaWI, NgoMIV AvaI (C/CCGGG), XmaI BsaWI, BspEI BsaWI, BspEI NgoMIV SgrAI	 AgeI, BsaWI, BsrFI, HpaII BsrFI, HpaII HpaII, NciI, ScrFI BsaWI, HpaII HpaII BsrFI, Cac8I, HpaII, NaeI BsrFI, HpaII
BsrGI* (T/GTACA)	Acc65I, BanI (G/GTACC), BsiWI	RsaI
BssHII (G/CGCGC)	MluI AscI	BstUI, HhaI BssHII, BstUI, Cac8I, HhaI
BstBI (TT/CGAA)	AccI (GT/CGAC), ClaI, TaqI-v2 AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	TaqI-v2 —

Compatible Cohesive Ends and Generation of New Restriction Sites (continued)

Enzyme	Ligated To	Recleaved By
BstYI (A/GATCY) (G/GATCY) (R/GATCY) (G/GATCY) (A/GATCY)	 BamHI, BglII BamHI BclI, DpnII BclI, DpnII BglII	 AlwI, BstYI, DpnII AlwI, BamHI, BstYI, DpnII, NlaIV DpnII AlwI, DpnII BglII, BstYI, DpnII
ClaI (AT/CGAT)	AccI (GT/CGAC), BstBI, TaqI-v2 AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	TaqI-v2 —
DpnII/MboI/ Sau3AI (/GATC)	BamHI, BstYI (R/GATCC) BclI, BglII, BstYI (R/GATCY)	AlwI, DpnII DpnII
EaeI (Y/GGCCR) (C/GGCCR) (T/GGCCR) (C/GGCCR) (T/GGCCR)	 PspOMI EagI EagI NotI NotI	 HaeIII, Sau96I BsiEI, EaeI, EagI, HaeIII EaeI, HaeIII AciI, BsiEI, EaeI, EagI, Fnu4HI, HaeIII AciI, EaeI, Fnu4HI, HaeIII
EagI* (C/GGCCG)	PspOMI EaeI (Y/GGCCR) EaeI (C/GGCCG) NotI	HaeIII, Sau96I EaeI, HaeIII BsiEI, EaeI, EagI, HaeIII AciI, BsiEI, EaeI, EagI, Fnu4HI, HaeIII
EcoRI* (G/AATTC)	ApoI (G/AATTC) ApoI (R/AATTY) MfeI, Tsp509I	ApoI, EcoRI, Tsp509I ApoI, Tsp509I Tsp509I
FatI (/CATG)	BspHI, NcoI, PciI	FatI, NlaIII
HinP1I (G/CGC)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI-v2 AciI, BsaHI (GR/CGCC), NarI BsaHI (GR/CGTC) HpaII	— HhaI HgaI AciI
HpaII/MspI (C/CGG)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI-v2 AciI, BsaHI (GR/CGCC), HinP1I, NarI	— AciI
KasI (G/GCGCC)	BanI (G/GCGCC)	BanI, BsaHI, HaeII, HhaI, KasI, NarI, NlaIV
MfeI* (C/AATTG)	ApoI (R/ATTTY), EcoRI, Tsp509I	Tsp509I
MluI (A/CGCGT)	AscI, BssHII	BstUI, HhaI
MseI (T/TAA)	AseI BfaI, Csp6I, NdeI	MseI —
NarI (GG/CGCC)	AccI (GT/CGAC), AclI, ClaI, BstBI, TaqI-v2 AciI, HinP1I BsaHI (GR/CGCC) BsaHI (GR/CGTC) HpaII	— HhaI BanI, BsaHI, HaeII, HhaI, NarI, NlaIV BsaHI, HgaI AciI
NcoI* (C/CATGG)	BspHI, FatI, PciI	FatI, NlaIII
NdeI (CA/TATG)	AseI, BfaI, Csp6I, MseI	—
NgoMIV (G/CCGGC)	AgeI, BsaWI, BsrFI (R/CCGGY), SgrAI AvaI (C/CCGGG), XmaI BsaWI, BspEI BsrFI (R/CCGGC), SgrAI	BsrFI, HpaII HpaII, NciI, ScrFI HpaII BsrFI, Cac8I, HpaII, NaeI
NheI* (G/CTAGC)	AvrII, SpeI, StyI (C/CTAGG), XbaI	BfaI
NlaIII (CATG/)	SphI, NspI	NlaIII
NotI* (GC/GGCCGC)	PspOMI EagI EaeI (Y/GGCCR)	AciI, EaeI, Fnu4HI, HaeIII AciI, BsiEI, EaeI, EagI, Fnu4HI, HaeIII AciI, BsiEI, EaeI, Fnu4HI, HaeIII

Enzyme	Ligated To	Recleaved By
NsiI* (ATGCA/T)	BsiHKAI (GTGCA/C), Bsp1286I (GTGCA/C), PstI, SbfI	—
NspI (RCATG/Y)	NlaIII, SphI	NlaIII, NspI
PacI (TTAAT/TAA)	AsiSI, BsiEI (CGAT/CG), PvuI	MseI
PciI (A/CATGT)	BspHI, FatI, NcoI	FatI, NlaIII
PpuMI (RG/GWCCY) (GG/GTCCY) (GG/GACCY)	 AvaII, RsrII AvaII, RsrII AvaII, RsrII	 AvaII, Sau96I AvaII, BsmFI, NlaIV, Sau96I AvaII, NlaIV, Sau96I
PspOMI (G/GGCCC)	EaeI (Y/GGCCR), EagI NotI	HaeIII, Sau96I AciI, Fnu4HI, HaeIII, Sau96I
PspXI (VC/TCGAGB)	XhoI, TliI SalI	XhoI, TliI TaqI-v2
PstI* (CTGCA/G)	BsiHKAI, Bsp1286I (GTGCA/C) NsiI SbfI	BsgI — PstI
PvuI* (CGAT/CG)	AsiSI PacI BsiEI (CGAT/CG)	DpnII, PvuI MseI BsiEI, DpnII, PvuI
RsrII (CG/GWCCG)	AvaII, PpuMI (RG/GACCY) PpuMI (RG/GACCY) PpuMI (RG/GTCCY)	AvaII, Sau96I AvaII, NlaIV, Sau96I AvaII, BsmFI, NlaIV, Sau96I
SacI* (GAGCT/C)	BanII (GAGCT/C), BsiHKAI, Bsp1286I (GAGCT/C)	AluI, BanII, BsiHKAI, Bsp1286I, SacI
SacII (CCGC/GG)	BsiEI (CGGC/CG)	AciI
SalI* (G/TCGAC)	PspXI, XhoI	TaqI-v2
SbfI* (CCTGCA/GG)	BsiHKAI, Bsp1286I (GTGCA/C) NsiI PstI	BsgI — PstI
SfcI (C/TGCAG)	ApaLI	BsgI
SgrAI (CR/CCGGYG)	See BsrFI	
SpeI* (A/CTAGT)	AvrII, NheI, StyI (C/CTAGG), XbaI	BfaI
SphI* (GCATG/C)	NlaIII, NspI	NlaIII, NspI
StyI* (C/CTAGG) (C/CATGG)	AvrII NheI, SpeI, XbaI BspHI NcoI	AvrII, BfaI, BsaJI, StyI BfaI NlaIII BsaJI, NcoI, NlaIII, StyI
TaqI-v2 (T/CGA)	AccI (GT/CGAC), ClaI, BstBI AciI, AclI, BsaHI (GR/CGYC), HinP1I, HpaII, NarI	TaqI-v2 —
Tsp509I (/AATT)	ApoI (R/AATTY), EcoRI, MfeI	Tsp509I
XbaI (T/CTAGA)	AvrII, NheI, SpeI, StyI (C/CTAGG)	BfaI
XhoI (TliI) (C/TCGAG)	PspXI SalI	XhoI, TliI TaqI-v2
XmaI (C/CCGGG)	AgeI, BsaWI, BspEI, BsrFI, NgoMIV, SgrAI AvaI (C/CCGGG)	HpaII, NciI, ScrFI AvaI, BsaJI, HpaII, NciI, ScrFI, SmaI, XmaI

*HF (high fidelity) versions of these enzymes are available.

Dam (GmATC), Dcm (CmCWGG) and CpG (mCG) Methylation

DNA methyltransferases (MTases) that transfer a methyl group from S-adenosylmethionine to either adenine or cytosine residues are found in a wide variety of prokaryotes and eukaryotes. Methylation should be considered when digesting DNA with restriction endonucleases because cleavage can be blocked or impaired when a particular base in the recognition site is methylated.

Prokaryotic Methylation

In prokaryotes, MTases have most often been identified as elements of restriction/modification systems that act to protect host DNA from cleavage by the corresponding restriction endonuclease. Most laboratory strains of *E. coli* contain three site-specific DNA methyltransferases.

- Dam methyltransferases– methylation at the N^6 position of the adenine in the sequence GATC (1,2).
- Dcm methyltransferases– methylation at the C^5 position of cytosine in the sequences CCAGG and CCTGG (1,3).
- EcoKI methylase– methylation of adenine in the sequences AAC(N^6A)GTGC and GCAC(N^6A)GTT.

Some or all of the sites for a restriction endonuclease may be resistant to cleavage when isolated from strains expressing the Dam or Dcm MTase if the methylase recognition site overlaps the endonuclease recognition site. For example, plasmid DNA isolated from *dam*$^+$ *E. coli* is completely resistant to cleavage by MboI, which cleaves at GATC sites.

Not all DNA isolated from *E. coli* is methylated to the same extent. While pBR322 DNA is fully modified (and is therefore completely resistant to MboI digestion), only about 50% of λ DNA Dam sites are methylated, presumably because the methylase does not have the opportunity to methylate the DNA fully before it is packaged into the phage head. As a result, enzymes blocked by Dam or Dcm modification will yield partial digestion patterns with λ DNA.

Restriction sites that are blocked by Dam or Dcm methylation can be unmethylated by cloning your DNA into a *dam*$^-$, *dcm*$^-$ strain of *E. coli*, such as *dam*$^-$/*dcm*$^-$ Competent *E. coli* (NEB #C2925).

Restriction sites can also be blocked if an overlapping site is present. In this case, part of the Dam or Dcm sequence is generated by the restriction enzyme sequence, followed by the flanking sequence. This situation should also be considered when designing restriction enzyme digests.

Eukaryotic Methylation

CpG MTases, found in higher eukaryotes (e.g., Dnmt1), transfer a methyl group to the C^5 position of cytosine residues. Patterns of CpG methylation are heritable, tissue specific and correlate with gene expression. Consequently, CpG methylation has been postulated to play a role in differentiation and gene expression (4).

Note: The effects of CpG methylation are mainly a concern when digesting eukaryotic genomic DNA. CpG methylation patterns are not retained once the DNA is cloned into a bacterial host.

Methylation Sensitivity

Information on methylation sensitivity for NEB restriction enzymes can be found in the Restriction Enzymes Performance Chart, as well as at **REBASE.neb.com**.

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(3) May, M.S. and Hattman, S. (1975) *J. Bacteriol.*, 123, 768–770.
(4) Siegfried, Z. and Cedar, H. (1997) *Curr. Biol.*, 7, –307.

Kathy joined NEB in 2019 as a Paralegal in our Legal Department. In her free time Kathy enjoys almost any outdoor activity, sewing, and a fine, craft beer.

Learn about dam, dcm and CpG methylation.

General Guidelines for PCR Optimization

New England Biolabs offers a diverse group of DNA Polymerases for PCR-based applications. Specific recommendations for PCR optimization can be found in the product literature or on the individual product webpages. However, these general guidelines will help to ensure success using New England Biolabs' PCR enzymes.

Setup Guidelines

DNA Template

- Use high quality, purified DNA templates whenever possible. Please refer to specific product information for amplification from unpurified DNA (e.g., colony PCR or direct PCR).
- For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg–10 ng of DNA per 50 µl reaction
- For higher complexity templates (e.g., genomic DNA), use 1 ng–1 µg of DNA per 50 µl reaction
- Higher DNA concentrations tend to decrease amplicon specificity, particularly for high numbers of cycles

Primers

- Primers should typically be 20–30 nucleotides in length, with 40–60% GC Content
- Primer Tm values should be determined with NEB's Tm Calculator (**TmCalculator.neb.com**)
- Primer pairs should have Tm values that are within 5°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between the primers
- Higher than recommended primer concentrations may decrease specificity
- When engineering restriction sites onto the end of primers, 6 nucleotides should be added 5´ to the site
- Annealing temperatures should be determined according to specific enzyme recommendations. *Please note that Q5® and Phusion®* annealing temperature recommendations are unique.*
- Final concentration of each primer should be 0.05–1 µM in the reaction. Please refer to the more detailed recommendations for each specific enzyme.
- When amplifying products > 20 kb in size, primers should be ≥ 24 nucleotides in length with a GC content above 50% and matched Tm values above 60°C
- To help eliminate primer degradation and subsequent non-specific product formation, use a hot-start enzyme (e.g., One*Taq*® Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)

Magnesium Concentration

- Optimal Mg^{2+} concentration is usually 1.5–2.0 mM for most PCR polymerases
- Most PCR buffers provided by NEB already contain sufficient levels of Mg^{2+} at 1X concentrations.
- NEB offers a variety of Mg-free reaction buffers to which supplemental Mg^{2+} can be added for applications that require complete control over Mg^{2+} concentration
- Further optimization of Mg^{2+} concentration can be done in 0.2-1 mM increments, if necessary. For some specific applications, the enzyme may require as much as 6 mM Mg^{2+} in the reaction.
- Excess Mg^{2+} may lead to spurious amplification; Insufficient Mg^{2+} concentrations may cause reaction failure

Deoxynucleotides

- Ideal dNTP concentration is typically 200 µM of each, however, some enzymes may require as much as 400 µM each. Please refer to specific product literature for more detailed recommendations.
- Excess dNTPs can chelate Mg^{2+} and inhibit the polymerase
- Lower dNTP concentration can increase fidelity, however, yield is often reduced
- The presence of uracil in the primer, template, or deoxynucleotide mix will cause reaction failure when using archaeal PCR polymerases. Use One*Taq* or *Taq* DNA Polymerases for these applications.

Enzyme Concentration

- Optimal enzyme concentration in the reaction is specific to each polymerase. Please see the product literature for specific recommendations.
- In general, excess enzyme can lead to amplification failure, particularly when amplifying longer fragments

Starting Reactions

- Unless using a hot start enzyme (e.g., One*Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase), assemble all reaction components on ice
- Add the polymerase last, whenever possible
- Transfer reactions to a thermocycler that has been pre-heated to the denaturation temperature. Please note that pre-heating the thermocycler is not necessary when using a hot start enzyme (e.g., One*Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase).

Cycling Guidelines

Denaturation

- Optimal denaturation temperature ranges from 94°–98°C and is specific to the polymerase in the reaction. Please refer to product information for recommended conditions.
- Avoid longer or higher temperature incubations unless required due to high GC content of the template
- For most PCR polymerases, denaturation of 5–30 seconds is recommended during cycling
- NEB's aptamer-based hot start enzymes do not require additional denaturation steps to activate the enzymes

Annealing

- Primer Tm values should be determined using the NEB Tm Calculator (**TmCalculator.neb.com**)
- For PCR polymerases other than Q5 High-Fidelity DNA Polymerase or Phusion High-Fidelity DNA Polymerase*, annealing temperatures are usually set at 2°–5°C below the lowest Tm of the primer pair
- When using Q5 High-Fidelity DNA Polymerase or Phusion High-Fidelity DNA Polymerase*, annealing temperatures should be set at 0°–3°C above the lowest Tm of the primer pair. Please refer to the product literature for detailed recommendations.
- Non-specific product formation can often be avoided by optimizing the annealing temperature or by switching to a hot start enzyme (e.g., One*Taq* Hot Start DNA Polymerase or Q5 Hot Start High-Fidelity DNA Polymerase)
- Annealing temperatures can be optimized by doing a temperature gradient PCR, starting at 5°C below the lowest Tm of the primer pair
- Ideally, primer Tm values should be less than the extension temperature. However, if Tm values are calculated to be greater than the extension temperature, a two-step PCR program (combining annealing and extension into one step) can be employed.

Extension

- Extension temperature recommendations range from 65°–72°C and are specific to each PCR polymerase. Please refer to the product literature for specific recommendations.
- Extension rates are specific to each PCR polymerase. In general, extension rates range from 15–60 seconds per kb. Please refer to the recommendations for each specific product.
- Longer than recommended extension times can result in higher error rates, spurious banding patterns and/or reduction of amplicon yields

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

PCR Troubleshooting Guide

The following guide can be used to troubleshoot PCR reactions. Additional tips for optimizing reactions can be found in the technical reference section of our website, **www.neb.com**.

Problem	Possible Cause	Solution
Sequence errors	Low fidelity polymerase	• Choose a higher fidelity polymerase such as Q5 High-Fidelity (NEB #M0491) or Phusion (NEB #M0530)* DNA Polymerases
	Suboptimal reaction conditions	• Reduce number of cycles • Decrease extension time • Decrease Mg^{2+} concentration in the reaction
	Unbalanced nucleotide concentrations	• Prepare fresh deoxynucleotide mixes
	Template DNA has been damaged	• Start with a fresh template • Try repairing DNA template with the PreCR® Repair Mix (NEB #M0309) • Limit UV exposure time when analyzing or excising PCR product from the gel
	Desired sequence may be toxic to host	• Clone into a non-expression vector • Use a low-copy number cloning vector
Incorrect product size	Incorrect annealing temperature	• Recalculate primer Tm values using the NEB Tm calculator (TmCalculator.neb.com)
	Mispriming	• Verify that primers have no additional complementary regions within the template DNA
	Improper Mg^{2+} concentration	• Adjust Mg^{2+} concentration in 0.2–1 mM increments
	Nuclease contamination	• Repeat reactions using fresh solutions
No product	Incorrect annealing temperature	• Recalculate primer Tm values using the NEB Tm calculator (TmCalculator.neb.com) • Test an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair
	Poor primer design	• Check specific product literature for recommended primer design • Verify that primers are non-complementary, both internally and to each other • Increase length of primer
	Poor primer specificity	• Verify that oligos are complementary to proper target sequence
	Insufficient primer concentration	• Primer concentration can range from 0.05–1 µM in the reaction. Please see specific product literature for ideal conditions
	Missing reaction component	• Repeat reaction setup
	Suboptimal reaction conditions	• Optimize Mg^{2+} concentration by testing 0.2–1 mM increments • Thoroughly mix Mg^{2+} solution and buffer prior to adding to the reaction • Optimize annealing temperature by testing an annealing temperature gradient, starting at 5°C below the lower Tm of the primer pair
	Poor template quality	• Analyze DNA via gel electrophoresis before and after incubation with Mg^{2+} • Check $A_{260/280}$ ratio of DNA template
	Presence of inhibitor in reaction	• Further purify starting template by alcohol precipitation, drop dialysis or commercial clean up kit (NEB #T1030) • Decrease sample volume
	Insufficient number of cycles	• Rerun the reaction with more cycles
	Incorrect thermocycler programming	• Check program, verify times and temperatures
	Inconsistent thermocycler block temperature	• Test calibration of heating block
	Contamination of reaction tubes or solutions	• Autoclave empty reaction tubes prior to use to eliminate biological inhibitors • Prepare fresh solutions or use new reagents
	Complex template	• Use Q5 High-Fidelity (NEB #M0491) or One*Taq* DNA Polymerase (NEB #M0482) • For GC-rich templates, use Q5 High-Fidelity DNA Polymerase (NEB #M0491) with the High GC Enhancer or One*Taq* DNA Polymerase (NEB #M0480) with One*Taq* GC Reaction Buffer (plus One*Taq* High GC Enhancer, if necessary) • For longer templates, we recommend LongAmp® Taq DNA Polymerase (NEB #M0323), Q5 or Q5 Hot Start High Fidelity DNA Polymerase (NEB #M0493)
Multiple or non-specific products	Premature replication	• Use a hot start polymerase, such as Q5 Hot Start High-Fidelity (NEB #M0493) or One*Taq* Hot Start (NEB #M0481) DNA Polymerases • Set up reactions on ice using chilled components and add samples to thermocycler preheated to the denaturation temperature
	Primer annealing temperature too low	• Recalculate primer Tm values using the NEB Tm Calculator (TmCalculator.neb.com) • Increase annealing temperature
	Incorrect Mg^{2+} concentration	• Adjust Mg^{2+} in 0.2–1 mM increments
	Poor primer design	• Check specific product literature for recommended primer design • Verify that primers are non-complementary, both internally and to each other • Increase length of primer • Avoid GC-rich 3´ ends
	Excess primer	• Primer concentration can range from 0.05–1 µM in the reaction. Please see specific product literature for ideal conditions.
	Contamination with exogenous DNA	• Use positive displacement pipettes or non-aerosol tips • Set-up dedicated work area and pipettor for reaction setup • Wear gloves during reaction setup
	Incorrect template concentration	• For low complexity templates (e.g., plasmid, lambda, BAC DNA), use 1 pg–10 ng of DNA per 50 µl reaction • For higher complexity templates (e.g., genomic DNA), use 1 ng–1 µg of DNA per 50 µl reaction

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

Optimization Tips for Luna® qPCR

New England Biolabs provides Luna products for your qPCR and RT-qPCR experiments. For more information on these products, visit **LUNAqPCR.com**.
The following tips can be used to help optimize qPCR. For RT-qPCR guidelines, please see next page.

Target Selection

- Short PCR amplicons, ranging from 70 to 200 bp, are recommended for maximum PCR efficiency
- Target sequences should ideally have a GC content of 40–60%
- Avoid highly repetitive sequences when possible

DNA Template

- Use high quality, purified DNA templates whenever possible. Luna qPCR is compatible with DNA samples prepared through typical nucleic acid purification methods.
- Template dilutions should be freshly prepared in either TE or water for each qPCR experiment
- Generally, useful concentrations of standard and unknown material will be in the range of 10^6 copies to 1 copy. For gDNA samples from large genomes, (e.g., human, mouse) a range of 50–1 pg of gDNA is typical. For small genomes, adjust as necessary using 10^6–1 copy input as an approximate range. Note that for dilutions in the single-copy range, some samples will contain multiple copies and some will have none, as defined by the Poisson distribution.
- To generate cDNA, use of the LunaScript® RT SuperMix Kit (NEB #M3010/E3010) is recommended. Up to 1 µg total RNA, 1 µg mRNA or 100 ng specific RNA can be used in a 20 µl reaction.
- cDNA does not need to be purified before addition to the Luna reaction but should be diluted at least 1:20 before addition to qPCR

Primers

- Primers should typically be 15–30 nucleotides in length
- Ideal primer content is 40–60% GC
- Primer Tm should be approximately 60°C
- Primer Tm calculation should be determined with NEB's TmCalculator (**TmCalculator.neb.com**) using the Hot Start *Taq* setting
- For best results in qPCR, primer pairs should have Tm values that are within 3°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between primers
- G homopolymer repeats ≥ 4 should be avoided
- Optimal primer concentration for dye-based experiments (250 nM) is lower than for probe-based experiments (400 nM). If necessary, the primer concentration can be optimized between 100–500 nM for dye-based qPCR or 200–900 nM for probe-based experiments.
- Higher primer concentrations may increase secondary priming and create spurious amplification products
- When using primer design software, enter sufficient sequence around the area of interest to permit robust primer design and use search criteria that permit cross-reference against relevant sequence databases to avoid potential off-target amplification.
- For cDNA targets, it is advisable to design primers across known exon-exon junctions in order to prevent amplification from genomic DNA
- Primers designed to target intronic regions can ensure amplification exclusively from genomic DNA

Hydrolysis Probes

- Probes should typically be 15–30 nucleotides in length to ensure sufficient quenching of the fluorophore
- The optimal probe concentration is 200 nM but may be optimized between 100 to 500 nM
- Both single or double-quenched probes may be used
- In general, non-fluorescence quenchers result in better signal-to-noise ratio than fluorescence quenchers
- Ideal probe content is 40–60% GC
- The probe Tm should be 5–10°C higher than the Tm of the primers to ensure all targeted sequences are saturated with probe prior to amplification by the primers
- Probes may be designed to anneal to either the sense or antisense strand
- Generally, probes should be designed to anneal in close proximity to either the forward or reverse primer without overlapping
- Avoid a 5´-G base which is known to quench 5´-fluorophores

Multiplexing

- Avoid primer/probe combinations that contain complementary sequences, and ensure target sequences do not overlap
- Probes should be designed such that each amplicon has a unique fluorophore for detection
- Select fluorophores based on the detection capabilities of the available real-time PCR instrument
- The emission spectra of the reporter fluorophores should not overlap
- Test each primer/probe combination in a singleplex reaction to establish a performance baseline. Ensure C_q values are similar when conducting the multiplex qPCR.
- Pair dim fluorescence dyes with high abundance targets and bright dyes with low abundance targets
- Optimization may require lower primer/probe concentrations to be used for high copy targets along with higher concentrations for low copy targets

Cycling Conditions

- Generally, best performance is achieved using the cycling conditions provided in the manual
- Longer amplicons (> 400 bp) can be used but may require optimization of extension times
- Due to the hot start nature of the polymerase, it is not necessary to preheat the thermocycler prior to use
- Select the "Fast" ramp speed where applicable (e.g., Applied Biosystems QuantStudio®)
- Amplification for 40 cycles is sufficient for most applications, but for very low input samples 45 cycles may be used

Reaction Setup

- For best results, keep reactions on ice prior to thermocycling
- A reaction volume of 20 µl is recommended for 96-well plates while a reaction volume of 10 µl is recommended for 384-well plates
- Reactions should be carried out in triplicate for each sample
- For each amplicon, ensure to include no template controls (NTC)
- To prevent carry-over contamination, treat reactions with 0.2 units/µl Antarctic Thermolabile UDG (NEB #M0372) for 10 minutes at room temperature prior to thermocycling
- The Luna reference dye supports broad instrument compatibility (High-ROX, Low-ROX, ROX-independent) so no additional ROX is required for normalization

Assay Performance

- Ensure 90–110% PCR efficiency for the assay over at least three $\log_{10}$ dilutions of template
- Linearity over the dynamic range (R^2) should ideally be ≥ 0.99
- Target specificity should be confirmed by product size, sequencing or melt-curve analysis

Optimization Tips for Luna® One-Step RT-qPCR

New England Biolabs provides Luna products for your qPCR and RT-qPCR experiments. For more information on these products, visit **LUNAqPCR.com**. The following tips can be used to help optimize your one-step RT-qPCR. For qPCR guidelines (DNA/cDNA starting material), please see previous page.

Target Selection

- Short PCR amplicons, ranging from 70 to 200 bp, are recommended for maximum PCR efficiency
- Target sequences should ideally have a GC content of 40–60%
- Avoid highly repetitive sequences when possible
- Target sequences containing significant secondary structure should be avoided

RNA Template

- Use high quality, purified RNA templates whenever possible. Luna RT-qPCR is compatible with RNA samples prepared through typical nucleic acid purification methods.
- Prepared RNA should be stored in an EDTA-containing buffer (e.g., 1X TE) for long-term stability
- Template dilutions should be freshly prepared in either TE or water for each qPCR experiment
- Treatment of RNA samples with DNase I (NEB #M0303) may minimize amplification from genomic DNA contamination
- Generally, useful concentrations of standard and unknown material will be in the range of 10^8 copies to 10 copies. Note that for dilutions in the single-copy range, some samples will contain multiple copies and some will have none, as defined by the Poisson distribution. For total RNA, Luna One-Step Kits can provide linear quantitation over an 8-order input range of 1 µg–0.1 pg. For most targets, a standard input range of 100 ng–10 pg total RNA is recommended. For purified mRNA, input of ≤ 100 ng is recommended. For *in vitro*-transcribed RNA, input of ≤ 10^9 copies is recommended.

Primers

- Primers should typically be 15–30 nucleotides in length
- Ideal primer content is 40–60% GC
- Primer Tm should be approximately 60°C
- Primer Tm calculation should be determined with NEB's TmCalculator. (**TmCalculator.neb.com**) using the Hot Start *Taq* setting.
- For best results in qPCR, primer pairs should have Tm values that are within 3°C
- Avoid secondary structure (e.g., hairpins) within each primer and potential dimerization between primers
- G homopolymer repeats ≥ 4 should be avoided
- The optimal primer concentration for dye-based experiments and probe-based experiments is 400 nM. If necessary, the primer concentration can be optimized between 100–900 nM.
- Higher primer concentrations may increase secondary priming and create spurious amplification products
- When using primer design software, enter sufficient sequence around the area of interest to permit robust primer design and use search criteria that permit cross-reference against relevant sequence databases to avoid potential off-target amplification
- It is advisable to design primers across known exon-exon junctions in order to prevent amplification from genomic DNA

Hydrolysis Probes

- Probes should typically be 15–30 nucleotides in length to ensure sufficient quenching of the fluorophore
- The optimal probe concentration is 200 nM but may be optimized between 100 to 500 nM
- Both single or double-quenched probes may be used
- In general, non-fluorescence quenchers result in better signal-to-noise ratio than fluorescence quenchers
- Ideal probe content is 40–60% GC
- The probe Tm should be 5–10°C higher than the Tm of the primers to ensure all targeted sequences are saturated with probe prior to amplification by the primers
- Probes may be designed to anneal to either the sense or antisense strand
- Generally, probes should be designed to anneal in close proximity to either the forward or reverse primer without overlapping
- Avoid a 5´-G base which is known to quench 5´-fluorophores

Multiplexing

- Avoid primer/probe combinations that contain complementary sequences, and ensure target sequences do not overlap
- Probes should be designed such that each amplicon has a unique fluorophore for detection
- Select fluorophores based on the detection capabilities of the available real-time PCR instrument
- The emission spectra of the reporter fluorophores should not overlap
- Test each primer/probe combination in a singleplex reaction to establish a performance baseline. Ensure C_q values are similar when conducting the multiplex qPCR.
- Pair dim fluorescence dyes with high abundance targets and bright dyes with low abundance targets
- Optimization may require lower primer/probe concentrations to be used for high copy targets along with higher concentrations for low copy targets

Reverse Transcription

- The default reverse transcription temperature is 55°C
- For difficult targets, the temperature of reverse transcription may be increased to 60°C for 10 minutes
- Due to the WarmStart feature of the Luna RT, reverse transcription temperatures lower than 50°C are not recommended

Cycling Conditions

- Generally, best performance is achieved using the cycling conditions provided in the manual
- Longer amplicons (> 400 bp) can be used but may require optimization of extension times
- Due to the dual WarmStart/Hot Start feature of the Luna kits, it is not necessary to preheat the thermocycler prior to use
- Select the "Fast" ramp speed where applicable (e.g., Applied Biosystems QuantStudio).
- Amplification for 40 cycles is sufficient for most applications, but for very low input samples 45 cycles may be used

Reaction Setup

- For best results, keep reactions on ice prior to thermocycling
- A reaction volume of 20 µl is recommended for 96-well plates while a reaction volume of 10 µl is recommended for 384-well plates
- Reactions should be carried out in triplicate for each sample
- For each amplicon, ensure to include no template controls (NTC)
- A no Luna RT control should be conducted to guarantee amplification is specific for RNA input and not due to genomic DNA contamination
- To prevent carry-over contamination, treat reactions with 0.2 units/µl Antarctic Thermolabile UDG (NEB #M0372) for 10 minutes at room temperature prior to thermocycling. Some Luna products (NEB #M3019, M3029, L4001) contain Thermolabile UDG, so no treatment is necessary.
- The Luna reference dye supports broad instrument compatibility (High-ROX, Low-ROX, ROX-independent) so no additional ROX is required for normalization
- No ROX versions (NEB #E3007, M3029) contain no reference dye and are compatible with any instrument that does not require ROX. If ROX normalization is needed, ROX can be added. Please refer to instrument manufacturer's instructions for details.

Assay Performance

- Ensure 90–110% PCR efficiency for the assay over at least three $\log_{10}$ dilutions of template.
- Linearity over the dynamic range (R^2) should ideally be ≥ 0.99
- Target specificity should be confirmed by product size, sequencing or melt-curve analysis

Luna® qPCR Troubleshooting Guide

Problem	Probable Cause(s)	Solution(s)
qPCR traces show low or no amplification	Reagent omitted from qPCR assay	• Verify all steps of the protocol were followed correctly
	Reagent added improperly to qPCR assay	
	Incorrect cycling protocol	• Refer to the proper qPCR cycling protocol in product manual
	Incorrect channel selected for the qPCR thermal cycler	• Verify correct optical settings on the qPCR instrument
	DNA template or reagents are contaminated or degraded	• Confirm the expiration dates of the kit reagents • Verify proper storage conditions provided in this user manual • Rerun the qPCR assay with fresh reagents • Confirm template input amount
Inconsistent qPCR traces for triplicate data	Improper pipetting during qPCR assay set-up	• Ensure proper pipetting techniques
	qPCR plate film has lost its seal, causing evaporation in the well. The resulting qPCR trace may show significantly different fluorescence values relative to its replicates	• Ensure the qPCR plate is properly sealed before inserting into the qPCR thermal cycler. • Exclude problematic trace(s) from data analysis.
	Poor mixing of reagents during qPCR set-up	• Make sure all reagents are properly mixed after thawing them
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler • Exclude problematic trace(s) from data analysis
DNA standard curve has a poor correlation coefficient/efficiency of the DNA standard curve falls outside the 90–110% range	Presence of outlying qPCR traces	• Omit data produced by qPCR traces that are clearly outliers caused by bubbles, plate sealing issues, or other experimental problems
	Improper pipetting during qPCR assay set-up	• Ensure that proper pipetting techniques are used
	Reaction conditions are incorrect	• Verify that all steps of the protocol were followed correctly
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler
	Poor mixing of reagents	• After thawing, make sure all reagents are properly mixed
	Threshold is improperly set for the qPCR traces	• Ensure the threshold is set in the exponential region of qPCR traces • Refer to the real-time instrument user manual to manually set an appropriate threshold
Melt curve shows different peaks for low input samples	Non-template amplification is occurring	• Compare melt curve of NTC to samples • Redesign primers with a Tm of 60°C or use our Tm calculator to determine the optimal annealing temperature of the primers • Perform a primer matrix analysis to determine optimal primer concentrations
	Infrequently, denaturation of a single species can occur in a biphasic manner, resulting in two peaks	
No template control qPCR trace shows amplification, NTC Cq is close to or overlapping lower copy standards	Reagents are contaminated with carried-over products of previous qPCR (melt curve of NTC matches melt curve of higher input standards)	• Replace all stocks and reagents • Clean equipment and setup area with a 10% chlorine bleach • Consider use of 0.2 U/l Antarctic Thermolabile UDG to eliminate carryover products
	Primers produce non-specific amplification (melt curve of NTC does not match melt curve of higher input standards)	• Redesign primers with a Tm of 60°C or use qPCR primer design software

Luna® One-Step RT-qPCR Troubleshooting Guide

Problem	Probable Cause(s)	Solution(s)
qPCR traces show low or no amplification	Incorrect RT step temperature or RT step omitted	• For typical use, a 55°C RT step temperature is optimal for the Luna WarmStart Reverse Transcriptase.
	Incorrect cycling protocol	• Refer to the proper RT-qPCR cycling protocol in product manual
	Reagent omitted from RT-qPCR assay	• Verify all steps of the protocol were followed correctly
	Reagent added improperly to RT-qPCR assay	
	Incorrect channel selected for the qPCR thermal cycler	• Verify correct optical settings on the qPCR instrument
	RNA template or reagents are contaminated or degraded	• Prepare high quality RNA without RNase/DNase contamination • Confirm template input amount • Confirm the expiration dates of the kit reagents • Verify proper storage conditions provided in product manual • Rerun the RT-qPCR assay with fresh reagents
Inconsistent qPCR traces for triplicate data	Improper pipetting during RT-qPCR assay set-up	• Ensure proper pipetting techniques
	qPCR plate film has lost its seal, causing evaporation in the well. The resulting qPCR trace may show significantly different fluorescence values relative to its replicates.	• Ensure the qPCR plate is properly sealed before inserting into the qPCR thermal cycler • Exclude problematic trace(s) from data analysis
	Poor mixing of reagents during RT-qPCR set-up	• Make sure all reagents are properly mixed after thawing them
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler • Exclude problematic trace(s) from data analysis
Standard curve has a poor correlation coefficient/ efficiency of the standard curve falls outside the 90–110% range	Cycling protocol is incorrect	• Refer to the proper RT-qPCR cycling protocol in product manual • Use a 55°C RT step temperature • For ABI instruments, use a 1 minute 60°C annealing/ extension step
	Presence of outlying qPCR traces	• Omit data produced by qPCR traces that are clearly outliers caused by bubbles, plate sealing issues, or other experimental problems
	Improper pipetting during RT-qPCR assay set-up	• Ensure that proper pipetting techniques are used
	Reaction conditions are incorrect	• Verify that all steps of the protocol were followed correctly
	Bubbles cause an abnormal qPCR trace	• Avoid bubbles in the qPCR plate • Centrifuge the qPCR plate prior to running it in the thermal cycler
	Poor mixing of reagents	• After thawing, make sure all reagents are properly mixed
	Threshold is improperly set for the qPCR traces	• Ensure the threshold is set in the exponential region of qPCR traces • Refer to the real-time instrument user manual to manually set an appropriate threshold
Melt curve shows different peaks for low input samples	Non-template amplification is occurring Infrequently, denaturation of a single species can occur in a biphasic manner, resulting in two peaks	• Compare melt curve of NTC to samples • Redesign primers with a Tm of 60°C or use our Tm calculator to determine the optimal annealing temperature of the primers • Perform a primer matrix analysis to determine optimal primer concentrations
No template control qPCR trace shows amplification/ NTC Cq is close to or overlapping lower copy standards	Reagents are contaminated with carried-over products of previous qPCR (Melt curve of NTC matches melt curve of higher input standards)	• Replace all stocks and reagents • Clean equipment and setup area with a 10% chlorine bleach • Consider use of 0.2 U/l Antarctic Thermolabile UDG to eliminate carryover products
	Primers produce non-specific amplification (Melt curve of NTC does not match melt curve of higher input standards)	• Redesign primers with a Tm of 60°C or use qPCR primer design software
Amplification in No-RT control	RNA is contaminated with genomic DNA	• Treat sample with DNase I • Redesign primer to span exon-exon junction

Cleavage Close to the End of DNA Fragments

Annealed 5´ FAM-labeled oligos were incubated with the indicated enzyme (10 units/ 1 pmol oligo) for 60 minutes at the recommended incubation temperature and NEBuffer. The digest was run on a TBE acrylamide gel and analyzed by fluorescent imaging. The double stranded oligos were designed to have the indicated number of base pairs from the end followed by the recognition sequence and an additional 12 bases. In some cases asymmetric cleavage was observed and interpreted as a negative result. Asymmetric cleavage decreased with increasing base pairs from the end.

Note: As a general rule and for enzymes not listed below, 6 base pairs should be added on on either side of the recognition site to cleave efficiently.

The extra bases should be chosen so that palindromes and primer dimers are not formed. In most cases there is no requirement for specific bases.

–	0%	+	0–20%
++	20–50%	+++	50–100%
NT	not tested		

Enzyme	Base Pairs From End 1 bp	2 bp	3 bp	4 bp	5 bp
AccI*	–	–	–	–	–
AciI	–	+	+	++	+++
AgeI-HF	++	+++	+++	+++	+++
AleI-v2	+++	+++	+++	+++	+++
AluI	–	+++	+++	+++	+++
ApaI	+++	+++	+++	+++	+++
AscI	+++	+++	+++	+++	+++
AvrII	++	++	+++	+++	+++
BamHI	+	++	+++	+++	+++
BamHI-HF	+	+	+++	+++	+++
BbsI-HF	+++	+++	+++	+++	+++
BclI-HF	–	–	+++	+++	+++
BglII	++	+++	+++	+++	+++
BmtI-HF	+++	+++	+++	+++	+++
BsaI-HFv2	+++	+++	+++	+++	+++
BsiWI	++	+++	+++	+++	+++
BsiWI-HF	+++	+++	+++	+++	+++
BsmBI-v2	+++	+++	+++	+++	+++
BsrGI-HF	+++	+++	+++	+++	+++
BssHII	+	+++	+++	+++	+++
BstZ17I-HF	+	+++	+++	+++	+++
ClaI	–	–	+	+++	+++
DdeI	+++	+++	+++	+++	+++
DpnI	–	++	++	NT	NT
DraIII-HF	+++	+++	+++	+++	+++
EagI-HF	+	+++	+++	+++	+++
EcoRI	+	+	++	++	+++
EcoRI-HF	+	+	++	+++	+++
EcoRV	++	++	++	++	+++
EcoRV-HF	+	++	++	++	+++
Esp3I	+++	+++	+++	+++	+++
FseI	+	++	+++	+++	+++
HindIII	–	+	+++	+++	+++
HindIII-HF	–	+	+++	+++	+++
HpaI	+++	+++	+++	+++	+++
KpnI-HF	+	+++	+++	+++	+++
MfeI-HF	+	++	+++	+++	+++
MluI-HF	+	++	+++	+++	+++

*AccI requires at least 13 base pairs beyond the end of its recognition sequence to cleave efficiently.

Enzyme	Base Pairs From End 1 bp	2 bp	3 bp	4 bp	5 bp
MseI	+++	+++	+++	+++	+++
NcoI	–	++	+++	+++	+++
NcoI-HF	+	++	+++	+++	+++
NdeI	+	+	+++	+++	+++
NheI-HF	++	++	+++	+++	+++
NlaIII	++	+++	+++	+++	+++
NotI	++	++	++	++	++
NotI-HF	++	++	++	++	++
NsiI	+	+	+++	+++	+++
NspI	–	–	+	+	+++
PacI	+++	+++	+++	+++	+++
PaqCI	++	+++	–	–	–
PciI	+++	+++	+++	+++	+++
PmeI	+++	+++	+++	+++	+++
PsiI-v2	+	+++	+++	+++	+++
PstI-HF	++	+++	+++	+++	+++
PvuI-HF	+++	+++	+++	+++	+++
PvuII	++	++	++	+++	+++
PvuII-HF	–	++	++	+++	+++
RsaI	+	+++	+++	+++	+++
SacI-HF	–	+	+++	+++	+++
SacII	+++	+++	+++	+++	+++
SalI	–	++	+++	+++	+++
SalI-HF	–	++	+++	+++	+++
SapI	+++	+++	+++	+++	+++
Sau3AI	+++	+++	+++	+++	+++
SbfI-HF	++	+++	+++	+++	+++
ScaI-HF	+	+++	+++	+++	+++
SfiI	+++	+++	+++	+++	+++
SmaI	+++	+++	+++	+++	+++
SpeI-HF	+	++	++	++	++
SphI	+++	+++	+++	+++	+++
SphI-HF	++	++	+++	+++	+++
SspI-HF	+	+++	+++	+++	+++
StuI	+++	+++	+++	+++	+++
StyI-HF	+	+++	+++	+++	+++
XbaI	++	++	++	++	++
XhoI	++	++	++	+++	+++
XmaI	+++	+++	+++	+++	+++

Activity of Restriction Enzymes in PCR Buffers

Frequently, a PCR product must be further manipulated by cleavage with restriction enzymes. This table summarizes the activity of restriction enzymes on the DNA in *Taq,* Q5, Phusion*, One*Taq* and LongAmp *Taq* PCR mixes. 50 µl reactions containing 5 units of restriction enzyme were incubated at the appropriate temperature for 1 hour in a PCR mix containing the following: 1 µg DNA, 1 unit of DNA Polymerase and 1X ThermoPol Reaction Buffer, Standard *Taq* Reaction Buffer, Phusion HF Buffer, One*Taq* Standard Reaction Buffer or LongAmp *Taq* Reaction Buffer. Reactions were supplemented with 200 µM dNTPs. Enzyme activity was analyzed by gel electrophoresis.

Notes: The polymerase is still active and can alter the ends of DNA fragments after cleavage, affecting subsequent ligation. Primers containing the restriction enzyme recognition site can act as competitive inhibitors in the cleavage reaction. The use of restriction enzymes under non-optimal conditions may increase the likelihood of star activity. If any problems are encountered, the DNA should be purified by spin column or phenol/chloroform extraction followed by alcohol precipitation.

Cleavage in extension mix with 5 units of enzyme:
+++ complete cleavage ++ ~50% cleavage
+ ~25% cleavage – no cleavage

Enzyme	*Taq* in Thermopol Rxn Buffer	Q5 in Q5 Buffer**	Phusion in Phusion HF Buffer	One*Taq* in One*Taq* Rxn Buffer	LongAmp *Taq* in LongAmp *Taq* Rxn Buffer
AatII	<+++	<+	+	++	+
AccI	<+++	<+	<+	+++	+++
Acc65I	+++	<+	<+	<+	+
AciI	++	++	+++	+++	+++
AclI	+++	<+	<+	+++	+++
AcuI	+++	<+	++	+++	+++
AfeI	+++	<+	++	+++	+++
AflII	+	<+	<+	+	<+
AflIII	<+	+++	+	<+	<+
AgeI-HF	+++	<+	++	+++	+++
AhdI	<+	–	–	<+	<+
AleI-v2	–	–	–	+	+
AluI	+++	+	+++	+++	+++
AlwI	–	<+	<+	<+	<+
AlwNI	<+++	+	+++	<+	+
ApaI	+++	<+	<+	<+	–
ApaLI	+++	<+	<+	+++	+++
ApeKI @75°C	<+++	++	+++	<+	+
ApoI-HF	+++	+	++	+++	+++
AscI	+++	<+	<+	<+	–
AseI	+++	<+	+	++	++
AsiSI	+++	<+	++	+++	+++
AvaI	+++	<+	+++	+++	+
AvaII	+++	<+	++	+++	+++
AvrII	+++	<+	<+	+++	+++
BaeGI	+++	<+	+++	+++	+++
BaeI	–	<+	++	<+	<+
BamHI	+++	<+	+++	+++	+++
BamHI-HF	+++	<+	–	<+	++
BanI	+++	<+	+++	+++	+++
BanII	+++	<+	+++	+++	+++
BbsI	+++	<+	<+	+++	+++
BbsI-HF	+	–	–	–	+
BbvCI	+++	–	–	<+	<+
BbvI	+++	<+	++	+++	+++
BccI	<+	<+	<+	<+	<+
BceAI	<+	<+	++	+	<+
BcgI	<+	<+	+	++	++
BciVI	–	–	–	<+	–
BclI @50°C	+++	++	+++	+++	+++
BclI-HF	+++	–	–	+	+
BcoDI	<+	<+	+	+	<+
BfaI	–	<+	–	–	–
BfuAI	<+++	–	+	<+	–
BglI	<+	++	+	<+	<+
BglII	<+	+	++	<+	<+
BlpI	<+++	<+	<+	<+	–
BmgBI	–	++	+	<+	<+
BmrI	<+++	<+	+++	+++	+++
BmtI-HF	++	<+	+	++	+++
BpmI	<+	<+	+++	<+++	<+++
BpuEI	+++	–	++	<+++	<+++
Bpu10I	<+	<+	+++	++	+++
BsaAI	+++	++	+++	+++	+++
BsaBI @60°C	+	<+	++	++	+++
BsaHI	+++	+	+++	+++	+++
BsaI-HFv2	+	<+	+	+	++

Enzyme	*Taq* in Thermopol Rxn Buffer	Q5 in Q5 Buffer**	Phusion in Phusion HF Buffer	One*Taq* in One*Taq* Rxn Buffer	LongAmp *Taq* in LongAmp *Taq* Rxn Buffer
BsaJI @60°C	+++	<+	++	+++	+++
BsaWI @60°C	<+++	<+	++	+	+
BsaXI	<+++	<+	<+	<+	<+
BseRI	+++	<+	++	++	+
BseYI	+++	++	++	+++	+++
BsgI	<+	<+	+	<+	<+
BsiEI @60°C	+++	<+	++	++	++
BsiHKAI @65°C	–	++	+	–	–
BsiWI @55°C	+++	<+	+++	+++	+++
BsiWI-HF	–	–	–	–	–
BsII @55°C	+++	++	+++	+++	+++
BsmAI @55°C	+++	++	+++	<+	<+
BsmBI-v2 @55°C	<+++	+	++	<+	<+
BsmFI @65°C	<+	+++	++	+	+
BsmI @65°C	+++	+	<+	+++	+
BsoBI	+++	+++	+++	++	+++
BspCNI	<+	<+	+	–	–
BspDI	<+++	<+	++	+++	+++
BspEI	–	<+	<+	–	–
BspHI	+++	<+	+++	+++	+++
Bsp1286I	<+	<+	<+	<+	<+
BspMI	+++	<+	++	<+	<+
BspQI @50°C	+	++	+++	+++	+++
BsrBI	+++	<+	+	+++	+++
BsrDI	<+	<+	+	<+	<+
BsrFI-v2	<+	–	–	–	–
BsrI	+++	<+	+++	++	+++
BssHII	+++	<+	+	+++	+++
BssSI-v2	+++	–	+	+++	+++
BstAPI @60°C	+++	<+	++	+++	+++
BstBI @65°C	+++	++	+++	+++	+++
BstEII-HF	+++	<+	<+	++	++
BstNI @60°C	+++	<+	<+	<+	<+
BstUI @60°C	+++	<+	<+	+++	+
BstXI	<+++	+	+	+	<+
BstYI @60°C	+++	<+	<+	++	+
BstZ17I-HF	+++	–	+	+++	+++
Bsu36I	<+	<+	<+	<+	+
BtgI	+++	<+	+	<+	<+
BtgZI @60°C	+++	+	++	++	++
BtsI-v2	+++	–	+	+++	+++
BtsCI @50°C	+++	<+	<+	+++	+++
Cac8I	+++	<+	<+	+++	++
ClaI	++	<+	<+	<+	++
CspCI	<+	–	+	<+	<+
CviAII @25°C	+++	<+	+	+++	+++
CviKI-1	+++	<+	++	+++	+++
CviQI	+++	+	+++	++	+++
DdeI	+++	++	+	+++	+++
DpnI	+++	++	+++	++	++
DpnII	+++	++	+++	+++	++
DraI	+++	<+	+++	+++	+++
DraIII-HF	++	++	+++	++	++
DrdI	+++	<+	+++	+++	+++
EaeI	+++	<+	–	<+	<+
EagI-HF	+	<+	+	++	++

* Phusion DNA Polymerase was developed by Finnzymes Oy, now a part of Thermo Fisher Scientific. This product is manufactured by New England Biolabs, Inc. under agreement with, and under the performance specifications of Thermo Fisher Scientific. Phusion® is a registered trademark and property of Thermo Fisher Scientific.

** It has been shown that the addition of 1X Restriction Enzyme Buffer may help to improve the ability of some enzymes to cleave.

Activity of Restriction Enzymes in PCR Buffers (continued)

Enzyme	*Taq* in Thermopol Rxn Buffer	Q5 in Q5 Buffer**	Phusion in Phusion HF Buffer	One*Taq* in One*Taq* Rxn Buffer	Longamp *Taq* in Longamp *Taq* Rxn Buffer
EarI	+++	<+	+++	+	<+
EciI	<+	++	+++	<++	<++
Eco53kI	+++	<+	<+	+++	+++
EcoNI	+++	<+	+	+++	+++
EcoO109I	+++	<+	–	<+	+
EcoP15I	<+	<+	+	<+	+
EcoRI	+	<+	+++	–	–
EcoRI-HF	+++	<+	+	+++	+++
EcoRV	<+	<+	+	–	<+
EcoRV-HF	+	<+	<+	+	++
Esp3I	+++	-	+++	+	+++
FatI @55°C	++	<+	+++	<+	+++
FauI @55°C	+	<+	++	+++	++
Fnu4HI	+++	<+	<+	++	+
FokI	+++	+	+	+++	+++
FseI	+	<+	++	+++	–
FspI	<++	<+	+	+	+
HaeII	+++	<+	+++	+++	+++
HaeIII	+++	<+	+++	+++	+++
HgaI	<+	<+	+	<++	<++
HhaI	+++	<+	+++	+++	+++
HincII	+++	<+	<+	+++	+++
HindIII	+++	<+	+	++	+++
HindIII-HF	+++	<+	<+	+++	+++
HinfI	+++	+++	+++	+	+++
HinP1I	+++	+	+++	+++	+++
HpaI	+++	<+	+++	+++	+++
HpaII	+++	<+	<+	<+	<+
HphI	<++	<+	<+	<+	<+
HpyAV	+++	–	++	+	++
HpyCH4III	<++	<+	+	<++	<++
HpyCH4IV	+++	<+	<+	+++	+++
HpyCH4V	+++	<+	<+	+++	+++
Hpy99I	+++	–	+	<+	<+
Hpy188I	+++	<+	+	++	++
Hpy166II	+++	+	++	+++	+++
Hpy188III	+	<+	<+	+	<+
KasI	+++	<+	+++	+++	–
KpnI-HF	++	–	++	<+	<+
MboI	+++	<+	+++	+++	+++
MboII	+++	+	++	+	+
MfeI-HF	+	–	–	+++	<+
MluCI	+	<+	<+	++	+
MluI-HF	++	–	++	++	++
MlyI	+++	+	++	<+	+
MmeI	<+	–	++	<+	<+
MnlI	+++	+	+	+	+
MscI	<+	<+	+	<+	<+
MseI	<+	<+	<+	<+	<+
MslI	+++	<+	+	+++	++
MspA1I	+++	<+	+++	++	+++
MspI	+++	<+	+++	++	+++
MwoI @60°C	+++	+++	+++	++	+++
NaeI	<+	<+	+	<+	<+
NarI	–	<+	++	+++	+++
NciI	+++	<+	<+	+	<+
NcoI	+++	<+	+	++	++
NcoI-HF	+++	<+	–	++	+
NdeI	<++	++	+++	++	<+
NgoMIV	–	<+	+	<+	<+
NheI-HF	+++	<+	–	++	++
NlaIII	<+	<+	+	++	<+
NlaIV	+++	<+	+++	+++	+++
NmeAIII	<+	–	+++	<+	<+
NotI	++	<+	+	<+	<+
NotI-HF	+++	<+	<+	<+	+
NruI-HF	++	–	–	+	–

Enzyme	*Taq* in Thermopol Rxn Buffer	Q5 in Q5 Buffer**	Phusion in Phusion HF Buffer	One*Taq* in One*Taq* Rxn Buffer	Longamp *Taq* in Longamp *Taq* Rxn Buffer
NsiI	+++	+	+++	++	+
NsiI-HF	+++	++	+++	+++	+++
NspI	+++	<+	<+	+++	++
PacI	+++	<+	<+	++	+++
PaeR7I	+++	<+	<+	+++	+++
PaqCI	–	–	–	+++	++
PciI	<+	<+	–	–	–
PflFI	+++	<+	<+	<+	+
PflMI	+	<+	+++	++	+++
PleI	+++	<+	<+	<+	<+
PluTI	+++	<+	+	+++	+++
PmeI	+++	<+	<+	+++	+++
PmlI	–	–	–	+	<+
PpuMI	+++	<+	+++	+++	+++
PshAI	+++	<+	<+	<+	<+
PsiI-v2	+++	–	–	+++	+++
PspGI @75°C	+++	+++	+++	+++	+++
PspOMI	+++	<+	+	+++	+++
PspXI	+++	<+	++	+++	+++
PstI	++	+	+	<+	<+
PstI-HF	+++	<+	++	++	+
PvuI-HF	+++	<+	+++	++	+++
PvuII	+++	<+	+	+++	+++
PvuII-HF	+	–	–	<+	<+
RsaI	+++	<+	++	+++	+++
RsrII	<++	–	–	<+	<+
SacI-HF	+++	<+	<+	<+	++
SacII	+++	<+	+++	++	+
SalI	<+	+	++	–	–
SalI-HF	+	<+	+++	+	+++
SapI	<++	<+	++	++	++
Sau3AI	+++	<+	<+	<+	<+
Sau96I	<++	+	+	+++	+++
SbfI-HF	+	–	–	<+	<+
ScaI-HF	+	<+	<+	–	–
ScrFI	+++	+++	+++	+++	+++
SexAI	+++	<+	+++	+++	+++
SfaNI	–	<+	++	<++	<++
SfcI	+++	<+	<+	+	+
SfiI @50°C	+++	–	–	+++	+++
SfoI	+++	<+	+++	+	+++
SgrAI	<++	<+	++	+	+++
SmaI	+++	<+	++	+++	+++
SmlI @55°C	<+	<+	+	+	+
SnaBI	<+	<+	<+	+++	+++
SpeI-HF	+++	–	<+	+++	+++
SphI	+++	+	++	<+	<+
SphI-HF	+++	<+	+	+++	+++
SrfI	<+	<+	+++	+	++
SspI-HF	++	<+	+	+++	+++
StuI	+++	<+	<+	+++	+++
StyD4I	<++	<+	+	<+	<+
StyI-HF	+	<+	<+	++	+++
SwaI @25°C	<+	<+	<+	<+	+++
TaqI-v2 @65°C	+++	<+	+	+++	+++
TfiI @65°C	<++	<+	<+	++	++
TseI @65°C	+++	+++	+++	+++	+++
Tsp45I @65°C	+++	–	–	+	<+
TspMI @75°C	+++	<+	+	+++	+++
TspRI @65°C	+	<+	<+	+++	+++
Tth111I @65°C	+++	<+	++	<+	+
XbaI	+++	–	<+	++	++
XcmI	+++	<+	+	+++	+++
XhoI	<+	<+	+++	++	+++
XmaI	+++	<+	+	–	–
XmnI	+++	<+	<+	++	+++
ZraI	+++	<+	<+	++	+

Getting Started with Molecular Cloning

Molecular cloning has traditionally used restriction enzymes to excise a fragment from source DNA, and to linearize a plasmid vector, while creating compatible ends. After purification, insert and vector are ligated to form a recombinant vector, which is transformed into an *E. coli* host. Alternatively, PCR can been used to generate both the vector and insert, which can be joined using a variety of techniques, such as standard DNA ligation, enzymatic joining using a recombinase or topoisomerase, homologous recombination, or synthetic biology (see NEBuilder HiFi DNA Assembly and Gibson Assembly).

Regardless of the method chosen, the process can be made more efficient and successful by following good practices in the lab. The following tips will help improve the success of your cloning experiments.

1. Take the time to plan your experiments

Pay attention to the junction sequences and the effect on reading frames of any translated sequences. Check both the vector and insert for internal restriction sites (we recommend NEBcutter at **NEBcutter.neb.com**) prior to designing PCR primers that contain similar sites to those used for cloning. Verify that the antibiotic selective marker in the vector is compatible with the chosen host strain.

2. Start with clean DNA at the right concentration

Ensure that your source DNA is free of contaminants, including nucleases and unwanted enzymatic activities. Use commercially-available spin columns to purify starting DNA, (e.g., Monarch Plasmid Miniprep Kit, NEB #T1010 for DNA plasmids, Monarch PCR & DNA Cleanup Kit, NEB #T1030 for DNA Fragments). Completely remove solvents, such as phenol, chloroform and ethanol, prior to manipulation of the DNA. Elute DNA from the spin columns with salt-free buffer to prevent inhibition of the downstream steps, either restriction digestion or PCR amplification.

3. Perform your restriction digests carefully

The reaction volume should be compatible with the downstream step (e.g., smaller than the volume of the well of an agarose gel used to resolve the fragments). For a typical cloning reaction, this is often between 20–50 µl. The volume of restriction enzyme(s) added should be no more than 10% of the total reaction volume, to ensure that the glycerol concentration stays below 5%; this is an important consideration to minimize star activity (unwanted cleavage).

4. Mind your ends

DNA ends prepared for cloning by restriction digest are ready for ligation without further modification, assuming the ends to be joined are compatible. If the ends are non-compatible, they can be modified using blunting reagents, phosphatases, etc.

DNA ends prepared by PCR for cloning may have a 3´ addition of a single adenine (A) residue following amplification using *Taq* DNA Polymerase (NEB #M0273). High-fidelity DNA polymerases, such as Q5 (NEB #M0491), leave blunt ends. PCR using standard commercial primers produces non-phosphorylated fragments, unless the primers were 5´ phosphorylated. The PCR product may need to be kinase treated to add a 5´ phosphate prior to ligation with a dephosphorylated vector.

5. Clean up your DNA prior to vector:insert joining

This can be done with gel electrophoresis or column purification (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030). Isolating the desired DNA from unwanted parent vectors and/or other DNA fragments can dramatically improve your cloning results.

Confirm digested DNA on an agarose gel prior to ligation. For a single product, run a small amount of the digest, and then column purify to isolate the remainder (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030). When multiple fragments are produced and only one is to be used, resolve the fragments on a gel and excise the desired fragment under UV light. Using longwave (365 nm) UV light will minimize any radiation-induced DNA damage to the fragment. Recover the DNA fragment from the agarose slice using a gel extraction kit (e.g., Monarch DNA Gel Extraction Kit, NEB #T1020) or β-Agarase I (NEB #M0392).

6. Quantitate your isolated material

Simple quantitation methods, such as gel electrophoresis with mass standards or spectroscopic quantitation on low-input spectrophotometers (such as a NanoSpec®), ensure that the proper amount of material is used for the downstream reactions.

7. Follow the manufacturer's guidelines for the joining reaction

For traditional cloning, follow the guidelines specified by the ligase supplier. If a 3:1 molar ratio of insert to vector is recommended, try this first for best results. Using a 3:1 mass ratio is not the same thing (unless the insert and vector have the same mass). Ligation usually proceeds quickly and, unless your cloning project requires the generation of a high-complexity library that benefits from the absolute capture of every possible ligation product, long incubation times are not necessary.

Follow the manufacturer's guidelines for the joining reactions in PCR cloning and seamless cloning. If you are performing a cloning protocol for the first time, adhere to the recommended protocol for optimal results. NEB recommends using NEBioCalculator to calculate ligation ratios.

8. Use competent cells that are suited to your needs

While some labs prepare their own competent cells "from scratch" for transformations, the levels of competence achieved rarely matches the high levels attained with commercially-available competent cells. Commercially-available competent cells save time and resources, and make cloning more reproducible.

Find an overview of traditional cloning.

Optimization Tips for Golden Gate Assembly

Looking to assemble multiple DNA fragments in a single reaction? Here are some tips to keep in mind when planning your Golden Gate Assembly experiments using one of our NEBridge Golden Gate Assembly kits for BsaI-HFv2 (NEB #E1601) or BsmBI-v2 (NEB #E1602), NEBridge Ligase Master Mix (NEB #M1100), or PaqCI (NEB #R0745), our newest Type IIS restriction enzyme optimized for use in assembly, featuring a 7 base recognition site which minimizes the need for domestication of internal sites in your sequences.

Check your sequences

- Always check your assembly sequences for internal sites before choosing which Type IIS restriction endonuclease to use for your assembly. While single insert Golden Gate assembly has such high efficiencies of assembly that the desired product is obtainable regardless of the presence of an internal site, this is not true for assemblies with multiple inserts. Options include choosing a different Type IIS restriction enzyme to direct your assembly, or eliminating internal sites through domestication. Our tutorial video on Golden Gate Assembly Domestication provides a full description of the many options available for internal site issues. Note the use of a Type IIS restriction enzyme with a 7 base recognition site, such as PaqCI, is less likely to have internal sites present in any given sequence.

Orient your primers

- When designing PCR primers to introduce Type IIS restriction enzyme sites, either for amplicon insert assembly or as an intermediate for pre-cloning the insert, remember that the recognition sites should always face inwards towards your DNA to be assembled. Consult the NEBridge Golden Gate Assembly Kit manuals or assembly videos for further information regarding the placement and orientation of the sites.

Choose the right plasmid

- Consider switching to the pGGAselect Destination Plasmid for your Golden Gate assembly. This versatile destination construct is included in all NEBridge Golden Gate Assembly kits and can be used for BsaI-HFv2, BsmBI-v2 or BbsI directed assemblies. It also features T7 and SP6 promoter sequences flanking the assembly site, and has no internal BsaI, BsmBI or BbsI sites. The pGGAselect plasmid can also be transformed into any *E. coli* strain compatible with pUC19 for producing your own plasmid preparation if so desired.

Choose the right buffer

- T4 DNA Ligase Buffer works best for Golden Gate Assembly with BsaI-HFv2, BsmBI-v2 and PaqCI. However, alternate buffers would be NEBuffer r1.1 for Bsa-HFv2, NEBuffer r2.1 for BsmBI-v2 & rCutSmart for PaqCI, if these buffers are supplemented with 1 mM ATP and 5-10 mM DTT.

Increase your complex assembly efficiency by increasing the Golden Gate cycling levels

- T4 DNA Ligase, BsaI-HFv2, BsmBI-v2 and PaqCI are very stable and continue to be active during extended cycling protocols; an easy way to increase assembly efficiencies without sacrificing fidelity is to increase the total cycles from 30 to 45-65, even when using long (5-minute) segments for the temperature steps.

Make sure your plasmid prep is RNA-free

- For pre-cloned inserts/modules, make sure your plasmid prep is free of RNA to avoid an overestimation of your plasmid concentrations.

Avoid primer dimers

- For amplicon inserts/modules, make sure your PCR amplicon is a specific product and contains no primer dimers. Primer dimers, with Type IIS restriction endonuclease sites (introduced in the primers used for the PCR reactions), would be active in the assembly reaction and result in mis-assemblies.

Avoid PCR-induced errors

- Do not over-cycle and use a proofreading high fidelity DNA polymerase, such as Q5 DNA High-Fidelity Polymerase.

Decrease insert amount for complex assemblies

- For complex assemblies involving >10 fragments, pre-cloned insert/modules levels can be decreased from 75 to 50 ng each without significantly decreasing the efficiencies of assembly.

Carefully design EVERY insert's overhang

- An assembly is only as good as its weakest junction. Research at NEB has led to an increased understanding of ligase fidelity, including the development of a comprehensive method for profiling end-joining ligation fidelity in order to predict which overhangs will result in improved accuracy. This ligase fidelity information can be paired with the appropriate Type IIS restriction enzyme chosen to direct your assembly to achieve high efficiencies and accurate complex assemblies. Please use the free NEBridge Golden Gate Assembly Tool to design primers for your Golden Gate Assembly reactions. Predict overhang fidelity or find optimal Golden Gate junctions for long sequences using our NEBridge Ligase Fidelity Tools.

Check for a sequence error if your assembly becomes non-functional

- Be aware that occasionally a pre-cloned insert/module can become corrupted by an error during propagation in *E. coli*, usually a frameshift due to slippage in a run of a single base (e.g., AAAA) by the *E. coli* DNA Polymerase. This should be suspected if previously functional assembly components suddenly become nonfunctional.

For more information on Golden Gate, visit **www.neb.com/GoldenGate**

How does Golden Gate Assembly work?

NEBridge® Ligase Master Mix Protocol Guidelines

This table provides guidance on using NEBridge Ligase Master Mix (MM) with NEB Type IIS restriction enzymes for Golden Gate Assembly. Recommendations differ based on the number of fragments to be assembled as well as the choice of Type IIS restriction enzyme. All incubations should be followed by an end soak for 5 min at 60°C prior to transformation. Store reactions at -20°C if not immediately being used for transformations.

	2 fragments*	3-6 fragments	7+ fragments**
	5 µl NEBridge Ligase MM 15 µl rxn volume 15-60 min 37°C *** -or- 15-30 cycles of 42°C X 1 min, 16°C X 1 min**** End soak 60°C X 5 min	5 µl NEBridge Ligase MM 15 µl rxn volume 30 cycles of 37°C X 1 min, 16°C X 1 min*** -or- 30 cycles of 42°C X 1 min, 16°C X 1 min**** End soak 60°C X 5 min	10 µl NEBridge Ligase MM 30 µl rxn volume 30-60 cycles of 37°C X 5 min, 16°C X 5 min*** -or- 30-60 cycles of 42°C X 5 min, 16°C X 5 min**** End soak 60°C X 5 min
BbsI-HF®	1 µl (20U)	1 µl (20U)	1 µl (50U)[a]
BsaI-HFv2	1 µl (20U)	1 µl (20U)	1 µl (20U)
BsmBI-v2	3 µl[b] (30U)	3 µl[b] (30U)	6 µl[b] (60U)
BspQI	1 µl (10U)	1 µl (10U)	2 µl (20U)
Esp3I	2 µl (20U)	3 µl (30U)	4 µl (40U)
PaqCI®	1 µl[c] (10U)	1 µl[c] (10U)	2.5 µl[c] (25U)
SapI	1 µl (10U)	1 µl (10U)	2 µl (20U)

* For 2 fragment assembly, 15 min or 15 cycles for single insert cloning; 60 min or 30 cycles for library construction

** For 7+ fragment assembly, 30 cycles for 7-13 fragment assembly; 60 cycles for 14+ fragment assembly

*** Reaction protocol for BbsI-HF, BsaI-HFv2, Esp3I, PaqCI and SapI

**** Reaction protocol for BsmBI-v2 and BspQI. Optimum reaction temperature is 42°C rather than 37°C.

a = Requires use of NEB #R3539M (50U/µl)

b = Use of less enzyme will reduce performance

c = Recommended PaqCI Activator : PaqCI ratio is 1:1 (pmol:U). Use 0.5 µl of PaqCI Activator (20 µM) for 2 and 3-6 fragments; 1.25 µl of PaqCI Activator (20 µM) for 7+ fragments

Kerri joined NEB in 2020 as an Administrative Assistant, and is now the International Business Coordinator, helping support our subsidiary offices and distribution partners across the globe. Outside of work, Kerri enjoys traveling and playing tennis.

Type IIS Restriction Enzymes

Type IIS restriction enzymes recognize asymmetric DNA sequences and cleave outside of their recognition sequence. They are useful for many applications, including Golden Gate Assembly. NEB currently offers over 50 Type IIS restriction enzymes.

Enzyme	Heat Inact.	NEBuffer	Reaction Temp.	Activity at 37°C	Storage Temp.	Recognition Sequence	Recognition Sequence Length	Overhang Length	Isoschizomers from NEB	Methylation Sensitivity**	Enzyme Sub-type
AcuI	Y	rCutSmart	37°C		-20°C	CTGAAG(16/14)	6	2			IIC
AlwI	N	rCutSmart	37°C		-20°C	GGATC(4/5)	5	1		dam	
BaeI SAM	Y	rCutSmart	25°C	100%	-20°C	(10/15)ACNNNNGTAYC(12/7)	7	5 & 5			IIC
BbsI *	Y	NEBuffer r2.1	37°C		-80°C	GAAGAC(2/6)	6	4			IIT
BbsI-HF *	Y	rCutSmart	37°C		-20°C	GAAGAC(2/6)	6	4			IIT
BbvI 2*site	Y	rCutSmart	37°C		-20°C	GCAGC(8/12)	5	4			
BccI	Y	rCutSmart	37°C		-20°C	CCATC(4/5)	5	1			
BceAI	Y	NEBuffer r3.1	37°C		-20°C	ACGGC(12/14)	5	2		CpG	
BcgI 2*site	Y	NEBuffer r3.1	37°C		-20°C	(10/12)CGANNNNNNTGC(12/10)	6	2 & 2		dam; CpG	IIC
BciVI	Y	rCutSmart	37°C		-20°C	GTATCC(6/5)	6	1			
BcoDI	N	rCutSmart	37°C		-20°C	GTCTC(1/5)	5	4	BsmAI	CpG	IIT
BfuAI 2*site	Y	NEBuffer r3.1	50°C	50%	-20°C	ACCTGC(4/8)	6	4	BspMI	CpG	
BmrI	Y	NEBuffer r2.1	37°C		-20°C	ACTGGG(5/4)	6	1			
BpmI 2*site	Y	NEBuffer r3.1	37°C		-20°C	CTGGAG(16/14)	6	2			IIC
BpuEI	Y	rCutSmart	37°C		-20°C	CTTGAG(16/14)	6	2			IIC
BsaI-HF®v2 *	Y	rCutSmart	37°C		-20°C	GGTCTC(1/5)	6	4		dcm; CpG	IIT
BsaXI	N	rCutSmart	37°C		-20°C	(9/12)ACNNNNNCTCC(10/7)	6	3 & 3			IIC
BseRI	Y	rCutSmart	37°C		-20°C	GAGGAG(10/8)	6	2			IIC
BsgI 2*site	Y	rCutSmart	37°C		-20°C	GTGCAG(16/14)	6	2			IIC
BsmAI	N	rCutSmart	55°C	50%	-20°C	GTCTC(1/5)	5	4	BcoDI	CpG	
BsmBI-v2 *	Y	NEBuffer r3.1	55°C	10%	-20°C	CGTCTC(1/5)	6	4	Esp3I	CpG	IIT
BsmFI	Y	rCutSmart	65°C	100%	-20°C	GGGAC(10/14)	5	4		CpG; dcm	IIC
BsmI	Y	rCutSmart	65°C	20%	-20°C	GAATGC(1/-1)	6	2			IIT
BspCNI	Y	rCutSmart	37°C		-20°C	CTCAG(9/7)	5	2			IIC
BspMI 2*site	Y	NEBuffer r3.1	37°C		-20°C	ACCTGC(4/8)	6	4	BfuAI		
BspQI *	Y	NEBuffer r3.1	50°C	50%	-20°C	GCTCTTC(1/4)	7	3	SapI		IIT
BsrDI	Y	NEBuffer r2.1	65°C	50%	-20°C	GCAATG(2/0)	6	2			IIT
BsrI	Y	NEBuffer r3.1	65°C	10%	-20°C	ACTGG(1/-1)	5	2			IIT
BtgZI *	Y	rCutSmart	60°C	50%	-20°C	GCGATG(10/14)	6	4		CpG	IIC
BtsCI	Y	rCutSmart	50°C	25%	-20°C	GGATG(2/0)	5	2			
BtsI-v2	Y	rCutSmart	37°C		-20°C	GCAGTG(2/0)	6	2			IIT
BtsIMutI	Y	rCutSmart	55°C	50%	-20°C	CAGTG(2/0)	5	2			IIT
CspCI 2*site	Y	rCutSmart	37°C		-20°C	(11/13)CAANNNNNGTGG(12/10)	7	2 & 2			IIC
EarI	Y	rCutSmart	37°C		-20°C	CTCTTC(1/4)	6	3		CpG	IIT
EciI	Y	rCutSmart	37°C		-20°C	GGCGGA(11/9)	6	2		CpG	IIC
Esp3I *	Y	rCutSmart	37°C		-20°C	CGTCTC(1/5)	6	4	BsmBI-v2	CpG	IIT
FauI	Y	rCutSmart	55°C	50%	-20°C	CCCGC(4/6)	5	2		CpG	
FokI 2*site	Y	rCutSmart	37°C		-20°C	GGATG(9/13)	5	4		dcm; CpG	
HgaI	Y	NEBuffer r1.1	37°C		-20°C	GACGC(5/10)	5	5		CpG	
HphI	Y	rCutSmart	37°C		-20°C	GGTGA(8/7)	5	1		dam; dcm	
HpyAV	Y	rCutSmart	37°C		-20°C	CCTTC(6/5)	5	1		CpG	
MboII 2*site	Y	rCutSmart	37°C		-20°C	GAAGA(8/7)	5	1		dam	
MlyI	Y	rCutSmart	37°C		-20°C	GAGTC(5/5)	5	0			
MmeI 2*site	Y	rCutSmart	37°C		-20°C	TCCRAC(20/18)	6	2		CpG	IIC
MnlI	Y	rCutSmart	37°C		-20°C	CCTC(7/6)	4	1			
NmeAIII 2*site	Y	rCutSmart	37°C		-20°C	GCCGAG(21/19)	6	2			IIC
PaqCI 2*site	Y	rCutSmart	37°C		-20°C	CACCTGC(4/8)	7	4		CpG	
PleI 2*site	Y	rCutSmart	37°C		-20°C	GAGTC(4/5)	5	1		CpG	
SapI *	Y	rCutSmart	37°C		-20°C	GCTCTTC(1/4)	7	3	BspQI		IIT
SfaNI	Y	NEBuffer r3.1	37°C		-20°C	GCATC(5/9)	5	4		CpG	

* Cited for use in Golden Gate Assembly according to current literature

** Methylation sensitivity applies to the recognition motif only

2*site Through suppression experiments and published reports, NEB has identified that these enzymes require more than one recognition site on the substrate to cleave optimally. For more information, see Restriction Enzyme Cleavage: 'single-site' enzymes and 'multi-site' enzymes.

Optimization Tips for NEBuilder® HiFi DNA Assembly and NEB Gibson Assembly®

Looking to assemble multiple DNA fragments in a single reaction? Here are some tips to keep in mind when planning your NEBuilder HiFi DNA Assembly or Gibson Assembly experiments.

Decide How You Want to Generate the Linearized Vector. You can Choose from Two Methods:

1) Restriction enzyme digestion: good for large plasmids you don't want to amplify; background may be higher if undigested vector is present.

2) PCR: achieves lower background versus restriction enzyme digestion, but is limited by the size of the vector. Typically, vectors up to 10 kb can be amplified; for amplicons greater than 10 kb, divide into 2 fragments.

Design the Primers

- Use the NEBuilder Assembly Tool (**NEBuilder.neb.com**) to design the primers and check the sequence of the final assembly. Primers will contain the overlap sequence. We recommend watching the tutorials before using the tool for the first time. There is one for restriction enzyme digestion and another for PCR. The videos can be found at **NEBuilderHiFi.com**
- Make sure the overlap is the correct length for the number of fragments in the assembly: Refer to the section titled "Use the Correct Amount of DNA" for more details.

Column Purify the PCR Products

- If you do not purify the PCR products, limit the unpurified PCR products to 20% of the reaction volume (4 µl for a standard 20 µl reaction).
- If PCR produces a single band of the correct size and the yield is good, DNA purification is not necessary.
- If PCR produces multiple products or a smear, it is best to optimize the PCR. If it is not possible to optimize, purify the products using gel extraction. Be careful, however, as gel extraction can introduce guanadine thiocynate (from the gel dissolving buffer) and can reduce the efficiency of the assembly reaction. To minimize this contamination, trim the gel slice so that a smaller amount of gel dissolving buffer is required. Due to the potential for residual guanidine salt being present in fragments isolated by gel-extraction, PCR or DNA column purification (NEB #T1030) is preferable to gel extraction (NEB #T1020).

Use the Correct Amount of DNA

- Make sure you calculate the optimum ratio of insert(s):vector. If the ratio is not ideal, we recommend using NEBioCalculator (**NEBioCalculator.neb.com**) to determine molar amounts.

  For NEBuilder HiFi DNA Assembly:

  2-3 fragments: 15-20 nt overlaps, total DNA = 0.03-0.2 pmol, 2 fold molar excess of each insert:vector

  4-6 fragments: 20-30 nt overlaps, total DNA = 0.2-0.5 pmol, 1:1 molar ratio of each insert:vector

  For NEB Gibson Assembly:

  2-3 fragments: 15-25 nt overlaps, total DNA = 0.02-0.5 pmol, 2-3 fold molar excess of each insert:vector

  4-6 fragments: 20-80 nt overlaps, total DNA = 0.2-1.0 pmol, 1:1 molar ratio of each insert:vector

Perform a PCR Assay to Determine if the Assembly is Successful

- Determine if the assembly works *in vitro* by amplifying the assembled product directly from the assembly reaction. Dilute 1 µl of the assembly reaction with 3 µl water then use 1 µl as a template in a 50 µl PCR. Use primers that anneal to the vector and amplify across the insert. Do not use primers that anneal across the assembly junction because this can lead to false positive results. If you can amplify the assembled product but cannot recover clones by transformation, then the problem is either with the transformation step, or the inability of the cells to maintain the transformed construct due to toxicity.

  Check the reaction conditions, DNA amounts, overlap sequences and perform the assembly control.

Always use High Competency Cells with a Transformation Efficiency of 10^8 – 10^9 cfu/µg

- We recommend NEB 5-alpha High Efficiency Competent *E. coli* (NEB #C2987) or NEB 10-beta High Efficiency Competent *E. coli* (NEB #C3019).

  More information can be found on NEBuilderHiFi.com

How does NEBuilder HiFi DNA Assembly work?

Preparation of Insert and Vectors

Insert From a Plasmid Source

- Digest plasmid with the appropriate restriction enzymes to produce a DNA fragment that can be cloned directly into a vector. Unidirectional cloning is achieved with restriction enzymes that produce non-compatible ends.

Insert From a PCR Product

- Design primers with appropriate restriction sites to clone unidirectionally into a vector
- Addition of 6 bases upstream of the restriction site is sufficient for digestion with most enzymes
- If fidelity is a concern, choose a proofreading polymerase such as Q5 High-Fidelity DNA Polymerase (NEB #M0491)
- Visit **www.NEBPCRPolymerases.com** for additional guidelines for PCR optimization
- Purify PCR product by running the DNA on an agarose gel and excising the band or by using a spin column (e.g., Monarch® DNA Gel Extraction Kit, NEB #T1020, Monarch PCR & DNA Cleanup Kit, NEB #T1030)
- Digest with the appropriate restriction enzyme

Standard Restriction Enzyme Protocol

DNA	1 µg
10X NEBuffer	5 µl (1X)
Restriction Enzyme	10 units is sufficient, generally 1 µl is used
Nuclease-free Water	To 50 µl
Incubation Time	1 hour*
Incubation Temperature	Enzyme dependent

** Can be decreased by using a Time-Saver qualified enzyme*

Time-Saver Restriction Enzyme Protocol

DNA	1 µg
10X NEBuffer	5 µl (1X)
Restriction Enzyme	1 µl
Nuclease-free Water	To 50 µl
Incubation Time	5–15 minutes*
Incubation Temperature	Enzyme dependent

** Time-Saver qualified enzymes can also be incubated overnight with no star activity*

Insert from Annealed Oligos

- Annealed oligos can be used to introduce a fragment (e.g., promoter, polylinker, etc.)
- Anneal two complementary oligos that leave protruding 5´ or 3´ overhangs for ligation into a vector cut with appropriate enzymes
- Non-phosphorylated oligos can be phosphorylated using T4 Polynucleotide Kinase (NEB #M0201)

Typical Annealing Reaction

Oligo 1	20 µM Final concentration
Oligo 2	20 µM Final concentration
NEBuffer r2.1	5 µl
Nuclease-free Water	To 50 µl
Incubation	95°C for 5 minutes, cool slowly to room temp.

Vector

- Digest vector with appropriate restriction enzymes. Enzymes that leave non-compatible ends are ideal as they prevent vector self-ligation

Dephosphorylation

- Dephosphorylation is sometimes necessary to prevent self-ligation. NEB offers four products for dephosphorylation of DNA:
- Quick CIP (NEB #M0525), Shrimp Alkaline Phosphatase (rSAP) (NEB #M0371) and Antarctic Phosphatase (AP) (NEB #M0289) are heat-inactivatable phosphatases. They work in all NEBuffers, but AP requires supplementation with Zn^{2+}.

Dephosphorylation of 5´ ends of DNA using Quick CIP

DNA	1 pmol of DNA ends
10X rCutSmart Buffer	2 µl
Quick CIP	1 µl
Nuclease-free Water	To 20 µl
Incubation	37°C for 10 minutes
Heat Inactivation	80°C for 2 minutes

Note: Scale larger reaction volumes proportionally.

Dephosphorylation of 5´ ends using Shrimp Alkaline Phosphatase (rSAP)

DNA	≥1 pmol of DNA ends
10X rCutSmart Buffer	2 µl
rSAP	1 µl
Nuclease-free Water	To 20 µl
Incubation	37°C for 30 minutes
Heat Inactivation	65°C for 5 minutes

Blunting

- In some instances, the ends of the insert or vector require blunting
- PCR with a proofreading polymerase will leave a predominantly blunt end
- T4 DNA Polymerase (NEB #M0203) or Klenow (NEB #M0210) will fill in a 5´ overhang and chew back a 3´ overhang
- The Quick Blunting Kit (NEB #E1201) is optimized to blunt and phosphorylate DNA ends for cloning in less than 30 minutes
- Analyze agarose gels with longwave UV (365 nM) to minimize UV exposure that may cause DNA damage

Blunting with the Quick Blunting Kit

DNA	Up to 5 µg
10X Blunting Buffer	2.5 µl
dNTP Mix (1 mM)	2.5 µl
Blunt Enzyme Mix	1 µl
Nuclease-free Water	To 25 µl
Incubation	room temperature; 15 min for RE-digested DNA; 30 min for sheared/nebulized DNA or PCR products*
Heat Inactivation	70°C for 10 minutes

** PCR-generated DNA must be purified before blunting using a purification kit (NEB #T1030), phenol extraction/ethanol precipitation, or gel extraction (NEB #T1020).*

Phosphorylation

- For ligation to occur, at least one of the DNA ends (insert or vector) should contain a 5´ phosphate
- Primers are usually supplied non-phosphorylated; therefore, the PCR product will not contain a 5´ phosphate
- Digestion of DNA with a restriction enzyme will always produce a 5´ phosphate
- A DNA fragment can be phosphorylated by incubation with T4 Polynucleotide Kinase (T4 PNK, NEB #M0201). T4 PNK can be inactivated at 65°C for 20 minutes.

Phosphorylation with T4 PNK

DNA (20 mer)	up to 300 pmol of 5´ termini
10X T4 PNK Buffer	5 µl
10 mM ATP	5 µl (1 mM final conc.)
T4 PNK	1 µl (10 units)
Nuclease-free Water	To 50 µl
Incubation	37°C for 30 minutes

Purification of Vector and Insert

- Purify the vector and insert by either running the DNA on an agarose gel and excising the appropriate bands or by using a spin column, such as Monarch DNA Gel Extraction Kit or PCR & DNA Cleanup Kit (NEB #T1020 or T1030)
- DNA can also be purified using β-Agarase I (NEB #M0392) with low melt agarose, or an appropriate spin column or resin
- Analyze agarose gels with longwave UV (360 nM) to minimize UV exposure that may cause DNA damage

Ligation of Vector and Insert

- Use a molar ratio of 1:3 vector to insert. Use NEBioCalculator to calculate molar ratios.
- If using T4 DNA Ligase (NEB #M0202) or the Quick Ligation Kit (NEB #M2200), thaw and resuspend the Ligase Buffer at room temp. If using Ligase Master Mixes, no thawing is necessary.
- The Quick Ligation Kit (NEB #M2200) is optimized for ligation of both sticky and blunt ends
- Instant Sticky-end Ligase Master Mix (NEB #M0370) is optimized for instant ligation of sticky/cohesive ends
- Blunt/TA Ligase Master Mix (NEB #M0367) is optimized for ligation of blunt or single base overhangs, which are the more challenging type of ends for T4 DNA Ligase
- Following ligation, chill on ice and transform
- DO NOT heat inactivate when using the Quick Ligation Buffer or Ligase Master Mixes, as this will inhibit transformation
- Electroligase (NEB #M0369) is optimized for ligation of both sticky and blunt ends and is compatible with electroporation (i.e., no cleanup step required)
- Improved Golden Gate Assembly can be achieved by selecting high fidelity overhangs [Potapov, V. et al. (2018) *ACS Synth. Biol.* 7(11), 2665–2674.

Ligation with the Quick Ligation Kit

Vector DNA (4 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	37.5 ng (0.060 pmol)
2X Quick Ligation Buffer	10 µl
Quick T4 DNA Ligase	1 µl
Nuclease-free Water	20 µl (mix well)
Incubation	Room temperature for 5 minutes

Ligation with Instant Sticky-end Ligase Master Mix

Vector DNA (3 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	50 ng
Master Mix	5 µl
Nuclease-free Water	To 10 µl
Incubation	None

Ligation with Blunt/TA Ligase Master Mix

Vector DNA (3 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	50 ng
Master Mix	5 µl
Nuclease-free Water	To 10 µl
Incubation	Room temperature for 15 minutes

Transformation

- To obtain transformants in 8 hrs., use NEB Turbo Competent *E. coli* (NEB #C2984)
- If recombination is a concern, then use the *recA*⁻ strains NEB 5-alpha Competent *E. coli* (NEB #C2987), NEB-10 beta Competent *E. coli* (NEB #C3019) or NEB Stable Competent *E. coli* (NEB #C3040)
- NEB 10-beta Competent *E. coli* works well for constructs larger than 5 kb
- NEB Stable Competent *E. coli* (NEB #C3040) can be used for constructs with repetitive sequences such as lentiviral constructs
- If electroporation is required, use NEB 10-beta (NEB #C3020) Electrocompetent *E. coli*
- Use pre-warmed selection plates
- Perform several 10-fold serial dilutions in SOC or NEB 10-beta/Stable Outgrowth Medium for plating

Transformation with NEB 5-alpha Competent *E. coli*

DNA	1–5 µl containing 1 pg – 100 ng of plasmid DNA
Competent *E. coli*	50 µl
Incubation	On ice for 30 minutes
Heat Shock	Exactly 42°C for exactly 30 seconds
Incubation	On ice for 5 minutes Add 950 µl room temperature SOC 37°C for 60 minutes, with shaking

Troubleshooting Guide for Cloning

We strongly recommend running the following controls during transformations. These controls may assist in identifying which step(s) in the cloning workflow has failed.

- Transform 100 pg – 1 ng of uncut vector to check cell viability, calculate transformation efficiency and verify the antibiotic resistance of the plasmid.
- Transform the cut vector to determine the amount of background due to undigested plasmid. The number of colonies in this control should be < 1% of the number of colonies in the uncut plasmid control transformation (from control #1).
- Transform a vector only ligation reaction. The ends of the vector should not be able to re-ligate because either they are incompatible (e.g., digested with two restriction enzymes that do not generate compatible ends) or the 5´ phosphate group has been removed in a dephosphorylation reaction (e.g., blunt ends treated with rSAP). This control transformation should yield the same number of colonies as control #2.
- Digest vector DNA with a single restriction enzyme, re-ligate and transform. The ends of the vector DNA should be compatible and easily joined during the ligation reaction, resulting in approximately the same number of colonies as control #1.

The cloning workflow often benefits from an accurate quantitation of the amount of DNAs that are being worked with. We recommend quantification of DNAs whenever possible.

Problem	Cause	Solution
Few or no transformants	Cells are not viable	• Transform an uncut plasmid (e.g., pUC19) and calculate the transformation efficiency of the competent cells. • If the transformation efficiency is low (< 10^4) re-make the competent cells or consider using commercially available high efficiency competent cells.
	Incorrect antibiotic or antibiotic concentration	• Confirm antibiotic and antibiotic concentration
	DNA fragment of interest is toxic to the cells	• Incubate plates at lower temperature (25–30°C). • Transformation may need to be carried out using a strain that exerts tighter transcriptional control over the DNA fragment of interest (e.g., NEB 5-alpha F´ I^q Competent *E. coli* (NEB #C2992))
	If using chemically competent cells, the wrong heat-shock protocol was used	• Follow the manufacturer's specific transformation protocol (Note: going above the recommended temperature during the heat shock can result in competent cell death)
	If using electrocompetent cells, PEG is present in the ligation mix	• Clean up DNA prior to transformation with the Monarch PCR & DNA Cleanup Kit (NEB #T1030) • Try NEB's ElectroLigase (NEB #M0369)
	If using electrocompetent cells, arcing was observed or no voltage was registered	• Clean up the DNA prior to ligation with the Monarch PCR & DNA Cleanup Kit (NEB T1030) • Tap the cuvette to get rid of any trapped air bubbles • Be sure to follow the manufacturer's specified electroporation parameters
	Construct is too large	• Select a competent cell strain that can be transformed efficiently with large DNA constructs [≥ 10 kb, we recommend trying NEB 10-beta Competent *E. coli* (NEB #C3019)] or NEB Stable Competent *E. coli* (NEB #C3040) • For very large constructs (> 10 kb), consider using electroporation
	Construct may be susceptible to recombination	• Select a *recA*⁻ strain such as NEB 5-alpha (NEB #C2987), NEB 10-beta (NEB #C3019) or NEB Stable (NEB #C3040) Competent *E. coli*
	The insert comes directly from mammalian or plant DNA and contains methylated cytosines, which are degraded by many *E. coli* strains	• Use a strain that is deficient in McrA, McrBC and Mrr, such as NEB 10-beta Competent *E. coli*
	Too much ligation mixture was used	• Use < 5 µl of the ligation reaction for the transformation
	Inefficient ligation	• Make sure that at least one fragment being ligated contains a 5´ phosphate moiety • Vary the molar ratio of vector to insert from 1:1 to 1:10. Use NEBioCalculator to calculate molar ratios • Purify the DNA to remove contaminants such as salt and EDTA with Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030) • ATP will degrade after multiple freeze-thaws; repeat the ligation with fresh buffer • Heat inactivate or remove the phosphatase prior to ligation • Ligation of single base-pair overhangs (most difficult) may benefit from being carried out with Blunt/TA Master Mix (NEB #M0367), Quick Ligation Kit (NEB #M2200) or concentrated T4 DNA Ligase (NEB #M0202) • Test the activity of the ligase by carrying out a ligation control with Lambda-HindIII digested DNA (NEB #N3012)
	Inefficient phosphorylation	• Purify the DNA prior to phosphorylation with Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030). Excess salt, phosphate or ammonium ions may inhibit the kinase. • If the ends are blunt or 5´ recessed, heat the substrate/buffer mixture for 10 minutes at 70°C. Rapidly chill on ice before adding the ATP and enzyme, then incubate at 37°C. • ATP was not added. Supplement the reaction with 1 mM ATP, as it is required by T4 Polynucleotide Kinase (NEB #M0201) • Alternatively, use 1X T4 DNA Ligase Buffer (NEB #B0202) (contains 1 mM ATP) instead of the 1X T4 PNK Buffer

Troubleshooting Guide for Cloning (continued)

Problem	Cause	Solution
Few or no transformants	Inefficient blunting	• Heat inactivate or remove the restriction enzymes prior to blunting • Clean up the PCR fragment prior to blunting with Monarch PCR & DNA Cleanup Kit (NEB #T1030) • Sonicated gDNA should be blunted for at least 30 minutes • Do not use > 1 unit of enzyme/µg of DNA • Do not incubate for > 15 minutes • Do not incubate at temperatures > 12°C (for T4 DNA Polymerase, NEB #M0203) or > 24°C (for Klenow, NEB #M0210) • Make sure to add a sufficient amount of dNTPs to the reaction (33 µM each dNTP for DNA Polymerase I, Large (Klenow) Fragment, NEB #M0210 and 100 µM each dNTP for T4 DNA Polymerase, NEB #M0203). • When using Mung Bean Nuclease (NEB #M0250), incubate the reaction at room temperature. Do not use > 1 unit of enzyme/µg DNA or incubate the reaction > 30 minutes.
	Inefficient A-Tailing	• Clean up the PCR prior to A-tailing. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). High-fidelity polymerases will remove any non-templated nucleotides.
	Restriction enzyme(s) didn't cleave completely	• Check the methylation sensitivity of the enzyme(s) to determine if the enzyme is blocked by methylation of the recognition sequence • Use the recommended buffer supplied with the restriction enzyme • Clean up the DNA to remove any contaminants that may inhibit the enzyme. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • When digesting a PCR fragment, make sure to have at least 6 nucleotides between the recognition site and the end of the DNA molecule
Colonies don't contain a plasmid	Antibiotic level used was too low	• Increase the antibiotic level on plates to the recommended amount • Use fresh plates with fresh antibiotics
	Satellite colonies were selected	• Choose large, well-established colonies for analysis
Colonies contain the wrong construct	Recombination of the plasmid has occurred	• Use a *recA*⁻ strain such NEB 5-alpha, NEB 10-beta or NEB Stable Competent *E. coli*
	Incorrect PCR amplicon was used during cloning	• Optimize the PCR conditions • Gel purify the correct PCR fragment. NEB recommends the Monarch DNA Gel Extraction Kit (NEB #T1020).
	Internal recognition site was present	• Use NEBcutter to analyze insert sequence for presence of an internal recognition site
	DNA fragment of interest is toxic to the cells	• Incubate plates at lower temperature (25–30°C) • Transformation may need to be carried out using a strain that exerts tighter transcriptional control of the DNA fragment of interest (e.g., NEB 5-alpha F´ I^q Competent *E. coli*) (NEB #C2992)
	Mutations are present in the sequence	• Use a high-fidelity polymerase (e.g., Q5 High-Fidelity DNA Polymerase, NEB #M0491) • Re-run sequencing reactions
Too much background	Inefficient dephosphorylation	• Heat inactivate or remove the restriction enzymes prior to dephosphorylation
	Kinase is present/active	• Heat inactivate the kinase after the phosphorylation step. Active kinase will re-phosphorylate the dephosphorylated vector.
	Restriction enzyme(s) didn't cleave completely	• Check the methylation sensitivity of the restriction enzyme(s) to be sure it is not inhibited by methylation of the recognition sequence • Use the recommended buffer supplied with the restriction enzyme • Clean up the DNA to remove contaminants. (e.g., too much salt). NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030).
	Antibiotic level is too low	• Confirm the correct antibiotic concentration
Ran the ligation on a gel and saw no ligated product	Inefficient ligation	• Make sure at least one DNA fragment being ligated contains a 5´ phosphate • Vary the molar ratios of vector to insert from 1:1 to 1:10. Use NEBioCalculator to calculate molar ratios. • Purify the DNA to remove contaminants such as salt and EDTA. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • ATP will degrade after multiple freeze-thaws; repeat the ligation with fresh buffer • Heat inactivate or remove the phosphatase prior to ligation • Ligation of single base-pair overhangs (most difficult) may benefit from being carried out with Blunt/TA Master Mix (NEB #M0367), Quick Ligation Kit (NEB #M2200) or concentrated T4 DNA Ligase (NEB #M0202) • Test the activity of the ligase by carrying out a ligation control with Lambda-HindIII digested DNA (NEB #N3012)
The ligated DNA ran as a smear on an agarose gel	The ligase is bound to the substrate DNA	• Treat the ligation reaction with Proteinase K (NEB #P8107) prior to running on a gel
The digested DNA ran as a smear on an agarose gel	The restriction enzyme(s) is bound to the substrate DNA	• Lower the number of units • Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the DNA or use Gel Loading Dye, Purple (6X) (NEB #B7024)
	Nuclease contamination	• Use fresh, clean running buffer • Use a fresh agarose gel • Clean up the DNA. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030).

Troubleshooting Guide for Cloning (continued)

Problem	Cause	Solution
Incomplete restriction enzyme digestion	Cleavage is blocked by methylation	• DNA isolated from a bacterial source may be blocked by Dam and Dcm methylation • DNA isolated from eukaryotic source may be blocked by CpG methylation • If the enzyme is inhibited by Dam or Dcm methylation, grow the plasmid in a *dam-/dcm-* strain (NEB #C2925) or use PCR DNA
	Salt inhibition	• Enzymes that have low activity in salt-containing buffers (NEBuffer r3.1) may be salt sensitive, so clean up the DNA prior to digestion. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. Monarch kits (NEB #T1010, #T1020, #T1030) use columns that have been designed to minimize salt carry over into the eluted DNA, so using them can minimize this issue. To prevent this, DNA solution should be no more than 25% of total reaction volume
	Inhibition by PCR components	• Clean up the PCR fragment prior to restriction digest. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030).
	Using the wrong buffer	• Use the recommended buffer supplied with the restriction enzyme
	Too few units of enzyme used	• Use at least 3–5 units of enzyme per µg of DNA
	Incubation time was too short	• Increase the incubation time
	Digesting supercoiled DNA	• Some enzymes have a lower activity on supercoiled DNA. Increase the number of enzyme units in the reaction.
	Presence of slow sites	• Some enzymes can exhibit slower cleavage towards specific sites. Increase the incubation time, 1–2 hours is typically sufficient.
	Two sites required	• Some enzymes require the presence of two recognition sites to cut efficiently. For more information, visit the table "Restriction Enzymes Requiring Multi-sites" on neb.com.
	DNA is contaminated with an inhibitor	• Assay substrate DNA in the presence of a control DNA. Control DNA will not cleave if there is an inhibitor present. Miniprep DNA is particularly susceptible to contaminants. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • Clean DNA with a spin column, we recommend the Monarch PCR & DNA Cleanup Kit (NEB #T1030), or increase volume to dilute contaminant
Extra bands in the gel	If larger bands than expected are seen in the gel, this may indicate binding of the enzyme(s) to the substrate	• Lower the number of units in the reaction • Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the substrate or add Gel Loading Dye, Purple (6X) (NEB #B7024)
	Star activity	• Use the recommended buffer supplied with the restriction enzyme • Decrease the number of enzyme units in the reaction • Make sure the amount of enzyme added does not exceed 10% of the total reaction volume. This ensures that the total glycerol concentration does not exceed 5% v/v • Decrease the incubation time. Using the minimum reaction time required for complete digestion will help prevent star activity. • Try using a High-Fidelity (HF) restriction enzyme. HF enzymes have been engineered for reduced star activity.
	Partial restriction enzyme digest	• Enzymes that have low activity in salt-containing buffers (e.g., NEBuffer r3.1) may be salt sensitive. Make sure to clean up the DNA prior to digestion. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • DNA purification procedures that use spin columns can result in high salt levels, which inhibit enzyme activity. Monarch kits (NEB #T1010, #T1020, #T1030) use columns that have been designed to minimize salt carry over into the eluted DNA, so using them can minimize this issue. To prevent this, DNA solution should be no more than 25% of total reaction volume • Clean-up the PCR fragment prior to restriction digest. NEB recommends the Monarch PCR & DNA Cleanup Kit (NEB #T1030). • Use the recommended buffer supplied with the restriction enzyme • Use at least 3–5 units of enzyme per g of DNA • Digest the DNA for 1–2 hours
No PCR fragment amplified	Used the wrong primer sequence	• Double check the primer sequence
	Incorrect annealing temperature	• Use the NEB Tm calculator to determine the correct annealing temperature (www.neb.com/TmCalculator)
	Incorrect extension temperature	• Each polymerase type has a different extension temperature requirement. Follow the manufacturer's recommendations.
	Too few units of polymerase	• Use the recommended number of polymerase units based on the reaction volume
	Incorrect primer concentration	• Each polymerase has a different primer concentration requirement. Make sure to follow the manufacturer's recommendations.
	Mg^{2+} levels in the reaction are not optimal	• Titrate the Mg^{2+} levels to optimize the amplification reaction. Follow the manufacturer's recommendations.
	Difficult template	• With difficult templates, try different polymerases and/or buffer combinations
The PCR reaction is a smear on a gel	If bands are larger than expected it may indicate binding of the enzyme(s) to the DNA	• Add SDS (0.1–0.5%) to the loading buffer to dissociate the enzyme from the DNA
Extra bands in PCR reaction	Annealing temperature is too low	• Use the NEB Tm calculator to determine the annealing temperature of the primers
	Mg^{2+} levels in the reaction are not optimal	• Titrate the Mg^{2+} levels to optimize the amplification reaction. Make sure to follow the manufacturer's recommendations.
	Additional priming sites are present	• Double check the primer sequence and confirm it does not bind elsewhere in the DNA template
	Formation of primer dimers	• Primer sequence may not be optimal. Additional primers may need to be tested in the reaction.
	Incorrect polymerase choice	• Try different polymerases and/or buffer combinations

Optimization Tips for Your Cloning Reactions

New England Biolabs offers a wide selection of reagents for your cloning experiments. For more information, visit **ClonewithNEB.com**. The following tips can be used to help optimize each step in your cloning workflow. Tips for restriction enzyme digestion and amplification can be found earlier in the technical reference section, or at www.neb.com.

cDNA Synthesis

Starting Material

- Intact RNA of high purity is essential for generating cDNA for cloning applications
- Total RNA or mRNA can be used in the reverse transcription reaction. Total RNA is generally sufficient for cDNA synthesis reactions. However, if desired, mRNA can be easily obtained using a PolyA Spin mRNA Isolation Kit (NEB #S1560) or Magnetic mRNA Isolation Kit (NEB #S1550).
- The amount of RNA required for cDNA cloning depends on the abundance of the transcript-of-interest. In general, 1 ng to 1 µg total RNA or 0.1–100 ng mRNA are recommended.

Product Selection

- Streamline your reaction setup by using the ProtoScript II First Strand cDNA Synthesis Kit (NEB #E6560). This kit combines ProtoScript II Reverse Transcriptase (NEB #M0360), a thermostable M-MuLV (RNase H^-) Reverse Transcriptase, and recombinant RNase Inhibitor in an enzyme Master Mix, along with a separate Reaction Mix containing dNTPs. Additionally, the kit contains two optimized reverse transcription primer mixes.

Yield

- ProtoScript II Reverse Transcriptase is capable of generating cDNA of more than 10 kb up to 48°C. We recommend 42°C for routine reverse transcription.
- You can increase the yield of a long cDNA product by doubling the amount of enzyme and dNTPs

Additives

- For most RT-PCR reactions, RNase H treatment is not required. But for some difficult amplicons or sensitive assays, add 2 units of *E. coli* RNase H to the reaction and incubate at 37°C for 20 minutes

Phosphorylation

Enzyme

- T4 Polynucleotide Kinase (NEB #M0201) and T4 DNA Ligase (NEB #M0202) can be used together in the T4 DNA Ligase Buffer
- T4 Polynucleotide Kinase is inhibited by high levels of salt (50% inhibition by 150 mM NaCl), phosphate (50% inhibition by 7 mM phosphate) and ammonium ions (75% inhibited by 7 mM $(NH_4)_2SO_4$)
- If using T4 Polynucleotide Kinase and working with 5´-recessed ends, heat the reaction mixture for 10 min at 70°C, chill rapidly on ice before adding the ATP (or Ligase Buffer containing ATP) and enzyme, then incubate at 37°C

Additives

- The addition of PEG 8000 (up to 5%) can improve results

Dephosphorylation

Enzyme

- When dephosphorylating a fragment following a restriction enzyme digest, a DNA clean up step is required if the restriction enzyme(s) used is NOT heat inactivatable. We recommend the Monarch PCR & DNA Cleanup Kit (NEB #T1030).
- When working with Quick CIP (NEB #M0525), rSAP (NEB #M0371) or AP (NEB #M0289), which are heat-inactivatable enzymes, a DNA clean-up step after dephosphorylation is not necessary prior to the ligation step.

Additives

- AP requires the presence of Zn^{2+} in the reaction, so don't forget to supplement the reaction with 1X Antarctic Phosphatase Reaction Buffer when using other NEBuffers

Blunting/End Repair

Enzyme

- Make sure that you choose the correct enzyme to blunt your fragment. The Quick Blunting Kit (NEB #E1201), T4 DNA Polymerase (NEB #M0203) and DNA Polymerase I, Large (Klenow) Fragment (NEB #M0210) will fill 5´ overhangs and degrade 3´ overhangs. Mung Bean Nuclease (NEB #M0250) degrades 5´ overhangs.
- T4 DNA Polymerase and DNA Polymerase I, Large (Klenow) Fragment are active in all NEBuffers. Please remember to add dNTPs.

Clean-up

- When trying to blunt a fragment after a restriction enzyme digestion, if the restriction enzyme(s) used are heat inactivatable, then a clean-up step prior to blunting is not needed. Alternatively, if the restriction enzyme(s) used are not heat inactivatable, a DNA clean-up step is recommended prior to blunting.
- When trying to blunt a fragment amplified by PCR, a DNA clean-up step is necessary prior to the blunting step to remove the nucleotides and polymerase
- When trying to dephosphorylate a fragment after the blunting step, you will need to add a DNA clean-up step (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030) after the blunting and before the addition of the phosphatase

Temperature

- When trying to blunt a fragment with Mung Bean Nuclease, the recommended temperature of incubation is room temperature, since higher temperatures may cause sufficient breathing of the dsDNA ends that the enzyme may degrade some of the dsDNA sequence. The number of units to be used and time of incubation may be determined empirically to obtain best results.

Heat Inactivation

- Mung Bean Nuclease reactions should not be heat inactivated. Although Mung Bean Nuclease can be inactivated by heat, this is not recommended because the DNA begins to "breathe" before the Mung Bean Nuclease is inactivated and undesirable degradation occurs at breathing sections. Purify DNA by phenol/chloroform extraction and ethanol precipitation or spin column purification [e.g., Monarch PCR & DNA Cleanup Kit (NEB #T1030)].

A-tailing

- If the fragment to be tailed has been amplified with a high-fidelity polymerase, the DNA needs to be purified prior to the tailing reaction. For this we recommend the Monarch PCR & DNA Cleanup Kit (NEB T1030). Otherwise, any high-fidelity polymerase present in the reaction will be able to remove any non-templated nucleotides added to the end of the fragments.

DNA Ligation

Reaction Buffers

- T4 DNA Ligase Buffer (NEB #B0202) should be thawed on the bench or in the palm of your hand, and not at 37°C (to prevent breakdown of ATP)
- Once thawed, T4 DNA Ligase Buffer should be placed on ice
- Ligations can be performed in any of the four standard restriction endonuclease NEBuffers or in T4 Polynucleotide Kinase Buffer (NEB #B0201) supplemented with 1 mM ATP
- When supplementing with ATP, use ribo-ATP (NEB #P0756). Deoxyribo-ATP will inhibit ligation.
- Before ligation, completely inactivate restriction enzyme by heat inactivation, spin column (e.g., Monarch PCR & DNA Cleanup Kit, NEB #T1030) or Phenol/EtOH purification

DNA

- Heat inactivate (AP, rSAP, Quick CIP) before ligation
- Keep total DNA concentration between 5–10 µg/ml
- Vector:Insert molar ratios between 1:1 and 1:10 are optimal for single insertions. Use NEBioCalculator at **NEBioCalculator.neb.com** to calculate molar ratios.
- For cloning more than one insert, we recommend the NEBuilder® HiFi DNA Assembly Master Mix (NEB #E2621) or Cloning Kit (NEB #E5520)
- If you are unsure of your DNA concentration, perform multiple ligations with varying ratios

Ligase

- For cohesive-end ligations, standard T4 DNA Ligase. Instant Sticky-end Ligase Master Mix or the Quick Ligation Kit are recommended.
- For blunt and single-base overhangs the Blunt/TA Ligase Master Mix is recommended
- For ligations that are compatible with electroporation, ElectroLigase is recommended
- Standard T4 DNA Ligase can be heat inactivated at 65°C for 20 minutes
- Do not heat inactivate the Quick Ligation Kit or the ligase master mixes

Transformation

- Add between 1–5 µl of ligation mixture to competent cells for transformation
- Extended ligation with PEG causes a drop off in transformation efficiency
- Electroporation is recommended for larger constructs (> 10,000 bp). Dialyze samples or use a spin column first if you have used the Quick Ligation Kit or ligase master mixes.
- ElectroLigase is recommended for ligations that will be electroporated

Transformation

Thawing

- Cells are best thawed on ice
- DNA should be added as soon as the last trace of ice in the tube disappears
- Cells can be thawed by hand, but warming decreases efficiency

DNA

- Up to 10 µl of DNA from a ligation mix can be used with only a 2-fold loss of efficiency

Incubation & Heat Shock

- Incubate on ice for 30 minutes. Expect a 2-fold loss in transformation efficiency for every 10 minutes this step is shortened.
- Both temperature and time are specific to the transformation volume and vessel. Typically, 30 seconds at 42°C is recommended, except when using BL21 (NEB #C2530) which requires exactly 10 seconds.

Outgrowth

- Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in transformation efficiency for every 15 minutes this step is shortened.
- SOC and NEB 10-beta/Stable Outgrowth Medium give 2-fold higher transformation efficiency than LB medium
- Incubation with shaking or rotation results in 2-fold higher transformation efficiency

Plating

- Selection plates can be used warm or cold, wet or dry with no significant effects on transformation efficiency
- Warm, dry plates are easier to spread and allow for the most rapid colony formation

DNA Contaminants to Avoid

Contaminant	Removal Method
Detergents	Ethanol precipitate
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspending
PEG	Column purify (e.g., Monarch PCR & DNA Cleanup Kit) or phenol/chloroform extract and ethanol precipitate

Troubleshooting Guide for DNA Cleanup & Plasmid Purification Using Monarch® Kits

Problem	Product	Possible Cause	Solution
No DNA purified	Monarch Plasmid Miniprep Kit (NEB #T1010)	Buffers added incorrectly	• Add buffers in the correct order so that the sample is bound, washed and eluted in the correct sequence • Ensure ethanol was added to Plasmid Wash Buffer 2
		Plasmid loss during culture growth	• Ensure proper antibiotic and concentration was used to maintain selection during culture growth
	Monarch DNA Gel Extraction Kit (NEB #T1020)	Ethanol not added to wash buffer	• Ensure the proper amount of ethanol was added to Monarch DNA Wash Buffer
	Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)		
Low DNA yield	Monarch Plasmid Miniprep Kit (NEB #T1010)	Incomplete lysis	• Pellet must be completely resuspended before addition of Plasmid Lysis Buffer (B2) – color should change from light to dark pink • Avoid using too many cells; this can overload the column. If culture volume is larger than recommended, scale up buffers B1-B3.
		Plasmid loss during culture growth	• Ensure proper antibiotic and concentration was used to maintain selection during culture growth
		Low-copy plasmid selected	• Increase amount of cells processed and scale buffers accordingly • Review our guidance for working with low copy plasmids
		Lysis of cells during growth	• Harvest culture during transition from logarithmic growth to stationary phase (~12-16 hours)
		Incomplete neutralization	• Invert tube several times until color changes to a uniform yellow color
		Incomplete elution	• Deliver Elution Buffer directly to center of column • Larger elution volumes and longer incubation times can increase yield • For elution of plasmids > 10 kb, heat the DNA Elution Buffer to 50°C and extend incubation time to 5 minutes
	Monarch DNA Gel Extraction Kit (NEB #T1020)	Reagents added incorrectly	• Be sure that buffers have been reconstituted correctly and that reagents have been added in the correct order
		Gel slice not fully dissolved	• Undissolved agarose may clog the column and interfere with binding. Incubate in Monarch Gel Dissolving Buffer for proper time and temperature.
		Gel dissolved above 60°C	• Dissolve gel slice in specified range (37-55°C). Higher temperatures can denature DNA
		Incomplete elution during preparation	• Deliver Elution Buffer directly to center of column • Larger elution volumes and longer incubation times can increase yield • For elution of DNA > 10 kb, heat the Elution Buffer to 50°C and extend incubation time to 5 minutes • Multiple rounds of elution can also be performed
	Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)	Reagents added incorrectly	• Be sure that buffers have been reconstituted correctly and that reagents have been added in the correct order
		Incomplete elution during preparation	• Deliver Elution Buffer directly to center of column • Larger elution volumes and longer incubation times can increase yield • For elution of DNA > 10 kb, heat the Elution Buffer to 50°C and extend incubation time to 5 minutes • Multiple rounds of elution can also be performed
Low DNA quality	Monarch Plasmid Miniprep Kit (NEB #T1010)	Plasmid degradation	• Be cautious of strains with high levels of endogenous endonuclease (e.g., HB101 and JM 100 series)
		Plasmid is denatured	• Limit incubation with Plasmid Lysis Buffer (B2) to two minutes, as NaOH in the buffer can denature the plasmid
		gDNA contamination	• Use careful inversion mixing after cell lysis to avoid shearing of host cell chromosomal DNA. Do not vortex.
		RNA contamination	• Incubate sample in neutralization buffer for the full 2 minutes. For cell culture volumes > 3 ml, increase the spin after neutralization to 5 minutes.
		Improper storage	• Elute DNA in DNA Elution Buffer or nuclease-free water, and store at -20°C. Do not store in solutions containing magnesium.
Low DNA purity	Monarch Plasmid Miniprep Kit (NEB #T1010)	Ethanol has been carried over	• Centrifuge final wash for 1 minute to ensure complete removal • Ensure column tip does not come in contact with flow through
		Excessive salt in sample	• Use both plasmid wash buffers and do not skip wash steps
		Excessive carbohydrate has been carried over	• Avoid strains with high amounts of endogenous carbohydrate (e.g., HB101 and JM 100 series). Be sure to follow protocol and include Plasmid Wash Buffer 1 step.
	Monarch DNA Gel Extraction Kit (NEB #T1020)	Gel stain not fully dissolved	• Undissolved agarose may leach salts into the eluted DNA
		Ethanol has been carried over	• Centrifuge final wash for 1 minute to ensure complete removal • Ensure column tip does not come in contact with flow through
		Trace amounts of salts have been carried over	• Ensure column tip does not come in contact with new tube for elution
	Monarch PCR & DNA Cleanup Kit (5 µg) (NEB #T1030)	Ethanol has been carried over	• Centrifuge final wash for 1 minute to ensure complete removal • Ensure column tip does not come in contact with flow through
		Trace amounts of salts have been carried over	• Ensure column tip does not come in contact with new tube

Guidelines for Choosing Sample Input Amounts When Using the Monarch® Genomic Purification Kit

Genomic DNA yield, purity and integrity vary immensely based on sample type, input amount and sample condition. Below, we have provided some empirical yield and DIN data from a wide variety of sample types, as well as guidance on the maximal input amounts for each of those samples when using the Monarch Genomic DNA Purification Kit (NEB #T3010). It is very important not to overload the column and the buffer system when extracting and purifying gDNA, as DNA yields, purity, integrity, and length may suffer.

Sample Type	Recommended Input Amount	Typical Yield (µg)	DIN	Maximum Input Amount
Tissue*				
Tail (mouse)	10 mg	12–20	8.5–9.5	25 mg
Ear (mouse)	10 mg	18–21	8.5–9.5	10 mg
Liver (mouse and rat)	10 mg	15–30	8.5–9.5	15 mg
Kidney (mouse)	10 mg	10–25	8.5–9.5	10 mg
Spleen (mouse)	10 mg	30–70	8.5–9.5	10 mg
Heart (mouse)	10 mg	9–10	8.5–9.5	25 mg
Lung (mouse)	10 mg	14–20	8.5–9.5	15 mg
Brain (mouse and rat)	10 mg	4–10	8.5–9.5	12 mg
Muscle (mouse and rat)	10 mg	4–7	8.5–9.5	25 mg
Muscle (deer)	10 mg	5	8.5–9.5	25 mg
Blood**				
Human (whole)	100 µl	2.5–4	8.5–9.5	100 µl
Mouse	100 µl	1–3	8.5–9.5	100 µl
Rabbit	100 µl	3–4	8.5–9.5	100 µl
Pig	100 µl	3.5–5	8.5–9.5	100 µl
Guinea pig	100 µl	3–8	8.5–9.5	100 µl
Cow	100 µl	2–3	8.5–9.5	100 µl
Horse	100 µl	4–7	8.5–9.5	100 µl
Dog	100 µl	2–4	8.5–9.5	100 µl
Chicken (nucleated)	10 µl	30–45	8.5–9.5	10 µl
Cells				
HeLa	1 x 10^6 cells	7–9	9.0–9.5	5 x 10^6 cells
HEK293	1 x 10^6 cells	7–9	9.0–9.5	5 x 10^6 cells
NIH3T3	1 x 10^6 cells	6–7.5	9.0–9.5	5 x 10^6 cells
Bacteria				
E. coli (gram-negative)	2 x 10^9 cells	6–10	8.5–9.0	2 x 10^9 cells
Rhodobacter sp. (gram-negative)	2 x 10^9 cells	6–10	8.5–9.0	2 x 10^9 cells
B. cereus (gram-positive)	2 x 10^9 cells	6–9	8.5–9.0	2 x 10^9 cells
Archaea				
T. kodakarensis	2 x 10^9 cells	3–5	8.5–9.0	2 x 10^9 cells
Yeast				
S. cerevisiae	5 x 10^7 cells	0.5–0.6	8.5–9.0	5 x 10^7 cells
Saliva/buccal cells***				
Saliva (human)	200 µl	2–3	7.0–8.0	500 µl
Buccal swab (human)	1 swab	5–7	6.0–7.0	1 swab

* Tissue gDNA yields are shown for frozen tissue powder, frozen tissue pieces and RNAlater-stabilized tissue pieces. Though frozen tissue powder results in highly-intact gDNA, lower yields can be expected than when using frozen or RNAlater-stabilized tissue pieces. Residual nuclease activity in tissue pieces will cut the gDNA, resulting in a slightly smaller overall size; however, this gDNA is optimal for silica-based purification.

** Human whole blood samples stabilized with various anticoagulants (e.g., EDTA, citrate and heparin) and various counter-ions were evaluated and results were comparable in all cases. Additionally, all indicated blood samples were tested both as fresh and frozen samples, yielding comparable results. Human samples were donated by healthy individuals; yields from unhealthy donors may differ.

***Buccal swabs and saliva samples partially consist of dead cell material with degraded gDNA. Therefore, the purified gDNA from those samples will naturally have lower DIN values.

Problem	Cause	Solution
Low Yield		
Cells	Frozen cell pellet was thawed and/or resuspended too abruptly	• Thaw cell pellets slowly on ice and flick tube several times to release the pellet from bottom of tube. Use cold PBS, and resuspend gently by pipetting up and down 5–10 times until pellet is dissolved
	Cell Lysis Buffer was added concurrently with enzymes	• Add Proteinase K and RNase A to sample and mix well before adding the Cell Lysis Buffer
Blood	Blood was thawed, allowing for DNase activity	• Keep blood samples frozen and add Proteinase K, RNase A and Blood Lysis Buffer directly to the frozen samples
	Blood sample is too old	• Fresh (unfrozen) whole blood should not be older than 1 week. Older samples will show progressive DNA degradation and loss of yield.
	Formation of hemoglobin precipitates	• Species with high hemoglobin content (e.g., guinea pig) may accumulate insoluble hemoglobin complexes that clog the membrane. Reduce Proteinase K lysis time from 5 to 3 minutes.
Tissue	Tissue pieces are too large	• Cut starting material to the smallest possible pieces or grind with liquid nitrogen. In large tissue pieces, nucleases will destroy the DNA before the Proteinase K can lyse the tissue.
	Membrane is clogged with tissue fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge lysate at maximum speed for 3 minutes, as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
	Sample was not stored properly	• Samples stored for long periods of time at room temperature, 4°C or -20°C, will show degradation and loss of gDNA. Flash freeze tissue samples with liquid nitrogen or dry ice and store at -80°C. Alternatively, use stabilizing reagents to protect the gDNA.
	Genomic DNA was degraded (common in DNase-rich tissues)	• Organ tissues (e.g., pancreas, intestine, kidney, liver) contain significant amounts of nucleases. Store properly to prevent DNA degradation. Keep on ice during sample preparation. Refer to the protocol for the recommended amount of starting material and Proteinase K to use.
	Column is overloaded with DNA	• Some organ tissues (e.g., spleen, kidney, liver) are extremely rich in genomic DNA. Using inputs larger than recommended will result in the formation of tangled, long-fragment gDNA that cannot be eluted from the silica membrane. Reduce the amount of input material.
	Incorrect amount of Proteinase K added	• Most samples are digested with 10 µl Proteinase K, but for brain, kidney and ear clips, use 3 µl.
DNA Degradation		
Tissue	Tissue samples were not stored properly	• Samples stored for long periods of time at room temperature, 4°C or -20°C, will show degradation and loss of gDNA. Flash freeze tissue samples with liquid nitrogen or dry ice and store at -80°C. Alternatively, use stabilizing reagents to protect the gDNA.
	Tissue pieces are too large	• Cut starting material to the smallest possible pieces or grind with liquid nitrogen. In large tissue pieces, nucleases will degrade the DNA before Proteinase K can lyse the tissue.
	High DNase content of soft organ tissue	• Organ tissues (e.g., pancreas, intestine, kidney, liver) contain significant amounts of nucleases. Store properly to prevent DNA degradation. Keep on ice during sample preparation. Refer to the protocol for the recommended amount of starting material and Proteinase K to use.
Blood	Blood sample is too old	• Fresh (unfrozen) whole blood should not be older than 1 week. Older samples will show progressive DNA degradation and loss of yield.
	Blood was thawed, allowing for DNase activity	• Keep frozen blood samples frozen and add enzymes and lysis buffer directly to the frozen samples
Salt Contamination		
	Guanidine salt was carried over into the eluate: • The binding buffer contains guanidine thiocyanate (GTC) which shows very strong absorbance at 200-230 nm. • The most common way that salt is introduced into the eluate is by allowing the buffer/lysate mixture to contact the upper column area.	• When transferring the lysate/binding buffer mix, avoid touching the upper column area with the pipet tip and always pipet carefully onto the silica membrane. • Avoid transferring any foam that may have been present in the lysate; foam can enter into the cap area of the spin column. • Close the caps gently to avoid splashing the mixture into the upper column area and move the samples with care in and out of the centrifuge. • If salt contamination is a concern, invert the columns a few times (or vortex briefly) with gDNA Wash Buffer as indicated in the protocol.

Troubleshooting Guide for Genomic DNA Purification When Using the Monarch® Kit (continued)

Problem	Cause	Solution
Protein Contamination		
Tissue	Incomplete digestion	• Cut samples to the smallest possible pieces. Incubate sample in the lysis buffer for an extra 30 minutes to 3 hours to degrade any remaining protein complexes.
	Membrane is clogged with tissue fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small, indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge the lysate at maximum speed for 3 minutes as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
Blood	High hemoglobin content	• Some blood samples (e.g., horse) are rich in hemoglobin, evidenced by their dark red color. Extend lysis time by 3–5 minutes for best purity results.
	Formation of hemoglobin precipitates	• Species with high hemoglobin content (e.g., guinea pig) may accumulate insoluble hemoglobin complexes that clog the membrane. Reduce Proteinase K lysis from 5 to 3 minutes.
RNA Contamination		
Tissue	Too much input material	• DNA-rich tissues (e.g., spleen, liver and kidney) will become very viscous during lysis and may inhibit RNase A activity. Do not use more than the recommended input amount.
	Lysis time was insufficient	• Extend lysis time by 30 minutes to 3 hours after the tissue piece has completely dissolved
Tissue Digestion Takes Too Long		
	Tissue pieces too large	• Cut tissue pieces to the smallest possible size or grind with liquid nitrogen before starting lysis
	Tissue pieces are stuck to bottom of tube	• Vortex to release pieces from the tube bottom, and immediately after adding Proteinase K and Tissue Lysis Buffer
	Too much starting material	• Use recommended input amount
Tissue Lysate Appears Turbid		
	Formation of indigestible fibers	• Proteinase K digestion of fibrous tissues (e.g., muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small indigestible protein fibers, which block the binding sites of the silica membrane. To remove fibers, centrifuge lysate at maximum speed for 3 minutes, as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material.
Ratio $A_{260}/A_{230} > 2.5$		
	Slight variations in EDTA concentration in eluates	• EDTA in elution buffer may complex with cations like Mg^{2+} and Ca^{2+} samples present in genomic DNA, which may lead to higher than usual A_{260}/A_{230} ratio. In some cases, this ratio exceeds a value of 3.0 and is consistent with highly pure samples. In these cases, the elevated value does not have any negative effect on downstream applications.

Guidelines for Choosing Sample Input Amounts When Using the Monarch® Total RNA Miniprep Kit

RNA yield, purity, and integrity vary immensely based on sample type, input amount and sample condition. Below, we have provided some empirical yield and RIN data from a wide variety of sample types, as well as guidance on the maximal input amounts for each of those samples when using the Monarch Total RNA Miniprep Kit (NEB #T2010). It is very important not to overload the column when extracting and purifying RNA, as yields, purity and integrity may suffer.

Sample Type [1]		Input	Average Yield (µg)	Observed RIN	Maximum Starting Material
Cultured Cells					
HeLa		1 x 10^6 cells	12–15	9-10	1 x 10^7 cells
HEK 293		1 x 10^6 cells	12–14	9-10	1 x 10^7 cells
NIH3T3		1 x 10^6 cells	8–12	9-10	1 x 10^7 cells
Mammalian Blood [2]					
Human	Fresh	200 µl	0.5–1.0	7-8	3 ml
	Frozen	200 µl	0.5–1.0	7-8	3 ml
	Stabilized	200 µl	0.5–1.0	7-8	3 ml
Rat	Frozen	100 µl	5.6	9	1 ml*
Blood Cells					
PBMC (isolated from 5 ml whole blood)		5 ml	3	7	1 x 10^7 cells
Tissue					
Rat liver	Frozen pulverized	10 mg	25	8–9	20 mg
	Stabilized solid	10 mg	50–60	8–9	20 mg
Rat spleen (stabilized solid with bead homogenizer)		10 mg	40–50	9	20 mg
Rat kidney (frozen pulverized)		10 mg	7–10	9	50 mg
Rat brain	Frozen pulverized	10 mg	2–3	8–9	50 mg
	Stabilized solid	10 mg	0.5–1.5	8–9	50 mg
	Stabilized solid with bead homogenizer	10 mg	5–8	8–9	50 mg
Rat muscle (frozen pulverized)		10 mg	2–3	8–9	50 mg
Mouse muscle	Frozen pulverized	10 mg	3	8–9	50 mg
	Powder with bead homogenizer	10 mg	5	7–8	50 mg
	Stabilized solid with bead homogenizer	10 mg	8–10	9	50 mg
Mouse heart (stabilized solid w/bead homogenizer)		10 mg	5–6	8–9	50 mg
Yeast					
S. cerevisiae	Frozen with bead homogenizer	1 x 10^7 cells	50	9–10**	5 x 10^7 cells
	Fresh with Zymolyase®	1 x 10^7 cells	60	9**	5 x 10^7 cells
Bacteria					
E. coli	Frozen	1 x 10^9 cells	5	10	1 x 10^9 cells
	Frozen with bead homogenizer	1 x 10^9 cells	10	10	1 x 10^9 cells
	Frozen with lysozyme	1 x 10^9 cells	70	10	1 x 10^9 cells
B. cereus	Frozen with lysozyme	1 x 10^8 cells	20–30	9	1 x 10^9 cells
	Frozen with bead homogenizer	1 x 10^8 cells	8	9–10	1 x 10^9 cells
Plant					
Corn leaf (frozen pulverized with bead homogenizer)		100 mg	45	8	100 mg
Tomato leaf (frozen pulverized with bead homogenizer)		100 mg	30	8	100 mg

[1] RNA for other blood samples, including drosophila, zebrafish embryos/larvae, plasma, serum, saliva, buccal swabs and nucleated blood have been successfully purified with this kit; protocols are available in the product manual.

[2] A protocol for nucleated blood (e.g., birds, reptiles) is also available.

* Mouse blood also has a maximum input of 1 ml.

** *S. cerevisiae* total RNA was run on an Agilent® Nano 6000 Chip using plant assay.

Find tips for RNA purification using Monarch.

Troubleshooting Guide for Total RNA Extraction & Purification Using Monarch® Kits

Problem	Cause	Solution
Clogged column	Insufficient sample disruption or homogenization	• Increase time of sample digestion or homogenization • Centrifuge sample after Proteinase K digestion or homogenization to pellet debris and use only supernatant for next steps • Use larger volume of DNA/RNA Protection Reagent (NEB #T2011) and/or RNA Lysis Buffer (NEB #T2012) for sample disruption and homogenization. See sample-specific protocols in the product manual.
	Too much sample	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. See Guidelines for Choosing Sample Input Amounts.
Low RNA yield	Incomplete elution	• After addition of Nuclease-free Water (NEB #B1500) to column matrix, incubate 5-10 min at room temperature and then centrifuge to elute • Perform a second elution (note: this will dilute sample)
	Sample is degraded	• Store input sample at -80°C prior to use • Use Monarch DNA/RNA Protection Reagent (NEB #T2011) to maintain RNA integrity during storage
	Insufficient disruption or homogenization	• Increase time of sample digestion or homogenization • Centrifuge sample after Proteinase K digestion or homogenization to pellet debris and use only supernatant for next steps • Use larger volume of DNA/RNA Reagent (NEB #T2011) and/or RNA Lysis Buffer (NEB #T2012) for sample disruption and homogenization. See sample specific protocol in the product manual. • For Proteinase K treated samples, doubling Proteinase K (from 5% to 10%) may lead to an increase in RNA yield
	Too much sample	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. See Guidelines for Choosing Sample Input Amounts.
RNA degradation	Starting material not handled/stored properly	• Store input sample at -80°C prior to use. Degradation of RNA may occur if sample is not flash frozen or protected by a preservation reagent. Use Monarch DNA/RNA Protection Reagent (NEB #T2011) to maintain RNA integrity during storage.
	Deviation from the stated protocol may expose RNA to unwanted RNase activities	• Refer to the General Guidelines for working with RNA in the product manual
	RNase contamination of eluted materials or kit buffers may have occurred	• See General Guidelines for working with RNA in the product manual for advice on reducing risks of contamination
Low OD ratios	Low $A_{260/280}$ values indicate residual protein in the purified sample	• Ensure the Proteinase K step was utilized for the recommended time. Ensure samples have no debris prior to addition of ethanol and loading onto RNA Purification Column.
	Low $A_{260/230}$ values indicate residual guanidine salts have been carried over during elution	• Ensure wash steps are carried out prior to eluting sample. Use care to ensure the tip of the column does not contact the flow-through after the final wash. If unsure, please repeat centrifugation. When reusing collection tubes, blot rim of tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer.
DNA contamination	Genomic DNA not removed by column	• Perform optional on-column DNase I treatment to remove unwanted gDNA from lysed sample • Perform in-tube/off-column DNase I treatment to remove gDNA
	Too much sample	• Reduce amount of starting material to match kit specifications to ensure buffer amounts are sufficient and column is not overloaded. See Guidelines for Choosing Sample Input Amounts.
Low performance of RNA in downstream steps	Salt and/or ethanol carryover has occurred	• Use care to ensure the tip of the RNA Purification Column does not contact the flow-through after the final wash. If unsure, please repeat centrifugation. • Be sure to spin the RNA Purification Column for 2 minutes following the final wash with RNA Wash Buffer • When reusing collection tubes, blot rim of tube on a Kimwipe prior to reattachment to the column to remove any residual wash buffer • Add additional wash step and/or extend spin time for final wash
Unusual spectrophotometric readings	RNA concentration is too low for spectrophotometric analysis	• For more concentrated RNA, elute with 30 µl of nuclease-free water • Increase amount of starting material (within kit specifications). See Guidelines for Choosing Sample Input Amounts.
	Silica fines in eluate	• Re-spin eluted samples and pipet aliquot from the top of the liquid to ensure the $A_{260/230}$ is unaffected by possible elution of silica particles

Guidelines for Choosing Sample Input Amounts When Using the Monarch® HMW DNA Extraction Kit for Cells & Blood

The table below provides data on minimum, maximum, and recommended input amounts for various cell lines and blood samples using the Monarch HMW DNA Extraction Kit for Cells & Blood. Data on yield, purity and RNA content is also provided. Samples that were successfully tested in standard ligation-based Oxford Nanopore Technologies® sequencing runs are indicated. RNA content was determined by HPLC analysis of nucleoside content after digestion of 1 µg of eluted nucleic acid with the Nucleoside Digestion Mix (NEB #M0649). Yields from blood samples vary by donor due to different leukocyte content; yield can vary up to 3-fold by donor. Similar yield and purity results were obtained with different anticoagulants (e.g., EDTA, citrate, heparin and PAXgene Blood DNA tubes were tested).

Using input amounts below the recommended minimum will reduce yields drastically. Exceeding maximum input amounts will result in DNA eluates that are highly viscous and difficult to dissolve and will reduce purity of the isolated DNA. Results are shown for samples that were lysed with agitation at 2,000 rpm.

Cells

	Minimum Input (Cells)	Maximum Input (Cells)*	Recommended Input Amount (Cells)	Yield (µg) FROM 1 x 10^6 cells	Purity Ratios		RNA content	Validated for ONT sequencing?
					$A_{260/280}$	$A_{260/230}$		
HEK293	1 x 10^5	1 x 10^7	1 x 10^6	11.5–13	1.86	2.4	≤ 1%	Yes
HeLa	1 x 10^5	1 x 10^7	1 x 10^6	12.9	1.86	2.4	≤ 1%	Yes
NIH3T3	1 x 10^5	1 x 10^7	1 x 10^6	9.4	1.86	2.4	≤ 1%	Yes
Jurkat	1 x 10^5	1 x 10^7	1 x 10^6	13.7	1.86	2.5	≤ 1%	Yes
K562 (suspension cells)	1 x 10^5	1 x 10^7	1 x 10^6	13.7	1.86	2.4	≤ 1%	Yes
HCT116	1 x 10^5	1 x 10^7	1 x 10^6	16.9	1.86	2.5	≤ 1%	Yes
A549	1 x 10^5	1 x 10^7	1 x 10^6	12.7	1.86	2.3	≤ 1%	Yes
U5Os	1 x 10^5	1 x 10^7	1 x 10^6	10.6	1.86	2.4	≤ 1%	Yes
HepG2	1 x 10^5	1 x 10^7	1 x 10^6	13.4	1.81	2.2	≤ 1%	Yes
NCI-460	1 x 10^5	1 x 10^7	1 x 10^6	9.5	1.86	2.4	≤ 1%	Yes
SK-N-SH	1 x 10^5	1 x 10^7	1 x 10^6	9.5	1.86	2.4	≤ 1%	Yes
Aa23	1 x 10^5	1 x 10^7	1 x 10^6	8.7	1.81	2.3	≤ 1%	Yes

Mammalian Blood

		Minimum Input (µl)	Maximum Input (µl)	Recommended Input (µl)	Yield (µg) for 500 µl**	Purity Ratios		RNA content	Validated for ONT sequencing?
						$A_{260/280}$	$A_{260/230}$		
Human***	Fresh	100	2,000	500	12–32	1.86	2.4	≤ 1%	Yes
	Frozen	100	2,000	500	9–30	1.86	2.4	≤ 1%	Yes
Mouse	Fresh	100	2,000	500	7–11	1.88	2.4	≤ 1%	Yes
	Frozen	100	2,000	500	16–17	1.88	2.4	≤ 1%	ND
Rat (fresh only)	Fresh	100	2,000	500	29–38	1.87	2.4	≤ 1%	Yes
Rabbit	Fresh	100	500	200	12–15	1.72	1.9	≤ 1%	Yes
	Fresh	100	500	200	200 µl: 4–5	1.89	2.4	≤ 1%	Yes
	Frozen	100	500	200	200 µl: 4–5	1.89	2.4	≤ 1%	Yes
Pig	Fresh	100	2,000	500	up to 42	1.86	2.4	≤ 1%	Yes
	Frozen	100	2,000	500	up to 40	1.86	2.4	≤ 1%	Yes
Horse	Fresh	100	2,000	500	16	1.86	2.3	≤ 1%	Yes
	Frozen	100	2,000	500	22.3	1.86	2.4	ND	ND
Cow	Fresh	200	2,000	500	7	1.86	2.4	≤ 1%	Yes
	Frozen	200	2,000	500	9.1	1.86	2.4	ND	ND
Rhesus monkey	Fresh	100	2,000	500	52	1.86	2.4	≤ 1%	Yes
	Frozen	100	2,000	500	52.6	1.86	2.5	ND	ND
Goat (fresh only)	Fresh	100	2,000	500	24	1.87	2.4	≤ 1%	Yes
Sheep (fresh only)	Fresh	100	2,000	500	15.3	1.87	2.4	ND	ND

Nucleated Blood

		Minimum Input (µl)	Maximum Input (µl)	Recommended Input (µl)	Yield (µg) for 5 µl**	Purity Ratios		RNA content	Validated for ONT sequencing?
						$A_{260/280}$	$A_{260/230}$		
Chicken	Fresh	2	20	5	33	1.86	2.5	ND	Yes
	Frozen	2	20	5	30	1.86	2.5	ND	ND
Turkey	Fresh	2	20	5	37	1.87	2.4	ND	Yes
	Frozen	2	20	5	28	1.87	2.5	ND	ND

ND = Not determined

* For low agitation speeds, do not exceed 5 x 106 cells

** Unless otherwise stated

***Compatible with K2-EDTA, Na-citrate, Na-heparin, PAXgene® Blood DNA

Guidelines for Choosing Sample Input Amounts When Using the Monarch® HMW DNA Extraction Kit for Tissue, Bacteria and Other Samples

The table below provides guidance on the minimum, maximum, and recommended input amounts for various sample types when using the Monarch HMW DNA Extraction Kit for Tissue. Data on yield, purity and RNA content is also provided. Samples that were successfully tested in standard ligation-based Oxford Nanopore Technologies sequencing runs are indicated. Using input amounts that exceed the maximum will lead to challenges in solubility and viscosity, and purity may be affected. If more starting material is required, splitting the sample and performing multiple preps is recommended. RNA content was determined by HPLC analysis of nucleoside content after digestion of 1 µg of eluted nucleic acid with the Nucleoside Digestion Mix (NEB #M0649). Using input amounts below the recommended minimums will reduce yields drastically.

		Minimum Input (mg)	Maximum Input (mg)	Recommended Input (mg)	Yield (µg) for recommended input (Yield per mg)	Purity Ratios		RNA Content	Validated for ONT Sequencing?
						$A_{260/280}$	$A_{260/230}$		
Mammalian Tissue									
Mouse brain	Fresh	2**	20	15	12–21	1.87	2.39	ND	Yes
	Frozen	2**	20	15	15–21 (1–1.5)	1.86	2.48	ND	Yes
Mouse liver	Fresh (w/NaCl)	2	15	10	7	1.84	2.10	1.2%	Yes
	Frozen (w/NaCl)	2	15	10	17–19 (1.7–1.9)	1.89	2.50	ND	Yes
	Fresh*	2	15	10	20	1.84	1.52+	8.7%	Yes
	Frozen*	2	15	10	27–31 (2.7–3.1)	1.89	1.93++	ND	Yes
Mouse muscle	Fresh	2**	25	20	8–9	1.87	2.25	2.1%	Yes
	Frozen	2**	25	20	12–16 (0.6–0.8)	1.87	2.30	ND	Yes
Mouse kidney	Fresh	2	15	10	23–34	1.86	2.44	ND	Yes
	Frozen	2	15	10	32–41 (3.2–4.1)	1.86	2.53	0.8%	Yes
Mouse tail	Frozen	2**	25	20	20 (1.8–2.1)	1.86	2.43	ND	Yes+++
Mouse ear punch	Fresh	2**	15	10	15–16 (1.5–1.6)	1.86	2.29	ND	Yes
Rat kidney	Frozen	2	15	10	20–25	1.87	2.40	ND	Yes
Bacteria									
E. coli (Gram-negative)	Frozen	5 x 10^8 cells	5 x 10^9 cells	1 x 10^9 cells	8–9	1.89	2.31	1.7%	Yes
B. cereus (Gram-positive)	Frozen	2 x 10^8 cells	4 x 10^8 cells	2 x 10^8 cells	4–5	1.86	2.20	3.9%	Yes
M. luteus (Gram-positive)	Frozen	ND	ND	1 x 10^8 cells	2.0	1.89	2.09	ND	ND
Amphibian									
X. laevis	Fresh	ND	ND	3–4	5	1.86	2.51	2.3%	ND
Yeast									
S. cerevisiae	Fresh	ND	ND	20 x 10^7 cells	3–6***	1.90	2.01	ND	ND
Insect									
A. aegypti	Frozen	ND	ND	15	6	1.84	2.53++	2.7%	ND
NEMATODE									
*C. elegans***	Frozen	ND	ND	2 plates	8.2	1.91	2.5	ND	ND

ND = Not determined

* Standard protocol without recommended NaCl treatment.

** If working with input amounts < 5 mg, refer to the product manual for guidance on reducing buffer volumes.

*** Total nucleic acid yields are 4-10 µg and 6-12 µg for haploid and diploid strains, respectively. Though an RNase A step is included, RNA is co-purified. Yields may vary depending on the strain.

**** Rotor-stator homogenization is recommended.

\+ Measured with Nanodrop One; systems that differentiate turbidity in the content profiling will give higher values.

++ Measured with Unchained Labs Lunatic (formerly Trinean DropSense16); devices without content profiling that differentiates turbidity may give lower values.

+++ Size selection is recommended.

Genetic Markers

A *genotype* indicates the genetic state of the DNA in an organism. It is a theoretical construct describing a genetic situation that explains the observed properties (phenotype, see below) of a strain. *E. coli* genotypes list only genes that are defective (1). If a gene is not mentioned, then it is not known to be mutated*, **. Prophages and plasmids that were present in the original K-12 strain (F, λ, e14, rac) are normally listed only if absent. However, for simplicity, we have not listed λ except when it is present, and we have listed F and its variants in all cases. Parentheses or brackets surround a prophage or plasmid when listed. Genes are given three-letter, lower-case, italicized names (e.g.,*dam*) that are intended to be mnemonics suggesting the function of the gene (here, **D**NA **a**denine **m**ethylase). If the same function is affected by several genes, the different genes are distinguished with uppercase italic letters (e.g., *recA, recB, recC, recD* all affect **rec**ombination). Proper notation omits superscript + or – in a genotype, but these are sometimes used redundantly for clarity, as with F´ *lac*$^{-}$ *proA*$^{+}$*B*$^{+}$. Deletion mutations are noted as Δ, followed by the names of deleted genes in parentheses, [e.g., Δ(*lac-pro*)]. All genes between the named genes are also deleted. Specific mutations are given allele numbers that are usually italic arabic numerals (e.g.,*hsdR17*) and may be characterized as *am*=amber (UAG) mutation or *ts*=inactive at high temperature, as appropriate. Some common alleles[e.g., Δ(*lac-pro*)X111] break the rules. If two strains' genotypes list a gene with the same allele number, they should carry exactly the same mutation.

The *phenotype* of a strain is an observable behavior, e.g., Lac^{-} fails to grow on lactose as a sole carbon source. Phenotypes are capitalized and in Roman type, and the letters are always followed by superscript + or – (or sometimes r, resistant, or s, sensitive). Although phenotypes do not, strictly speaking, belong in a genotype, they are sometimes included following the genotype designation when the former is not obvious from the latter [e.g., *rpsL104* (Str^{r})– gene name from **r**ibosomal **p**rotein, **s**mall subunit, S12, confers resistance to streptomycin].

Some common genes of interest are described below and on the next page; a catalogue of genetically defined genes can be found in reference 2 and on the very useful internet site maintained by the *E. coli* Genetic Stock Center (CGSC) at Yale University <http://cgsc.biology.yale.edu/>. Additional information from CGSC can be obtained from curator Mary Berlyn by e-mail <cgsc@yale.edu>.

* Most *E. coli* laboratory strains have been heavily mutagenized over forty years of study, and different lines may carry different, so far undiscovered, mutations that may or may not affect your situation. For this reason, it is sometimes useful to try more than one line or strain background in your experiments.

** *E. coli* B and its derivatives are naturally Lon^{-} and Dcm^{-}. We have listed this in brackets even though it is the wild type state for these strains.

dam	Endogenous adenine methylation at GATC sequences is abolished. *dam* strains have a high recombination frequency, express DNA repair functions constitutively, and are poorly transformed by Dam-modified plasmids. Used for making DNA susceptible to cleavage by some restriction enzymes (e.g., BclI).
dcm	Endogenous cytosine methylation at CCWGG sequences is abolished. Used for making DNA susceptible to cleavage by some restriction enzymes (e.g., AvaII).
dnaJ	One of several "chaperonins" is inactive. This defect has been shown to stabilize certain mutant proteins expressed in *E. coli*.
dut	dUTPase activity is abolished. This mutation, in combination with *ung*, allows incorporation of uracil into DNA. Used for oligonucleotide mutagenesis.
endA	Activity of nonspecific Endonuclease I is abolished. DNA preparations are thought to be of higher quality when prepared from *endA* strains.
e14	An excisable prophage-like element, present in K-12 but missing from many derivatives. e14 carries the *mcrA* gene among others, therefore e14– strains are McrA–.
F	A low-copy number self-transmissible plasmid. F´ factors carry portions of the *E. coli* chromosome, most notably the lac operon and *proAB* on F´ *lac*$^{-}$ *proA*$^{+}$*B*$^{+}$.
fhuA	An iron uptake receptor is mutated. This mutation confers resistance to phage T1 (ferric hydroxamate uptake). Former name is *tonA*.
gal	The ability to metabolize galactose is abolished.
glnV	See ***supE***.
gyrA	A point mutation in DNA gyrase, subunit A. This mutation confers resistance to the antibiotic nalidixic acid.
hflA	This mutation results in high frequency lysogenization by λ.
hsdR, hsdS	DNA that does not contain methylation of certain sequences is recognized as foreign by EcoKI or EcoBI and restricted (degraded). These enzymes recognize different sequences and are encoded by different alleles of *hsdRMS*. *hsdR* mutations abolish restriction but not protective methylation (r^{-}m^{+}), while *hsdS* mutations abolish both (r^{-}m^{-}). DNA made in the latter will be restricted when introduced into a wild-type strain.
lacIq	The *lac* repressor is overproduced, turning off expression from P*lac* more completely.
lacZ	β-galactosidase activity is abolished.
lacZ:: T7gene 1	The phage T7 RNA polymerase (= gene 1) is inserted into the lacZ gene.
lacY	Lactose permease activity is abolished.
	Δ(*lac*) = deletion; there are four common deletions involving *lac*:
	Δ(*lacZ*)M15 expresses a fragment that complements the *lac* α-fragment encoded by many vectors. These vectors will yield blue color on X-Gal only if the host carries ΔM15.
	ΔU169, ΔX111, and ΔX74 all delete the entire *lac* operon from the chromosome,in addition to varying amounts of flanking DNA. ΔX111 deletes *proAB* as well, so that the cell requires proline for growth on minimal medium, unless it also carries F´ *lac proA*$^{+}$*B*$^{+}$.
lon	Activity of a protease responsible for degrading aberrant proteins is abolished. Some eukaryotic proteins are stabilized in *lon* strains. *E. coli* B naturally lacks Lon.
lysY	The lysozyme gene from the T7 bacteriophage is mutated. The mutation K128Y eliminates lysozyme activity, but the mutant protein still binds to and inhibits T7 RNA polymerase.

References
(1) Demerec et al. (1966) *Genetics*, 54, 61–76.
(2) Berlyn, M.K.B. (1996). In F. C. Niedhardt et al. (Ed.), *Escherichia coli and Salmonella: cellular and molecular biology*, (2nd ed.), Vol. 2, (pp. 1715–1902). ASMPress.
(3) Raleigh, E.A. et al. (1991) *J. Bacteriol.*, 173, 2707–2709.

Genetic Markers (continued)

malB	The *malB* region encompasses the genes *malEFG* and *malK lamB malM*. Δ(*malB*) deletes most or all of this region and eliminates expression of Maltose Binding Protein (MalE).
mcrA, mcrBC	A restriction system that requires methyl mcrBC cytosine is abolished. DNA containing methylcytosine in some sequences is restricted by Mcr$^+$. *dcm*-modified DNA is not restricted by Mcr$^+$. Δ(*mcrC-mrr*) deletes six genes: *mcrC-mcrB-hsdS-hsdM-hsdR-mrr; mcrA* is lost with e14.
mrr	A restriction system that requires cytosine or adenine methylation is abolished; however, *dam*$^-$, *dcm*$^-$ or EcoKI-modified DNA is not restricted by Mrr$^+$. The methylcytosine-dependent activity is also known as McrF (3).
mtl	The ability to metabolize the sugar alcohol mannitol is abolished.
ompT	Activity of outer membrane protease (protease VII) is abolished.
phoA	Activity of alkaline phosphatase is abolished.
prc	See ***tsp***.
recA	Homologous recombination is abolished; particularly desirable when working with sequences containing direct repeats > 50 bp.
recB, recC	Exonuclease and recombination activity of Exonuclease V is abolished. Homologous recombination is much reduced in *recB recC* strains that are not also *sbcB* or *sbcA*. Stability of inverted repeat sequences is enhanced in *recB recC* strains, especially if they are also *sbcB sbcC*. Plasmid replication may be aberrant.
recD	Exonuclease activity of ExoV is abolished, but recombination activity is elevated. Inverted repeat sequences in λ can be propagated in *recD* strains. Plasmid replication is aberrant.
recF	Plasmid-by-plasmid homologous recombination is abolished.
recJ	Plasmid-by-plasmid homologous recombination is abolished.
relA1	Lacks ppGpp synthesis during the stringent response to amino acid starvation; activity of ATP:GTP 3´-pyrophosphotransferase (EC2.7.6.5) is abolished.
rfbD	Lacks functional TDP-rhamnose synthetase, and thus does not synthesize the cell surface O-antigen.
rpoH	(also known as *htpR*) Lack of this heat-shock transcription factor abolishes expression of some stress-induced protease activities in addition to *lon*. Some cloned proteins are more stable in *rpoH*am *sup*Cts strains at high temperature.
sbcB	Exo I activity is abolished. Strains carrying *recB recC* and *sbcB* are usually also *sbcC*. These quadruple mutant strains are recombination-proficient and propagate inverted repeats in λ, but plasmid replication is aberrant.
sbcC	Usually found with *recB recC sbcB*. However, strains carrying *sbcC* alone are recombination-proficient and stably propagate inverted repeats both in λ and in plasmids.
sulA	Mutations in this gene allows cells to divide and recover from DNA damage in a lon mutant background (suppressor of Lon).
supC(ts)	A thermosensitive tyrosine-inserting ochre (UAA) and amber (UAG) suppressor tRNA. Nonsense mutations in the same strain are suppressed only at low temperatures. Now called ***tyrT***.
supE	A glutamine-inserting amber (UAG) suppressor tRNA; required for growth of some phage vectors. Now called ***glnV***.
supF	A tyrosine-inserting amber (UAG) suppressor tRNA; required for lytic growth of S7 or S100 λ phage, such as λgt11. Now called ***tyrT***.
thi-1	The ability to synthesize thiamine is abolished (vitamin B1).
traD	The self-transmissibility of the F factor is severely reduced.
tsp	A periplasmic protease that may degrade secreted or cytoplasmically overexpressed proteins after lysis is abolished. Now called ***prc***.
tsx	Confers resistance to bacteriophage T6.
tyrT	See ***supC***, ***supF***.
ung	Uracil N-glycosylase activity is abolished. Uracil incorporated into DNA is removed by Ung$^+$, leaving baseless site. See *dut*.
xyl	The ability to metabolize the sugar xylose is abolished.
(P1)	The cell carries a P1 prophage. Such strains express the P1 restriction system.
(P2)	The cell carries a P2 prophage. This allows selection against Red$^+$ Gam$^+$ λ (Spi$^-$ selection).
(ϕ80)	The cell carries the lambdoid prophage ϕ80. A defective ϕ80 prophage carrying the *lac* M15 deletion is present in some strains.
(Mu)	Mu prophage; Mud means the phage is defective.

Enhancing Transformation Efficiency

Transformation efficiency is defined as the number of colony forming units (cfu) that would be produced by transforming 1 µg of plasmid into a given volume of competent cells. However, 1 µg of plasmid is rarely transformed. Instead, efficiency is routinely calculated by transforming 100 pg–1 ng of highly purified supercoiled plasmid under ideal conditions. Transformation Efficiency (TE) is calculated as: TE = Colonies/µg/Dilution. Efficiency calculations can be used to compare cells or ligations. Our recommended protocols and tips are presented here to help you achieve maximum results.

Recommended Protocols

High Efficiency Transformation Protocol

1. Thaw cells on ice for 10 minutes
2. Add 1 pg–100 ng of plasmid DNA (1–5 µl) to cells and mix without vortexing
3. Place on ice for 30 minutes
4. Heat shock at 42°C for 10–30 seconds or according to recommendations. For BL21, use exactly 10 seconds.
5. Place on ice for 5 minutes
6. Add 950 µl of room temperature SOC or NEB 10-beta/Stable Outgrowth Medium.
7. Place at 37°C for 60 minutes. Shake vigorously (250 rpm) or rotate.
8. Mix cells without vortexing and perform several 10-fold serial dilutions in SOC or NEB 10-beta/Stable Outgrowth Medium.
9. Spread 50–100 µl of each dilution onto pre-warmed selection plates and incubate overnight at 37°C (30°C for SHuffle® strains) or according to recommendations

5 Minute Transformation Protocol

(10% efficiency compared to above protocol)

1. Thaw cells in your hand
2. Add 1 pg–100 ng of plasmid DNA (1–5 µl) to cells and mix without vortexing
3. Place on ice for 2 minutes
4. Heat shock at 42°C for 30 seconds or according to recommendations.
5. Place on ice for 2 minutes
6. Add 950 µl of room temperature SOC or NEB 10-beta/Stable Outgrowth Medium. Immediately spread 50–100 µl onto a selection plate and incubate overnight at 37-42°C. (30°C for SHuffle strains) NOTE: Selection using antibiotics other than ampicillin may require some outgrowth prior to plating.

Transformation Tips

Thawing

- Cells are best thawed on ice
- DNA should be added as soon as the last trace of ice in the tube disappears
- Cells can be thawed by hand, but warming above 0°C decreases efficiency

Incubation of DNA with Cells on Ice

- Incubate on ice for 30 minutes. Expect a 2-fold loss in TE for every 10 minutes this step is shortened.

Heat Shock

- Both temperature and time are specific to the transformation volume and vessel. Typically, 30 seconds at 42°C is recommended.

Outgrowth

- Outgrowth at 37°C for 1 hour is best for cell recovery and for expression of antibiotic resistance. Expect a 2-fold loss in TE for every 15 minutes this step is shortened.
- SOC gives 2-fold higher TE than LB medium
- Incubation with shaking or rotation results in 2-fold higher TE

Find tips for successful transformation.

Plating

- Selection plates can be used warm or cold, wet or dry with no significant effects on TE
- Warm, dry plates are easier to spread and allow for the most rapid colony formation

DNA

- DNA should be purified and resuspended in water or TE Buffer
- Up to 10 µl of DNA from a ligation mix can be used with only a 2-fold loss of efficiency
- Purification by either a spin column or phenol/chloroform extraction and ethanol precipitation is ideal
- The optimal amount of DNA is lower than commonly recognized. Using clean, supercoiled pUC19, the efficiency of transformation is highest in the 100 pg–1 ng range. However, the total colonies which can be obtained from a single transformation reaction increase up to about 100 ng.

DNA Contaminants to Avoid

Contaminant	Removal Method
Detergents	Ethanol precipitate
Phenol	Extract with chloroform and ethanol precipitate
Ethanol or Isopropanol	Dry pellet before resuspending
PEG	Column purify or phenol/chloroform extract and ethanol precipitate
DNA binding proteins (e.g., ligase)	Column purify or phenol/ chloroform extract and ethanol precipitate

Electroporation Tips

NEB 10-beta (NEB #C3020) Competent *E. coli* is available as electrocompetent cells. The following tips will help maximize transformation efficiencies.

- Pre-chill electroporation cuvettes and microcentrifuge tubes on ice
- Thaw cells on ice and suspended well by carefully flicking the tubes
- Once DNA is added, electroporation can be carried out immediately. It is not necessary to incubate DNA with cells. The maximum recommended volume of a DNA solution to be added is 2.5 µl. Addition of a large volume of DNA decreases transformation efficiency.
- DNA should be purified and suspended in water or TE. Transformation efficiency is > 10-fold lower for ligation mixtures than the control pUC19 plasmid due to the presence of ligase and salts. If used directly, ligation reactions should be heat-inactivated at 65°C for 20 min and then diluted 10-fold. For optimal results, spin columns are recommended for clean up of ligation reactions.
- Electroporation conditions vary with different cuvettes and electroporators. If you are using electroporators not specified in the protocol, you may need to optimize the electroporation conditions. Cuvettes with 1 mm gap are recommended (e.g., BTX Model 610/613 and Bio-Rad #165-2089). Higher voltage is required for cuvettes with 2 mm gap.
- Arcing may occur due to high concentration of salts or air bubbles
- It is essential to add recovery medium to the cells immediately after electroporation. One minute delay can cause a 3-fold reduction in efficiency.
- Cold and dry selection plates lead to lower transformation efficiency. Pre-warm plates at 37°C for 1 hour. Using 37°C pre-warmed recovery medium increases the efficiency by about 20%.
- Refreeze unused cells in a dry ice/ethanol bath for 5 min and then store at -80°C. Do not use liquid nitrogen. Additional freeze-thaw cycles result in lower transformation efficiency.

T7 Protein Expression

1. Transform expression plasmid into a T7 expression strain. Plate out on antibiotic selection plates and incubate overnight at 37°C (24 hours at 30°C for SHuffle strains).
2. Resuspend a single colony in 10 ml liquid culture with antibiotic
3. Incubate at 37°C until OD_{600} reaches 0.4–0.6
4. Induce with 40 µl of a 100 mM stock of IPTG (final conc. = 0.4 mM) and induce for 2 hours at 37°C (4 hours at 30°C or 16°C overnight for SHuffle strains)
5. Check expression by Coomassie stained protein gel, Western Blot or activity assay. Check expression in the total cell extract (soluble + insoluble) and the soluble fraction alone.
6. For large scale, inoculate 1 L of liquid medium (with antibiotic) with a freshly grown colony or 10 ml of freshly grown culture. Incubate at 37°C (30°C for SHuffle strains) until OD_{600} reaches 0.4–0.6. Add IPTG to 0.4 mM. Induce 2 hours at 37°C or 15°C overnight (4 hours at 30°C or 16°C overnight for SHuffle strains).

Troubleshooting Tips

No Colonies or No Growth in Liquid Culture

- Even though T7 expression is tightly regulated, there may be a low level of basal expression in the T7 Express host. If toxicity of the expressed protein is likely, transformation of the expression plasmid should be carried out in a more tightly controlled expression strain:
  - In *I*^{q} strains over-expression of the *LacI*^{q} repressor reduces basal expression of the T7 RNA polymerase
  - In *lysY* strains, mutant T7 lysozyme is produced which binds to T7 RNA polymerase, reducing basal expression of the target protein. Upon induction, newly made T7 RNA polymerase titrates out the lysozyme and results in expression of the target protein.
- Incubation at 30°C or room temperature may also alleviate toxicity issues
- Check antibiotic concentration (test with control plasmid)

No Protein Visible on Gel or No Activity

- Check for toxicity - the cells may have eliminated or deleted elements in the expression plasmid. If this is the case, test *I*^{q} and/or *lysY* strains to reduce basal expression.
- Culture cells for protein induction. Just before induction, plate a sample on duplicate plates with and without antibiotic selection. If toxicity is an issue, significantly fewer colonies will be seen on plates containing antibiotic (indicating that the plasmid has been lost) compared to plates without antibiotic.

Induced Protein is Insoluble

T7 expression often leads to very high production of protein that can result in the target protein becoming insoluble. In this case:

- Induce at lower temperatures (12–15°C overnight)
- Reduce IPTG concentration to 0.01 mM – 0.1 mM
- Induce for less time (as little as 15 minutes)
- Induce earlier in growth (OD_{600} = 0.3 or 0.4)

Salam joined NEB in 2020 and is currently a Production Scientist at NEB's manufacturing facility in Rowley, MA, which is used to manufacture GMP-grade materials.

Troubleshooting Guide for NEBNext® DNA Library Prep Kits

Troubleshooting guides are available at www.neb.com for NEBNext products including NEBNext RNA Library Prep, NEBNext Ultra II FS DNA Library Prep, NEBNext DNA Library Prep, NEBNext rRNA Depletion Kit (Bacteria) and NEBNext Custom RNA Depletion Design Tool with NEBNext RNA Depletion Core Reagent Set

Problem	Cause	Solution
Failed Library Prep **For example:** **• You may see nothing on the Bioanalyzer, or similar instrument** **• After amplification, you may see library fragments that are still the same size as the starting input DNA rather than ~120 bp longer than the input DNA**	Input DNA contains an inhibitor	• Ensure DNA does not contain inhibitor • Consider additional cleanup step
	Failed step - Any of the enzymatic steps can fail if a critical reagent is omitted, or if the reagent has become inactive	• Confirm reagents were added for each step in the protocol
Low Library Yield	Input DNA is damaged	• Shear input DNA on a Covaris® instrument in 1X TE Buffer, and/ or use the NEBNext FFPE DNA Repair Mix (NEB #M6630) after shearing and prior to library prep
	Adaptor is denatured	• When diluting NEBNext adaptors, use 10 mM Tris HCl (pH 7.5-8.0) with 10 mM NaCl • Keep the adaptor on ice until use
	Insufficient mixing	• Mix samples well with 80-90% of the total volume in the well or tube by pipetting up and down. Keep the tip in the liquid to avoid the formation of bubbles. • For enzymatic steps, follow the manual recommendations (usually 10 mix cycles) • Try to avoid losing sample in the pipette tip or on the source tube during transfer
	SPRI beads have dried out before elution	• Add Elution Buffer and mix before the beads turn lighter brown and start cracking • For additional tips about SPRI beads view our video
	Incomplete ethanol removal during SPRI bead wash	• Quickly spin the tube after the last ethanol wash at each SPRI bead step, keep the tube on the magnet and remove residual ethanol with a p10 tip • For additional tips about SPRI beads view our video (neb.com/tools-and-resources/video-library)
	SPRI bead sample loss	• Mix slowly to avoid droplets clinging to the inside of the tip, which may not combine with the sample before the tip is ejected. Dispense the last mix slowly into the sample tube so that the liquid stays together. Wait 1 second before pushing the pipette to the second stop. • When removing the supernatant, take care not to remove any beads. Check your tip over a white piece of paper. If beads are visible, dispense everything back into the tube and allow beads to resettle. • For additional tips about SPRI beads view our video
	Sample storage after A-tailing	• Avoid prolonged storage of sample before moving to ligation. If sample inputs are low, avoid overnight storage and move immediately from end prep to adaptor ligation.
	Adaptor self-ligation (Adaptor dimer formation)	• Do not add adaptor to the ligation master mix. This can cause increased adaptor dimer formation. • For best results, add the adaptor to the sample, mix and then add ligase master mix and ligation enhancer
	Ligation incubation temperature is too warm	• If ligation incubation occurs above 20˚C the DNA ends may breathe, which could reduce ligation
Adaptor Dimer Formation (sharp 127 bp peak on Bioanalyzer)		• To recover the samples, repeat the bead cleanup using a 0.9 x bead ratio.
	Adaptor concentration too high	• Optimize adaptor dilution based on your sample input, quality and type using an adaptor titration experiment • Adaptor titration may need to be repeated if the source of the sample input changes (e.g., extraction method, tissue type, etc.)
	Adaptor self-ligation (Adaptor Dimer formation)	• Do not add adaptor to ligation master mix. This can cause increased adaptor dimer formation. • For best results, add adaptor to sample, mix and then add ligase master mix and ligation enhancer. Mix again.
Adaptor or primers remaining after PCR (e.g., visible on Bioanalyzer or similar instrument after PCR)	Excess adaptor or primer used or inefficient cleanup	• Perform another 0.9 x SPRI cleanup
Overamplification (Once PCR primers are depleted, library fragments will become single stranded and/ or form heteroduplexes. These appear as high molecular weight fragments on a Bioanalyzer or similar instrument)	Too many PCR Cycles	• We recommend starting with the number of PCR cycles recommended in the product manual. The ideal number of PCR cycles for your samples may vary. • Reduce the number of PCR cycles if you are seeing overamplification • Data quality may be compromised if overamplified libraries are sequenced
	Not enough PCR primer	• Check primer concentration and ensure that you are adding the primer volume recommended in the manual • Store primers at the correct temperature to prevent degradation • Data quality may be compromised overamplified libraries are sequenced
	Too much input DNA	• The higher the input of template for the PCR, the sooner the primers will be depleted • NEBNext adaptor and PCR primers require a minimum of 3 PCR cycles. If you cannot further reduce the number of PCR cycles, consider a size selection step, or using only a fraction of the ligated library as input for PCR.

DNA Sample Input Guidelines

Integrity of DNA

- The quality of the input material directly affects the quality of the library. Absorbance measurements can be used as an indication of DNA purity. Ideally, the ratio of the absorbance at 260 nm to 280 nm should be between 1.8 – 2.0. However, measurements can be affected by the presence of RNA or small nucleic acid fragments. A DNA Integrity Number can be determined using the Agilent TapeStation® or similar instrumentation, and qPCR-based methods can also provide a measurement of DNA integrity.

Quantitation of DNA

- It is important to quantify accurately the DNA sample prior to library construction. Fluorescence-based detection which utilizes dsDNA-specific dyes, such as the Qubit® from Thermo Fisher Scientific®. Qubit is more accurate than UV spectrometer-based measurements, as the presence of RNA or other contaminants can result in overestimation of the amount of the DNA sample by the latter. This can result in use of non-optimal adaptor dilutions and numbers of PCR cycles, compromising library prep efficiency.

RNA Sample Input Guidelines

Integrity of RNA

- We recommend determining the RNA sample input using the RNA Integrity Number (RIN) estimated by the Agilent TapeStation or similar instrumentation. Ideally the RNA sample will have a RIN value of 7 or higher but NEBNext RNA products are compatible for use even with samples with low RIN values.
- RNA should be completely free of DNA, and DNA digestion of the purified RNA using RNase-free DNase I (such as that provided with the Monarch Total RNA Miniprep Kit) is recommended

Quantitation of RNA

- It is important to quantify accurately the RNA sample prior to library construction. The concentration can be estimated with the Agilent Bioanalyzer or similar instrumentation, using a pico or nano chip. Alternatively, RNA concentration can be determined by measuring the absorbance at 260 nm (A_{260}) in a spectrophotometer such as a NanoDrop®. Note that free nucleotides or organic compounds used in some RNA extraction methods also absorb UV light near 260 nm and will cause an over-estimation of RNA concentration.

Library Quantitation

- Library quantity and quality can be determined using the Agilent TapeStation or similar instrumentation, or qPCR-based methods. The NEBNext Library Quant Kit for Illumina is an ideal method for highly accurate library quantitation, that is especially important for PCR-free workflows.

Bead-based Clean-ups and Size Selection

- Be sure to vortex the beads well just before use. They should form a uniform suspension. If beads have settled for a long time period without being agitated a tight bead sediment can form. When beads have not been used for several weeks, plan for extra time for bead vortexing and agitation.
- Do not over-dry the beads. Beads should still be dark brown and glossy looking when eluting. Over-drying can make resuspension difficult and reduce yield.
- Take care not to remove beads after separation. If beads are accidentally aspirated, dispense everything back, allow the beads to settle and then try again.
- Remove all of the supernatant after the bind step. After removing most of the liquid with a p200 pipette, aspirate any remaining drops with a p10 pipette if necessary. Incomplete supernatant removal can cause leftover adaptor dimer or PCR primers to remain in the libraries.
- Bead ratios for cleanup and size selection after NEBNext ligation steps are appropriate just for the ligation step. Different ratios would apply if size selection is done after PCR or at any other step in the workflow.
- When adding beads to the sample, aspirate slowly to make sure the correct volume of beads is drawn into the tip. Remove any droplets of beads from the outside of the tip and make sure you dispense the full volume into the sample.

Indices/Barcodes

- When using a subset of the indices supplied in a kit, or using indices from more than one kit, it is important to optimize the combination of indices used, in order to ensure balanced sequencing reads. We provide recommendations for NEBNext index combinations in the manuals for NEBNext Oligos products.
- For index primers provided in vials, open only one vial at a time, to minimize the risk of contamination
- Be sure to change pipette tips for each index primer
- For 96-well plate formats, NEBNext index primers are provided in single-use plates with pierceable foil lids. To avoid risk of contamination, do not pipette from a well more than one time.

Labeling with SNAP-tag® Technology-Troubleshooting Guide

Application	Problem	Cause	Solution
Cellular Labeling	No labeling	Fusion protein not expressed	• Verify transfection • Check expression of fusion protein via Western blot or SDS-PAGE with an appropriate fluorescent substrate
	Weak labeling	Poor expression and/or insufficient exposure of fusion protein to substrate	• Increase substrate concentration • Increase incubation time
		Rapid turnover of fusion protein	• Analyze samples immediately or fix cells directly after labeling • Label at lower temperature (4°C or 16°C)
	High background	Non-specific binding of substrates	• Reduce substrate concentration and/or incubation time • Allow final wash step to proceed for up to 2 hours • Include fetal calf serum or BSA during labeling
	Signal strongly reduced after short time	Instability of fusion protein	• Fix cells • Switch tag from N-terminus to C-terminus or vice versa
		Photobleaching	• Add commercially available anti-fade reagent • Reduce illumination time and/or intensity
Labeling in Solution	Precipitation	Insoluble fusion	• Test from pH 5.0 to 10.0 • Optimize salt concentration [50 to 250 mM] • Add 0.05 to 0.1% Tween 20
	Weak or no labeling	Exhaustive labeling has not been achieved	• Increase incubation time to 2 hrs at 25°C or 24 hrs at 4°C • Reduce the volume of protein solution labeled • Check expression of fusion protein via SDS-PAGE with an appropriate fluorescent substrate
	Loss of activity	Instability of fusion protein	• Reduce labeling time • Decrease labeling temperature (4°C or 16°C)

Cellular Imaging & Analysis FAQs

Q. How does SNAP-tag® labeling differ from using GFP fusion proteins?

A. GFP and SNAP-tag are both valuable technologies used to visualize proteins in live cells. GFP is an intrinsically fluorescent protein derived from *Aequorea victoria* while SNAP-tag is derived from hAGT, a human DNA repair protein. In contrast to GFP, the fluorescence of SNAP-tag fusions can be readily turned on with the addition of a variety of fluorescent probes added directly to the culture media. Substituting different fluorophores or other functionalities (biotin, magnetic beads, blocking agents) requires no new cloning or expression, merely incubation of the appropriate substrate with cells, cell lysates or recombinant proteins.

Q. What is the difference between SNAP- and CLIP-tag™?

A. SNAP-tag and CLIP-tag are both derived from O6 -alkylguanine-DNA-alkyltransferase (hAGT). SNAP-tag recognizes O^6-labeled benzylguanine substrates while CLIP-tag recognizes O^2-labeled benzylcytosine substrates. Each tag transfers the label from the substrate to itself, resulting in specific covalent labeling. In creating the tags, hAGT has been engineered to no longer interact with DNA, but rather with derivatives of the free benzylguanine or benzylcytosine substrates. The tags exhibit no cross-reactivity with one another, enabling researchers to simultaneously label fusion proteins containing SNAP- and CLIP-tags with different fluorophores in live cells.

Q. Can I clone my protein as a fusion to the N- or C-terminus of the tags?

A. Yes. SNAP- and CLIP-tags can be fused to either the N- or C-terminus of a protein of interest. However, to label surface proteins on the outside of cells, the SNAP-tag or CLIP-tag must be cloned so that it is oriented to the extracellular surface of the plasma membrane. In this orientation, the tag is accessible to its fluorophore conjugated substrate.

Q. Are the substrates toxic to cells?

A. No toxicity has been noted by proliferation or viability assays when using up to 20 µM substrate for 2 hours. Most of the substrates can be incubated with cells for 24 hours up to a concentration of 20 µM without significant toxicity.

Q. How stable is the labeled protein in mammalian cells?

A. The stability of the tagged protein in the cell is dependent upon the stability of protein of interest. Labeled SNAP-tag fusion protein has been detected for up to 2 days in mammalian cells.

Q. Are SNAP-tag substrates stable to fixation?

A. Yes. SNAP-tag substrates are derived from organic fluorophores which are stable to fixation. Fluorescently-labeled SNAP-tag fusion proteins do not lose signal intensity in contrast to some GFP spectral variants. After labeling the SNAP-tag fusion proteins, the cells can be fixed with standard fixation methods such as para-formaldehyde, ethanol, methanol, methanol/acetone etc. without loss of signal.

Q. What conditions are recommended for SNAP-tag labeling *in vitro*?

A. The SNAP-tag labeling reaction is tolerant of a wide range of buffers. The requirements of the fusion partner should dictate the buffer selected. The following buffer guidelines are recommended: pH between 5.0 and 10.0, monovalent salts (e.g. sodium chloride) between 50 mM and 250 mM and at least 1 mM DTT. Non-ionic detergents can be added to 0.5% v/v if required, but SDS and other ionic detergents should be avoided entirely because they inhibit the activity of the SNAP-tag. Metal chelating reagents (e.g., EDTA and EGTA) also inhibit SNAP-tag activity and should be avoided.

Lambda Map

48,502 base pairs
GenBank Accession #: NC_001416

For ordering information, see Cloning Plasmids and DNAs in DNA Modifying Enzymes & Cloning Technologies.

There are no restriction sites for the following:

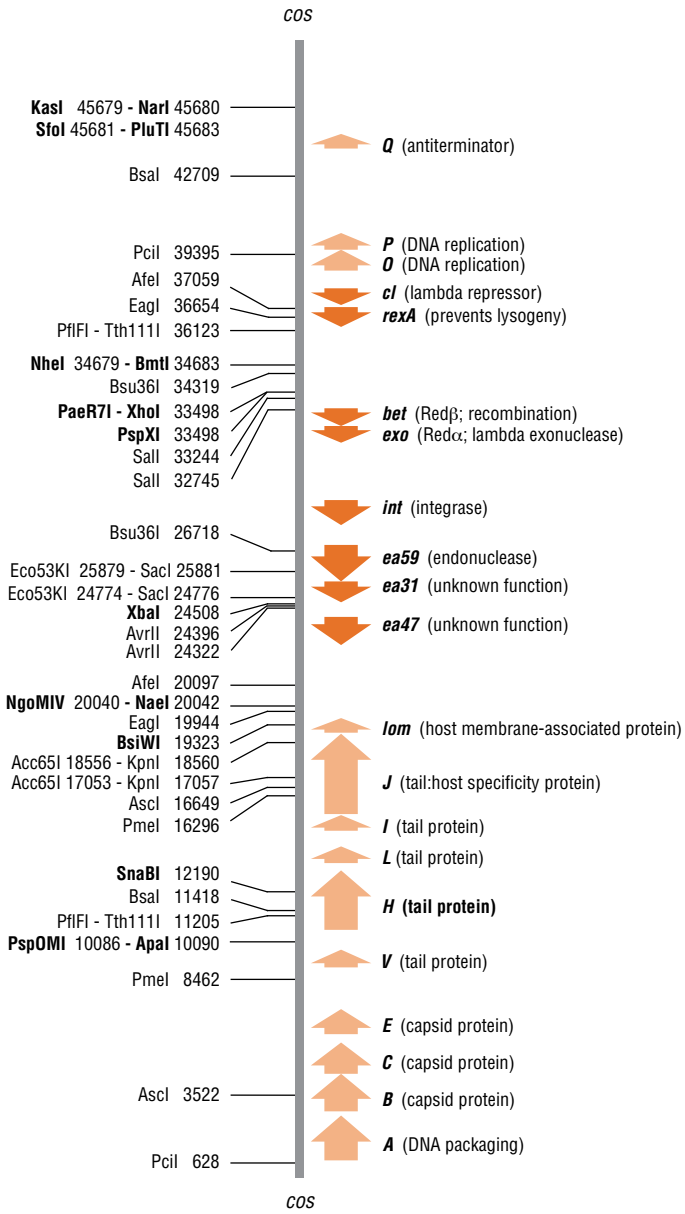
AbsI(x), AsiSI, FseI, MauBI(x), MreI(x), NotI, PacI, SfiI, SpeI, SrfI, SwaI

Lambda (λ) is a large, temperate *E. coli* bacteriophage with a linear, largely double-stranded DNA genome (1-5). At each end, the 5´ strand overhangs the 3´ strand by 12 bases. These single-stranded overhangs are complementary and anneal to form a cos site following entry into a host cell. Once annealed, the genome is a circular, completely double-stranded molecule which serves as a template for rolling-circle replication.

Many laboratory strains of lambda are derivatives of the strain λ *cI*857*ind*1 *Sam*7, which contains four point mutations relative to the wild type strain. The ind1 mutation in the cI gene creates a new HindIII site at 37584 not present in the wild type. All lambda products sold by NEB are λ *cI*857*ind*1 *Sam*7.

Numbering of the genome sequence begins at the first (5´-most) base of the left end and continues rightward from late genes nu1 and A towards the early genes. The map below shows the positions of all known ORFs larger than 200 codons.

Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand.**



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M13mp18 Map

GenBank Accession #: X02513

Revised sequence file available at www.neb.com.
For ordering information, see Cloning Plasmids and DNAs in DNA Modifying Enzymes & Cloning Technologies.

There are no restriction sites for the following:

AatII, AbsI(x), AcuI, AflII, AgeI, AhdI, AjuI(x), ApaI, ApaLI, AscI, AsiSI, AvrII, BbsI, BcgI, BciVI, BclI, BlpI, BmgBI, BmtI, BplI(x), BsaI, BsgI, BsiWI, BspEI, BspQI, BssHII, BssSI, BstAPI, BstBI, BstEII, BstXI, BstZ17I, EagI, EcoNI, EcoO109I, EcoRV, FseI, FspAI(x), HpaI, KflI(x), MauBI(x), MfeI, MluI, MreI(x), MteI(x), NcoI, NheI, NmeAIII, NotI, NruI, NsiI, PaeR7I, PaqCI, PasI(x), PflFI, PflMI, PfoI(x), PmeI, PmlI, PpuMI, PshAI, PspOMI, PspXI, PsrI(x), RsrII, SacII, SanDI, SapI, ScaI, SexAI, SfiI, SgrAI, SgrDI(x), SpeI, SrfI(x), StuI, StyI, Tth111I, XcmI, XhoI, ZraI

(x) = enzyme not available from NEB

Feature	Description	Coordinates
gene II	replication	6848-831 (cw)
gene X	replication	496-831
gene V	replication	843-1106
gene VII	minor coat protein	1108-1209
gene IX	minor coat protein	1206-1304
gene VIII	major coat protein	1301-1522
gene III	minor coat protein	1578-2852
gene VI	minor coat protein	2855-3193
gene I	phage assembly	3195-4241
gene XI (I*)	phage assembly	3915-4241
gene IV	phage assembly	4219-5499
ori	M13 origin (+) of replication	5487-5867
*lacZ*α	for α-complementation	6216-6722
MCS	multiple cloning site	6230-6286

(cw) = clockwise

M13 is a filamentous *E. coli* bacteriophage specific for male (F factor-containing) cells. Its genome is a circular, single-stranded DNA molecule 6407 bases in length, and contains 10 genes. A double-stranded form (RF) arises as an intermediate during DNA replication.

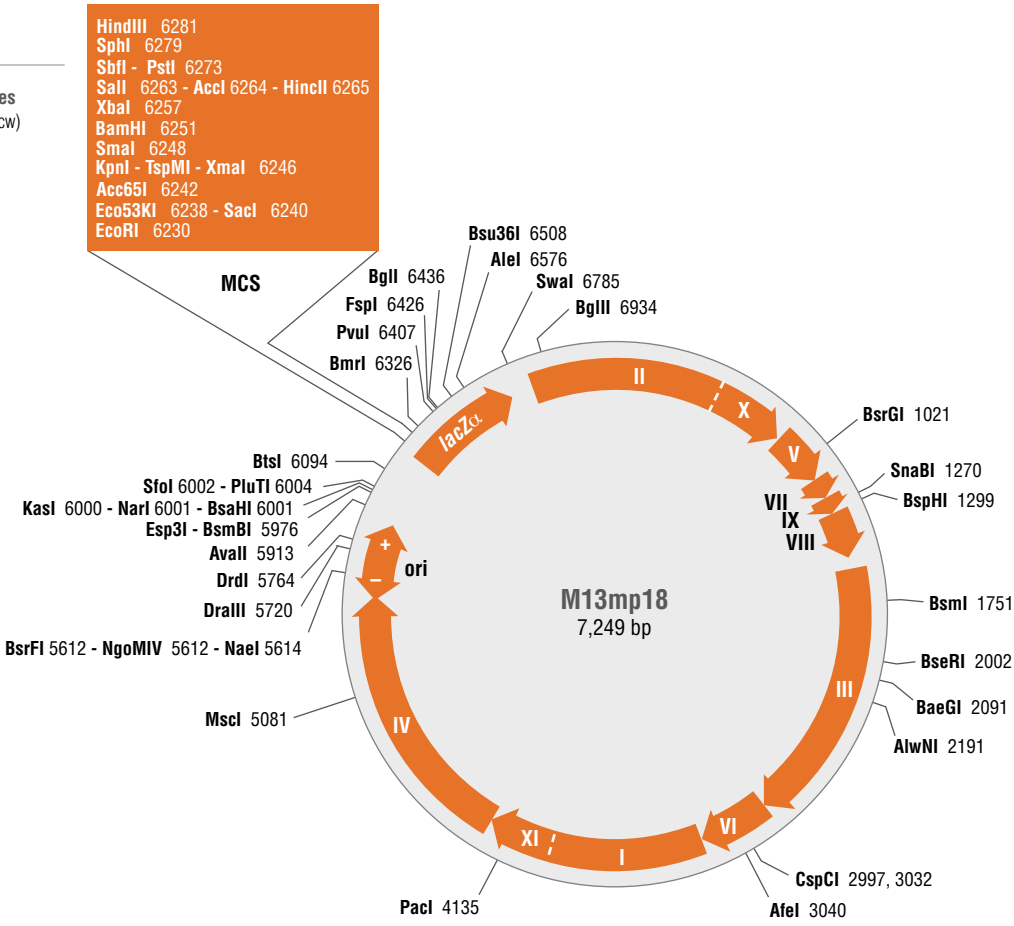
The M13mp phage vectors, derived from M13, contain the *lacZ*α gene and differ from each other by the cloning sites embedded within it. The location of cloning sites inside this gene allows screening for insertions using α-complementation. The map of M13mp18, whose multiple cloning site (MCS) was later employed to construct the plasmid pUC19, is shown below. M13mp19 is identical to M13mp18 except that the MCS region (6231-6288) is inverted.

The complete nucleotide sequences of M13mp18 and M13mp19 have been determined at New England Biolabs (1), resulting in several nucleotide changes relative to the previous sequence data (2,3).

Enzymes with unique restriction sites are shown in **bold** type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

M13 origin of replication arrows indicate the direction of synthesis of both the (+) and (–) strands.



References

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pBR322 Map

GenBank Accession #: J01749

For ordering information, see Cloning Plasmids and DNAs in DNA Modifying Enzymes & Cloning

There are no restriction sites for the following:

AbsI(x), Acc65I, AflII, AgeI, AjuI(x), AleI, AloI(x), ApaI, ArsI(x), AscI, AsiSI, AvrII, BaeI, BarI(x), BbvCI, BclI, BglII, BlpI, BmgBI, BpiI(x), BsaXI, BseRI, BsiWI, BsrGI, BssHII, BstBI, BstEII, BstXI, Bsu36I, CspCI, DraIII, Eco53KI, FalI(x), FseI, HpaI, KflI(x), KpnI, MauBI(x), MfeI, MluI, MreI(x), MteI(x), NcoI, NotI, NsiI, PacI, PaeR7I, PaqCI, PasI(x), PmeI, PmlI, PsiI, PspOMI, PspXI, PsrI(x), RsrII, SacI, SacII, SbfI, SexAI, SfiI, SgrDI(x), SmaI, SnaBI, SpeI, SrfI, StuI, SwaI, TspMI, XbaI, XcmI, XhoI, XmaI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
tet (Tc^R)	86-1276	pSC101
bla (Ap^R)	4153-3293	*Tn3*
rop	1915-2106	pMB1
origin	3122-2534	pMB1

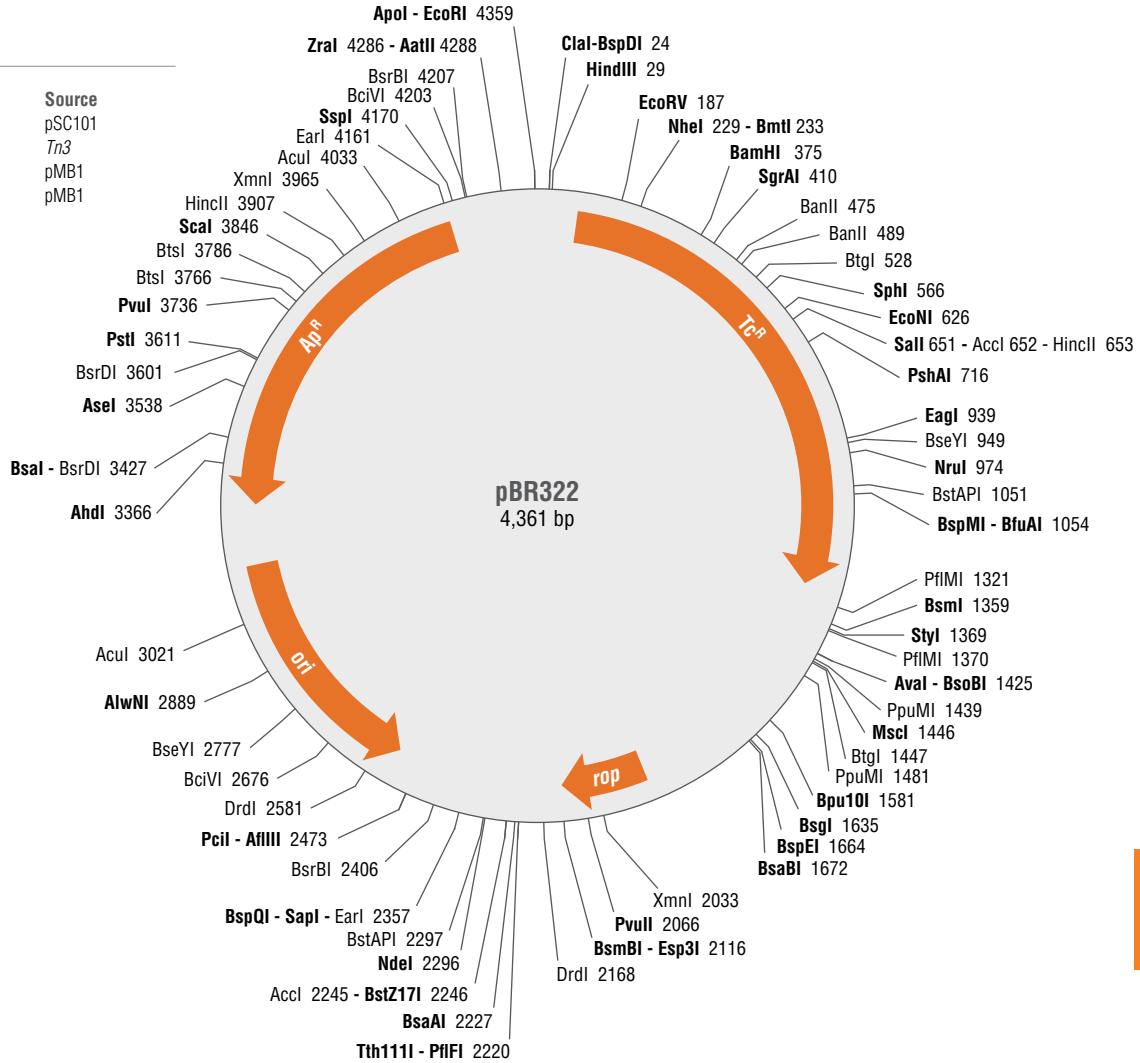
ori = origin of replication
Ap = ampicillin
Tc = tetracycline

pBR322 is an *E. coli* plasmid cloning vector containing the origin of replication from pMB1 (a plasmid in the ColE1 compatibility group; 1–3). The *rop* gene product, which regulates plasmid replication by stabilizing the interaction between RNAI and RNAII transcripts, maintains the copy number at about 20 per cell. However, pBR322 can be amplified with chloramphenicol or spectinomycin (4).

Enzymes with unique restriction sites are shown in **bold** type and enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence.



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(3) Watson, N. (1988) *Gene*, 70, 399–403.
(4) Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*, (2nd ed.), Cold Spring Harbor, Cold Spring Harbor Laboratory Press.

pKLAC2 Map

GenBank Accession #: EU196354

Sequence file available at www.neb.com.
For more information, see the *K.lactis* Protein Expression Kit (NEB #E1000).

There are no restriction sites for the following:

AatII, AbsI(x), Acc65I, AfeI, AflII, ApaI, AscI, AsiSI, AvrII, BbvCI, BlpI, Bpu10I, BsiWI, FseI, FspAI(x), KflI(x), KpnI, MauBI(x), MluI, MreI(x), MscI, MteI(x), PacI, PaqCI, PasI(x), PmeI, PmlI, PspOMI, PspXI, PsrI(x), RsrII, SfiI, SgrAI, SpeI, SrfI, SwaI, ZraI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
expression region:		
α-mating factor		
leader sequence	14-349	*K. lactis*
MCS	257-354	–
LAC4 TT region	371-953	*K. lactis*
AdH1 promoter region	1010-1712	*S. cerevisiae*
amdS	1713-3359	*A. nidulans*
LAC4 promoter region		
(5´ end)	4068-4648	*K. lactis*
origin	5102-5690	pMB1
bla (Ap^R)	6721-5861	Tn3
LAC4 promoter region		
(3´ end)	7475-9107	*K. lactis* (modified)

ori = origin of replication
Ap = ampicillin
TT = transcription terminator

pKLAC2 is an expression vector capable both of replication in *E. coli* and stable integration into the genome of the yeast *Kluyveromyces lactis* (1). It is designed for high-level expression of recombinant protein in *K. lactis* using the *K. lactis* Protein Expression Kit (NEB #E1000). pKLAC2 contains a universal multiple cloning site (MCS) that is compatible with all NEB expression systems.

In *E. coli*, it replicates using the pMB1 origin of replication from pBR322 (although the *rop* gene is missing) and carries the *bla* (Ap^R) marker for selection with ampicillin. Upon transformation of *K. lactis* GG799 competent cells (NEB #C1001), SacII- or BstXI-linearized pKLAC2 integrates into the *K. lactis* chromosome at the *LAC4* locus. Yeast transformants can be selected using the acetamidase selectable marker (*amdS*), which is expressed from the yeast *ADH1* promoter. Acetamidase expressed from pKLAC2 permits transformed cells to utilize acetamide as a sole nitrogen source on defined medium (2).

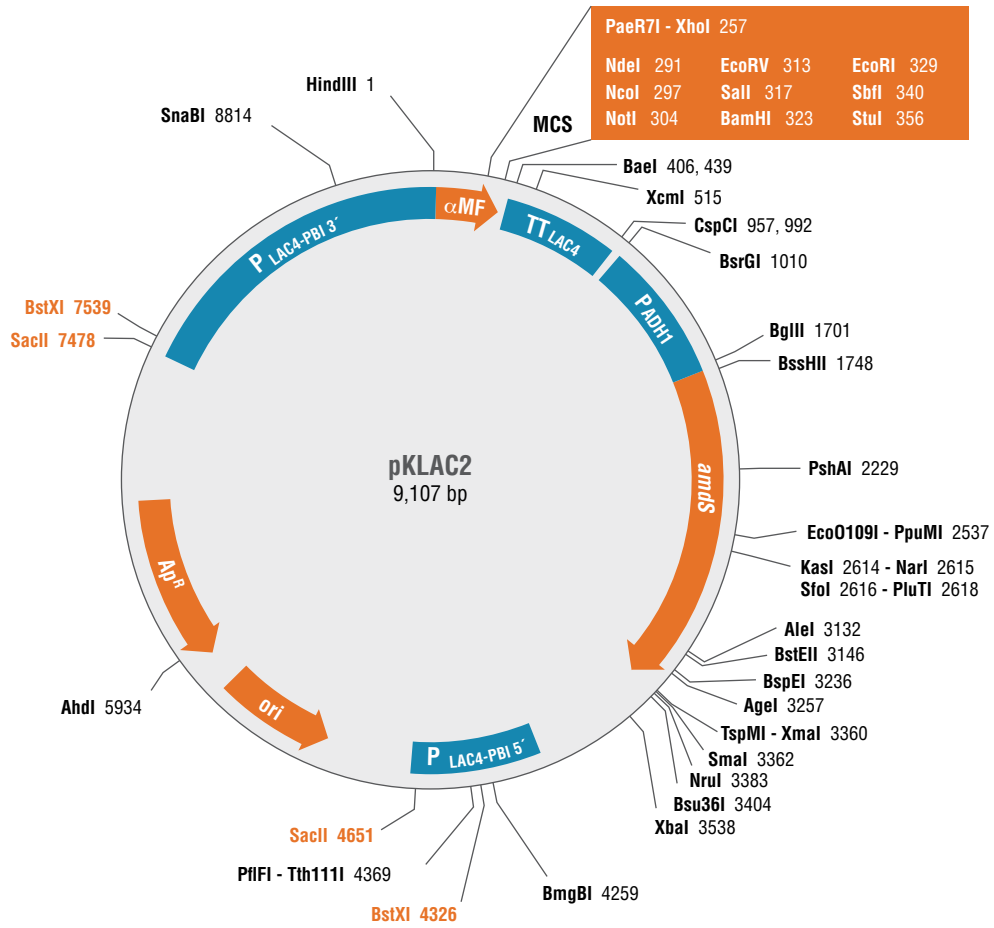
The multiple cloning site (MCS) is positioned to allow translational fusion of the K. lactis α-mating factor secretion domain (α-MF) to the N-terminus of the recombinant target protein. This directs the fusion protein to the general secretory pathway, but the α-MF domain is cleaved off in the Golgi apparatus by the Kex protease, resulting in secretion of the recombinant protein alone.

Expression of the recombinant fusion protein is driven by the *K. lactis LAC4* promoter, which has been modified to be transcriptionally silent in *E. coli* (1). This facilitates the cloning of proteins that are toxic to *E. coli*. This promoter is split such that when pKLAC2 is cleaved with SacII or BstXI, the recombinant protein and selectable marker are flanked by the two halves of the promoter. When these ends recombine with the *LAC4* promoter in the K. lactis chromosome, the result is integration of the recombinant fusion protein (driven by the *LAC4* promoter) and *amdS* upstream of the *LAC4* gene (driven by a duplicate copy of the *LAC4* promoter) (2).

Enzymes with unique restriction sites are shown in **bold** type and selected enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Components of coordinated regions are indented below the region itself.

pMB1 origin of replication coordinates include the region from the –35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. Promoter and transcription terminator coordinates represent cloned regions and not necessarily the precise functional elements.



References

(1) Colussi, P.A. and Taron, C.H. (2005) *Appl. Environ. Microbiol.*, 71, 7092–7098.

(2) van Ooyen, A.J. et al. (2006) *FEMS Yeast Res.*, 6, 381–392.

pMAL-c6T Map

Sequence file available at www.neb.com.
For ordering information, see Protein Expression & Purification.

There are no restriction sites for the following:

AatII, AbsI(x), Acc65I, AflII, AgeI, AjuI(x), AleI, ArsI(x), AscI, AsiSI, AvrII, BaeI, BarI(x), BbvCI, BmtI, BpiI(x), BsaAI, BseRI, BsmFI, BspDI, BsrGI, BstBI, BstZ17I, ClaI, CspCI, DraIII, EcoNI, FalI(x), FseI, FspAI(x), KfiI(x), KpnI, MauBI(x), MreI(x), MscI, MteI(x), NaeI, NcoI, NdeI, NgoMIV, NheI, NruI, NsiI, PacI, PaeR7I, PaqCI, PasI(x), PmeI, PmlI, PshAI, PspXI, PsrI(x), SacII, SexAI, SfiI, SgrAI, SmaI, SnaBI, SpeI, SphI, SrfI, StuI, StyI, SwaI, TspMI, XbaI, XhoI, XmaI, XmnI, ZraI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
lacI^q	80-1162	*E. coli*
Ptac	1405-1432	–
expression ORF	1527-2761	–
malE	1527-2721	*E. coli*
MCS	2722-2761	–
bla (ApR)	3101-3961	*Tn3*
origin	4049-4637	pMB1
rop	5007-5198	pMB1

pMAL-c6T is an *E. coli* plasmid cloning vector designed for recombinant protein expression and purification using the NEBExpress MBP Fusion and Purification System (NEB #E8201) (1–3). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322.

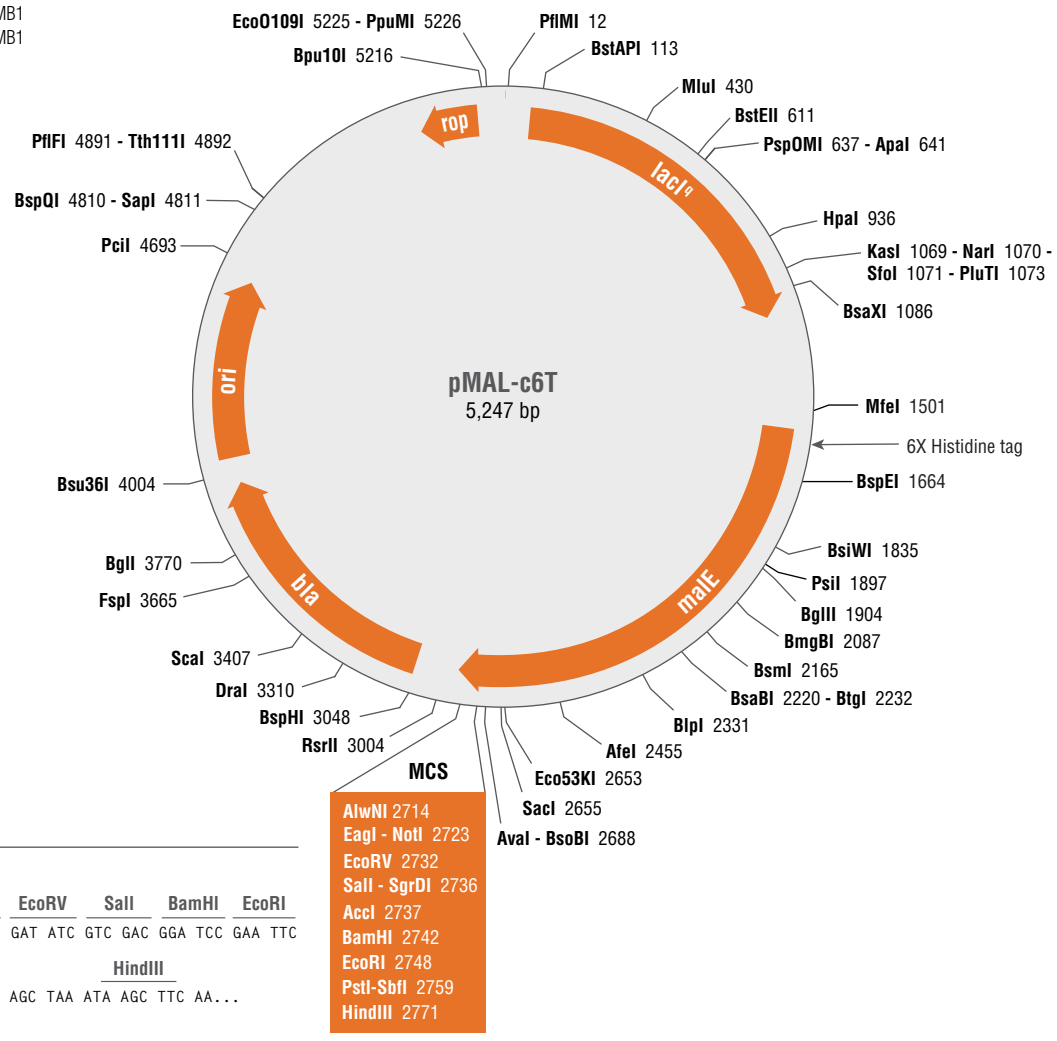
The multiple cloning site (MCS) is positioned to allow translational fusion of the *E. coli* maltose binding protein (MBP, encoded by the malE gene lacking its secretory signal sequence) to the *N*-terminus of the cloned target protein, thus resulting in an MBP-fusion protein localized in the cytoplasm. The pMAL-c6T vector contains a multiple cloning site (MCS) that is compatible with other NEB expression systems and is followed by stop codons in all three reading frames. In this vector, MBP has been engineered for tighter binding to amylose. This allows easy purification of the fusion protein, and the MBP domain can be subsequently removed using TEV Protease (NEB #P8112).

Transcription of the gene fusion is controlled by the inducible "tac" promoter (P_{tac}). Basal expression from P_{tac} is minimized by the binding of the Lac repressor, encoded by the *lacI*^{q} gene, to the *lac* operator immediately downstream of P_{tac}. A portion of the *rrnB* operon containing two terminators, derived from the vector pKK233-2, prevents transcription originating from P_{tac} from interfering with plasmid functions.

Enzymes with unique restriction sites are shown in **bold** type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

The pMB1 origin of replication includes the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point (labeled "ori") and the rop gene, which controls expression of the RNAII transcript. *bla* (Ap^R) gene coordinates include the signal sequence.



pMAL-c6T Polylinker:

```
                AlwNI      NotI/EagI   EcoRV     SalI      BamHI     EcoRI
5´ malE ...ATG CTG ATG GGC GGC CGC GAT ATC GTC GAC GGA TCC GAA TTC

            PstI/SbfI                          HindIII
CCT GCA GGT AATTAA ATA AGC TAA ATA AGC TTC AA...
```


References

(1) Guan, C. et al. (1987) *Gene*, 67, 21–30.

(2) Maina, C.V. et al. (1988) *Gene*, 74, 365–373.

(3) Riggs, P.D. (1992). In F.M. Ausubel, et al. (Eds.), *Current Prot. in Molecular Biol.* New York: John Wiley & Sons, Inc.

pMiniT 2.0 Map

Sequence available at www.neb.com
For more information, see the NEB PCR Cloning Kit (NEB #E1202, #E1203).

There are no restriction sites for the following:

AbsI(x), Acc65I, AccI, AflII, AgeI, AjuI(x), AleI, AloI(x), ApaI, ArsI(x), AscI, AsiSI, AvrII, BaeI, BanII, BarI(x), BbsI, BbvCI, BclI, BglII, BlpI(x), BmgBI, BmtI, BpiI(x), Bpu10I, BsaI, BsaAI, BsaBI, BseRI, BsgI, BsiWI, BsmFI, BsmI, BspDI, BspEI, BsrGI, BssHII, BstAPI, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, ClaI, CspCI, DraIII, Eco53kI, EcoNI, EcoO109I, EcoRV, FalI(x), FseI, FspAI(x), HinclI, HindIII, HpaI, KasI, KflI(x), KpnI, MauBI(x), MfeI, MluI, MreI(x), MscI, MteI(x), NaeI, NarI, NcoI, NgoMIV, NheI, NsiI, PasI(x), PflFI, PflMI, PfoI(x), PluTI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PsrI(x), PvuII, RsrII, SacI, SacII, SalI, SexAI, SfiI, SfoI, SgrAI, SgrDI(x), SmaI, SnaBI, SpeI, SphI, SrfI, StuI, StyI, SwaI, TspMI, Tth111I, XbaI, XcmI, XmaI

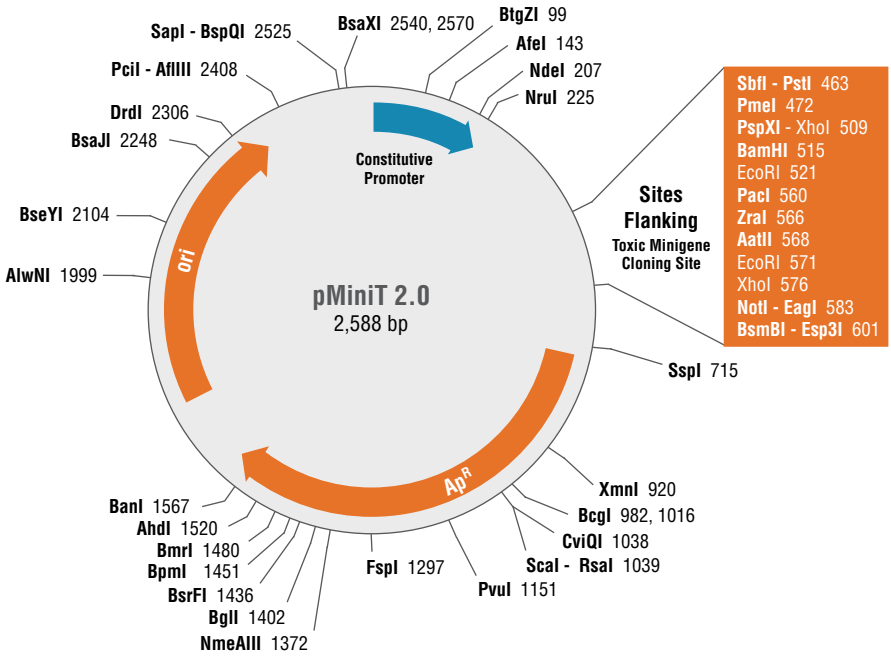
(x) = enzyme not available from NEB

Feature	Coordinates	Source
Constitutive promoter	1-214	pNK2138
SP6 promoter	479-496	SP6
Toxic minigene	541-549	–
Synthetic T7 promoter	619-602	T7
bla (Ap^R)	733-1593	*Tn3*
origin	1764-2352	pUC19

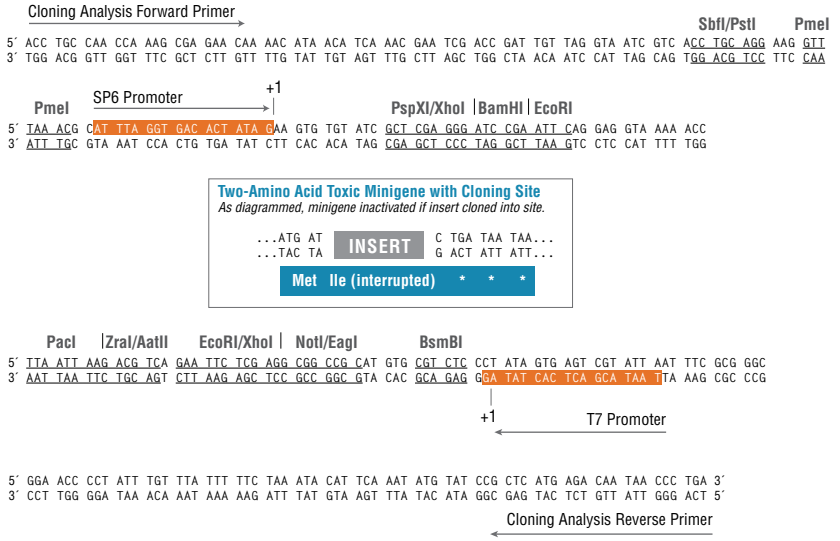
pMiniT 2.0 is an *E. coli* plasmid cloning vector designed for cloning blunt-ended or single-base overhang PCR products, or amplicons, using the NEB PCR Cloning Kit (NEB #E1202, #E1203). The pMiniT 2.0 also enables in vitro transcription using SP6 and T7 promoters. It is compatible with Golden Gate Assembly as the BsaI site has been removed from the Ampicillin resistance gene.

In *E. coli*, it replicates using the pMB1 origin of replication from pUC19 and carries the bla (Ap^R) marker for selection with ampicillin. pMiniT2.0 contains a toxic minigene that is under the control of a constitutive promoter. If the pMiniT 2.0 vector recircularizes without an insert, the toxic minigene it will cause lethal inhibition of protein synthesis and no colony will result. If the pMiniT 2.0 Vector carries an insert, a colony will grow.

The map shown below displays the construct formed if no insert is present. Unique restriction sites are shown in **bold**. Additional restriction sites that can be used for subcloning are also shown. Expanded box below shows location of sequencing primers, restriction sites for subcloning or linearization for *in vitro* transcription, RNA Polymerase promoter sequences and placement of insertion site within the toxic minigene. **Coordinates indicate position of cutsite on the top strand.**



Features within Sequence Flanking the Toxic Minigene/Cloning Site:



pNEB206A Map

Sequence file available at www.neb.com.

There are no restriction sites for the following:

AbsI(x), Acc65I, AccI, AfeI, AflII, AgeI, AjuI(x), AleI, AloI(x), ApaI, ArsI(x), AsiSI, AvaI, AvrII, BaeI, BarI(x), BbsI, BclI, BfuAI, BglII, BlpI, BmgBI, BmtI, BpiI(x), BsaAI, BsaBI, BsgI, BsiWI, BsmFI, BsmI, BsoBI, BspDI, BspEI, BspMI, BsrGI, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, BtgZI, ClaI, CspCI, DraIII, EagI, EcoNI, EcoRV, FaiI(x), FseI, FspAI(x), HincII, HpaI, KflI(x), KpnI, MauBI(x), MfeI, MluI, MreI(x), MscI, MteI(x), NaeI, NcoI, NgoMIV, NheI, NotI, NruI, NsiI, PaeR7I, PaqCI, PasI(x), PflFI, PflMI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PspXI, PsrI(x), RsrII, SacII, SalI, SexAI, SfiI, SgrAI, SgrDI(x), SmaI, SnaBI, SpeI, SphI, SrfI, StuI, StyI, SwaI, TspMI, Tth111I, XcmI, XhoI, XmaI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
*lacZ*α	505-146	–
cloning site	430-461	–
origin	1491-903	pUC19
bla (Ap^R)	2522-1662	Tn3

ori = origin of replication
Ap = ampicillin

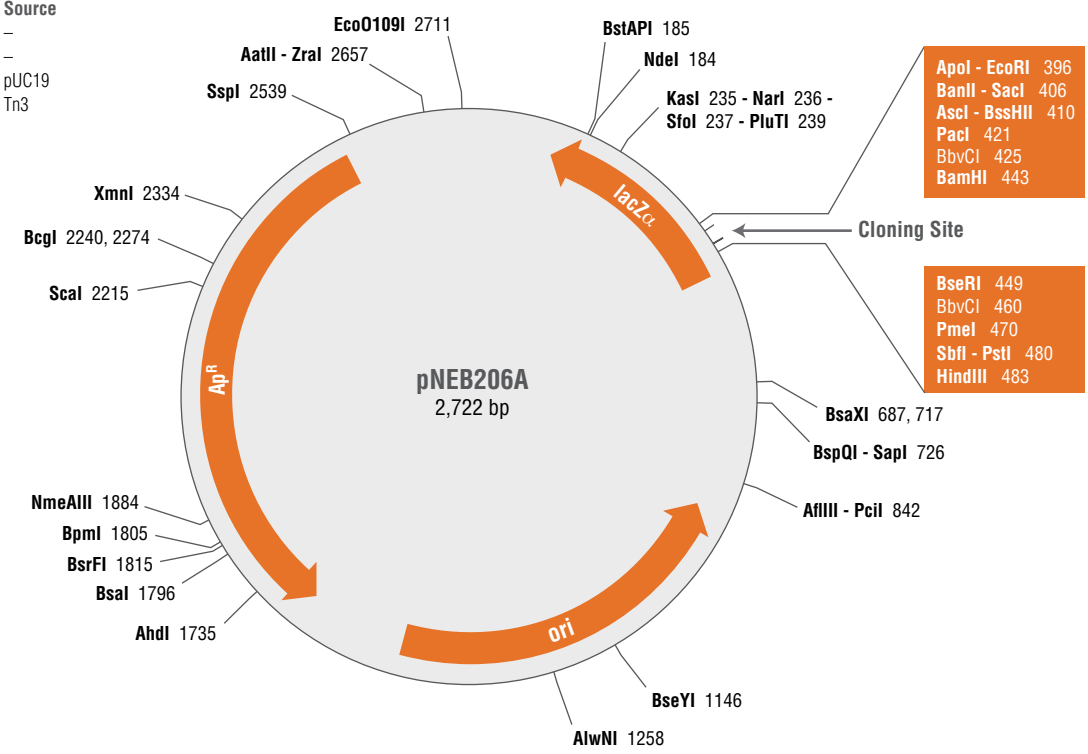
pNEB206A is an *E. coli* plasmid vector designed for fast and efficient cloning of PCR products to be used in conjunction with USER Enzyme (NEB #M5505; 1). It is derived from pNEB193 containing the high-copy pUC19 origin of replication and lacZα gene for screening of insertions at the cloning site using α-complementation (2).

The plasmid is supplied in a linearized form 2,706 bp in length (with bp 438-453 excised from the circular form), flanked by two noncomplementary 8-base 3´ overhangs at the intended cloning site. Amplification with deoxyuridine-containing primers and subsequent treatment (as defined in the protocol "Cloning with USER Enzyme" found on our website), results in PCR products with 5´ overhangs complementary to those in pNEB206A. These products can be directionally cloned into pNEB206A at high efficiency without the use of restriction enzymes or DNA ligase, forming recombinant circular molecules.

Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence. Cloning site coordinates include those bases in the circular form that are single-stranded in or missing from the supplied linear form.



pNEB206A (linearized form) cloning site:

```
        Eco53KI
          SacI        BssHII
   EcoRI            AscI       PacI       BbvCI
agtgAATTCGAGCTCAGGCGCGCCTTAATTAAGCTGAGGGAAAGT
tcacTTAAGCTCGAGTCCGCGCGGAATTAATTCGACT
      .          .          .          .
      400        410        420        430
...S  N  S  S  L  R  A  K  I  L  S  L  S  L
```

```
                                                  SbfI
                BbvCI    PmeI       PstI     HindIII
                  TCAGCGTTTAAACCCTGCAGGAAGCTTGGcgtaatcatggtcat
TACAGAGGAGTCGCAAATTTGGGACGTCCTTCGAACCgcattagtaccagta
                .          .          .          .          .
                460        470        480        490        500
                T  E  E  A  N  L  G  Q  L  F  S  P  T  I  M  T  M
```

*lacZ*α translational start

References
(1) Bitinaite, J. and Vaiskunaite, R. (2003) unpublished observations.
(2) Yanisch-Perron, C. et al. (1985) *Gene*, 33, 103–119.

pSNAP$_f$ Map

Sequence file available at www.neb.com.
For ordering information, see Cloning Vectors in Cellular Analysis.

There are no restriction sites for the following:

AbsI(x), AfeI, AflII, AjuI(x), AlfI(x), AloI(x), AsiSI, BaeI, BarI(x), BbvCI, BlpI, BplI(x), BsiWI, BsmBI, BspDI, BspEI, BstAPI, BstBI, BstEII, ClaI, EcoNI, Esp3I, FseI, FspAI(x), KflI(x), MauBI(x), MreI(x), MteI(x), PasI(x), PfoI(x), PshAI, PsrI(x), SexAI, SgrAI, SrfI, StuI, XcmI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
CMV promoter	251-818	–
expression region	915-1564	–
MCS1	915-965	–
SNAP$_f$	969-1514	–
MCS2	1515-1564	–
IRES	1910-2500	*ECMV*
Neo^R	2536-3339	*Tn5*
origin	4094-4682	pUC19
bla (Ap^R)	4853-5713	*Tn3*

ori = origin of replication
Ap = ampicillin
Neo = neomycin
IRES = internal ribosomal entry site

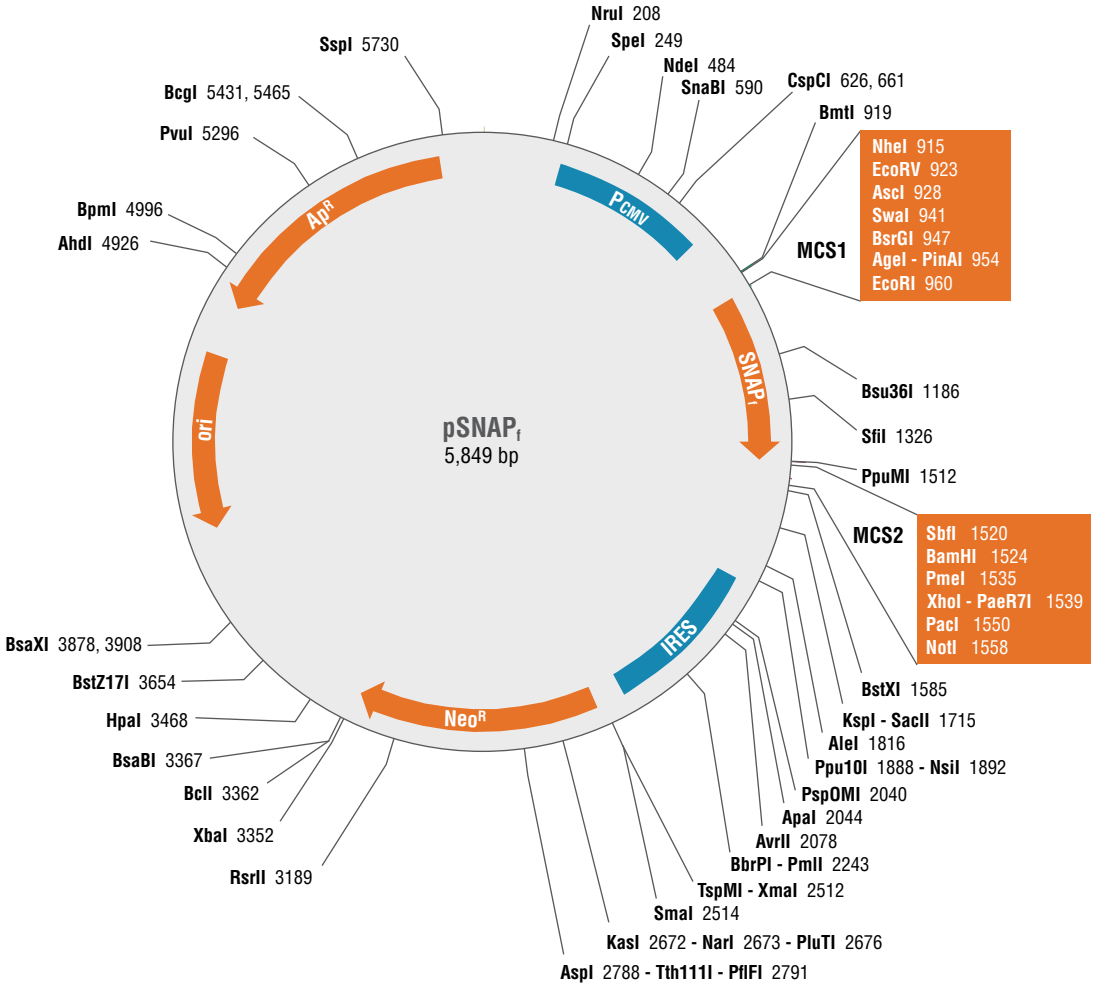
pSNAP$_f$ Vector is a mammalian expression plasmid intended for the cloning and stable or transient expression of SNAP-tag® protein fusions in mammalian cells. This plasmid encodes SNAP$_f$, a SNAP-tag protein, which is expressed under control of the CMV promoter. SNAPf is an improved version of the SNAP-tag which exhibits faster labeling kinetics. The SNAP-tag is a novel tool for protein research, allowing the specific, covalent attachment of virtually any molecule to a protein of interest. The SNAP-tag is a small protein based on human O6-alkylguanine-DNA-alkyltransferase (hAGT). SNAP-tag substrates are derivatives of benzyl purines and benzyl pyrimidines. In the labeling reaction, the substituted benzyl group of the substrate is covalently attached to the SNAP-tag. Use of this system involves two steps: sub-cloning and expression of the protein of interest as a SNAP-tag fusion, and labeling of the fusion with the SNAP-tag substrate of choice.

Codon usage of the gene is optimized for expression in mammalian cells. pSNAP$_f$ contains two multiple cloning sites to allow cloning of the fusion partner as a fusion to the N- or C-terminus of the SNAP-tag. The expression vector has an Internal Ribosome Entry Site (IRES) and a neomycin resistance gene downstream of the SNAP-tag for the efficient selection of stable transfectants.

Enzymes with unique restriction sites are shown in **bold** type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Component genes or regions of fusion ORFs are indented below the ORF itself.

pUC19 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap^R) gene coordinates include the signal sequence.



MCS1

```
     NheI        AscI        SwaI   BsrGI        EcoRI
...GCTAGC GATATCGGCG CGCCAGCATT TAAATCTGTA CAGACCGGTG AATTC
   CGATCG CTATAGCCGC GCGGTCGTAA ATTTAGACAT GTCTGGCCAC TTAAG...
```

MCS2

```
                                  XhoI
     SbfI       BamHI      PmeI        PacI        NotI
...CCTGCA GGCGGATCCG CGTTTAAACT CGAGGTTAAT TAATGAGCGG CCGC
   GGACGT CCGCCTAGGC GCAAATTTGA GCTCCAATTA ATTACTCGCC GGCG...
```

pTXB1 Map

Sequence file available at www.neb.com.
For ordering information, see the IMPACT Kit (NEB #E6901).

There are no restriction sites for the following:

Acc65I, AflII, AjuI(x), AleI, ArsI(x), AscI, AsiSI, AvrII, BaeI, BbvCI, BglII, BlpI(x), BmgBI, Bpu10I, BseRI, BspDI, BstBI, Bsu36I, ClaI, CspCI, Eco53KI, FalI(x), FseI, FspAI(x), HindIII, KflI(x), KpnI, MauBI(x), MscI, MteI(x), NcoI, NsiI, PacI, PaqCI, PasI(x), PmlI, PpuMI, PsrI(x), RsrII, SacI, SanDI(x), SbfI, SexAI, SfiI, SgrDI(x), SmaI, SnaBI, SrfI, TspMI, XmaI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
bla (Ap^R)	140-1000	*Tn3*
M13 origin	1042-1555	M13
origin	1666-2254	pMB1
rop	2814-2623	pMB1
lacI	4453-3371	*E. coli*
T7 promoter	5637-5654	T7
expression ORF	5725-6558	–
MCS	5722-5775	–
Mxe GyrA intein	5776-6369	*M. xenopi*
CBD	6400-6558	*B. circulans*

ori = origin of replication
Ap = ampicillin

pTXB1 is an *E. coli* plasmid cloning vector designed for recombinant protein expression, purification, and ligation using the IMPACT™ Kit (NEB #E6901) (1,2). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, pTXB1 also contains an M13 origin of replication.

The multiple cloning site (MCS) is positioned to allow translational fusion of the Mxe GyrA intein tag to the C-terminus of the cloned target protein (2,3). The chitin binding domain (CBD) from *B. circulans*, fused to the C-terminus of the intein, facilitates purification of the intein-target protein precursor.

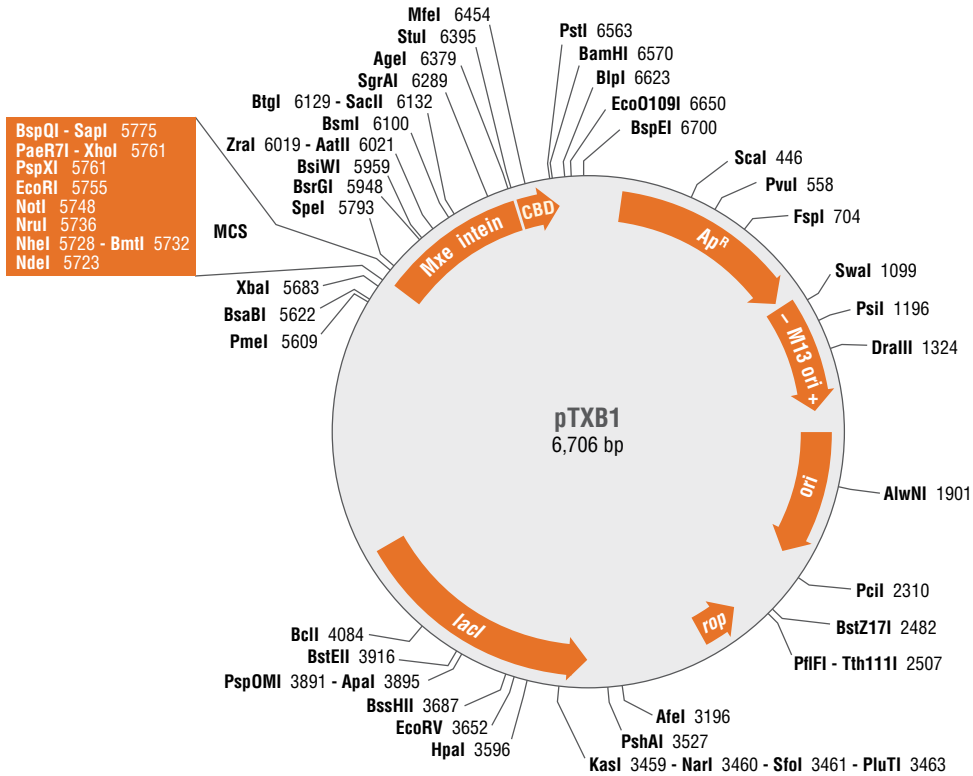
Transcription of the gene fusion is controlled by the inducible T7 promoter, requiring E. coli strains containing integrated copies of the T7 RNA polymerase gene [e.g., C2566 or BL21(DE3)] for expression. Basal expression from the T7 promoter is minimized by the binding of the Lac repressor, encoded by the lacI gene, to the lac operator immediately downstream of the T7 promoter (4). Translation of the fusion utilizes the translation initiation signal (Shine Dalgarno sequence) from the strongly expressed T7 gene 10 protein (φ10).

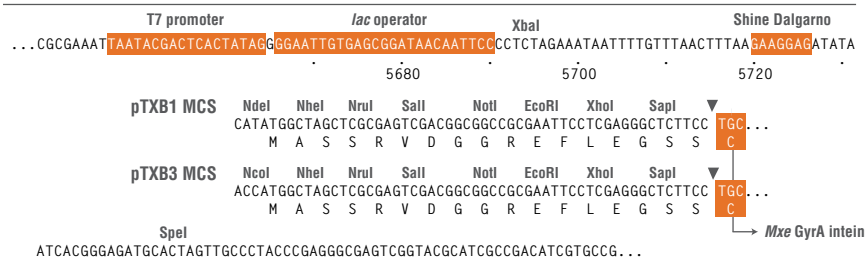
pTXB1 and pTXB3 are identical except for the MCS regions: pTXB1 contains an NdeI site, and pTXB3 an NcoI site, overlapping the initiating methionine codon of the intein fusion gene. The N-terminal cysteine residue ("Cys1") of the intein is shaded.

Enzymes with unique restriction sites are shown in **bold** type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Component genes or regions of fusion ORFs are indented below the ORF itself.

pMB1 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. For the M13 origin, the arrow shows the direction of synthesis of the (+) strand, which gets packaged into phage particles. *bla* (Ap^R) gene coordinates include the signal sequence.





References

(1) Chong, S. et al. (1997) *Gene*, 192, 271–281.
(2) Evans, T.C., Benner and Xu, M.-Q. (1998) *Protein Sci.*, 7, 2256–2264.
(3) Southworth, M.W. et al. (1999) *Biotechniques*, 27, 110–120.
(4) Dubendorff, J.W. and Studier, F.W. (1991) *J. Mol. Biol.*, 219, 45–59.

pTYB21 Map

Sequence file available at www.neb.com.
For ordering information, see the IMPACT Kit (NEB #E6901).

There are no restriction sites for the following:

AatII, AbsI(x), AflII, AgeI, AjuI(x), AscI, AsiSI, AvrII, BbvCI, BmgBI, BpiI(x), BseRI, BsiWI, BsmI, BspDI, Bsu36I, ClaI, CspCI, FalI(x), FseI, FspAI(x), KflI(x), MauBI(x), MreI(x), MteI(x), NruI, NsiI, PacI, PaeR7I, PaqCI, PasI(x), PpuMI, PspXI, PsrI(x), RsrII, SexAI, SfiI, SgrAI, SmaI, SnaBI, SrfI, TspMI, XhoI, XmaI, ZraI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
bla (Ap^R)	140-1000	*Tn3*
M13 origin	1042-1555	M13
origin	1666-2254	pMB1
rop	2814-2623	pMB1
lacI	4453-3371	*E. coli*
T7 promoter	5637-5654	T7
expression ORF	5725-7368	–
MCS	7301-7361	–
Sce VMA intein	5770-7299	*S. cerevisiae*
CBD	6595-6747	*B. circulans*

ori = origin of replication
Ap = ampicillin

pTYB21 is an *E. coli* plasmid cloning vector designed for recombinant protein expression and purification using the IMPACT™ Kit (NEB #E6901) (1,2). It contains the pMB1 origin of replication from pBR322 and is maintained at a similar copy number to pBR322; in addition, pTYB21 also contains an M13 origin of replication.

The multiple cloning site (MCS) is positioned to allow translational fusion of the *Sce* VMA intein tag to the N-terminus of the cloned target protein (2). The chitin binding domain (CBD) from *B. circulans*, facilitates purification of the intein-target protein precursor.

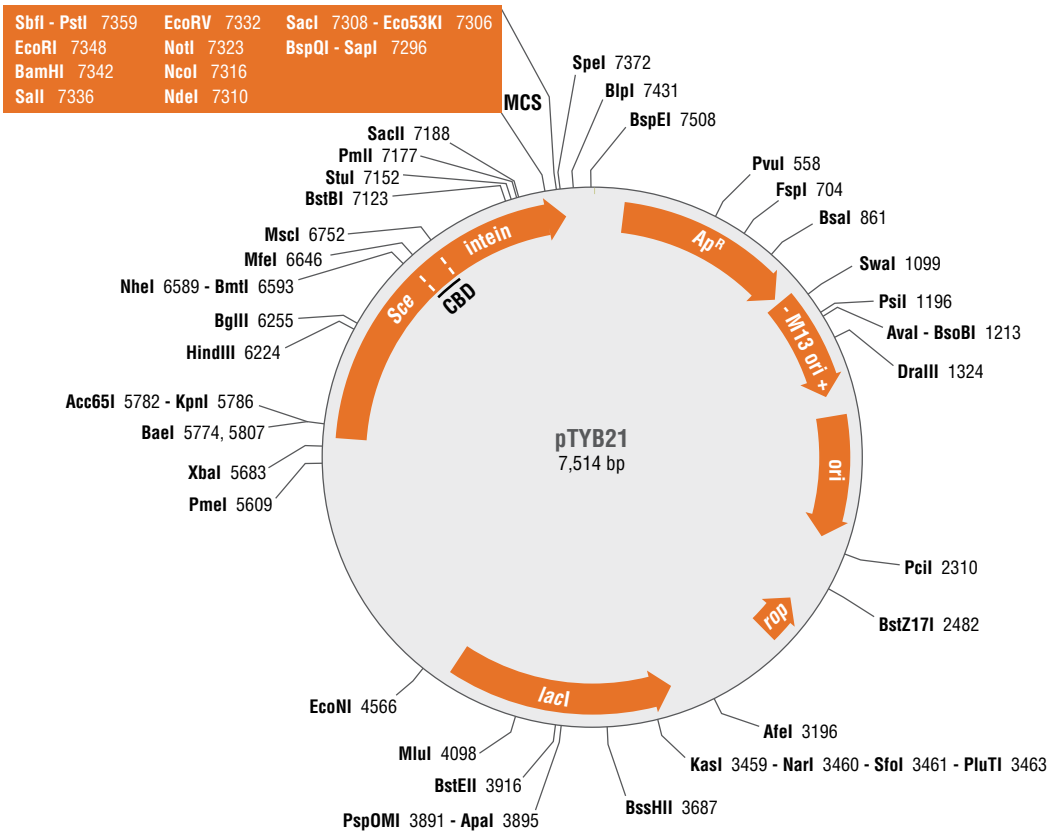
Transcription of the gene fusion is controlled by the inducible T7 promoter, requiring *E. coli* strains containing integrated copies of the T7 RNA polymerase gene [e.g., C2566 or BL21(DE3)] for expression. Basal expression from the T7 promoter is minimized by the binding of the Lac repressor, encoded by the *lacI* gene, to the *lac* operator immediately downstream of the T7 promoter (3). Translation of the fusion utilizes the translation initiation signal (Shine Dalgarno sequence) from the strongly expressed T7 gene 10 protein (φ10).

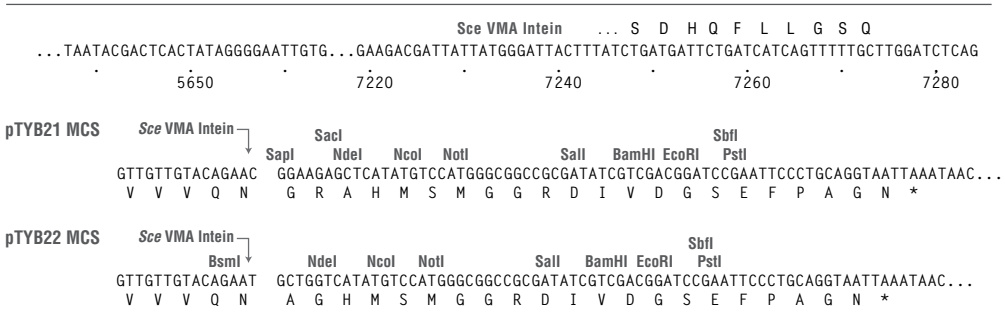
pTYB21 contains a SapI site which allows for cloning of a target gene without any extra amino acids. pTYB22 is identical to pTYB21 except for the MCS regions (see below). pTYB22 contains an NdeI site overlapping the initiating methionine codon of the intein fusion gene. pTYB21 differs from pTYB11 in that it contains a universal MCS that is compatible with all NEB expression systems.

Enzymes with unique restriction sites are shown in **bold** type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons. Component genes or regions of fusion ORFs are indented below the ORF itself.

pMB1 origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. For the M13 origin, the arrow shows the direction of synthesis of the (+) strand, which gets packaged into phage particles. *bla* (Ap^R) gene coordinates include the signal sequence.





References

(1) Chong et al. (1996) *J. Biol. Chem.*, 271, 22159–22168

(2) Chong et al. (1998) *NAR*, 26, 5109–5115.

(3) Dubendorff, J.W. and Studier, F.W. (1991) *J. Mol. Biol.*, 219, 45–59.

pUC19 Map

GenBank Accession #: L09137

For ordering information, see Cloning Plasmids and DNAs in DNA Modifying Enzymes & Cloning Technologies.

There are no restriction sites for the following:

AbsI(x), AfeI, AflII, AgeI, AjuI(x), AleI, AloI(x), ApaI, ArsI(x), AscI, AsiSI, AvrII, BaeI, BarI(x), BbsI, BbvCI, BclI, BglII, BlpI, BmgBI, BmtI, BplI(x), Bpu10I, BsaAI, BsaBI, BseRI, BsgI, BsiWI, BsmFI, BsmI, BspDI, BspEI, BsrGI, BssHII, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, BtgZI, ClaI, CspCI, DraIII, EagI, EcoNI, EcoRV, FalI(x), FseI, FspAI(x), HpaI, KflI(x), MauBI(x), MfeI, MluI, MreI(x), MscI, MteI(x), NaeI, NcoI, NgoMIV, NheI, NotI, NruI, NsiI, PacI, PaeR7I, PaqCI, PasI(x), PflFI, PflMI, PmeI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PspXI, PsrI(x), RsrII, SacII, SexAI, SfiI, SgrAI, SgrDI(x), SnaBI, SpeI, SrfI, StuI, StyI, SwaI, Tth111I, XcmI, XhoI

(x) = enzyme not available from NEB

Feature	Coordinates	Source
*lacZ*α	469-146	–
origin	1455-867	pMB1 (mutant)
bla (Ap[R])	2486-1626	*Tn3*

ori = origin of replication
Ap = ampicillin

pUC19 is a small, high-copy number E. coli plasmid cloning vector containing portions of pBR322 and M13mp19 (1). It contains the pMB1 origin of replication from pBR322, but it lacks the *rop* gene and carries a point mutation in the RNAII transcript (G 2975 in pBR322 to A 1308 in pUC19; 2). These changes together result in a temperature-dependent copy number of about 75 per cell at 37°C and > 200 per cell at 42°C (2,3). The multiple cloning site (MCS) is in frame with the *lacZ*α gene, allowing screening for insertions using α-complementation.

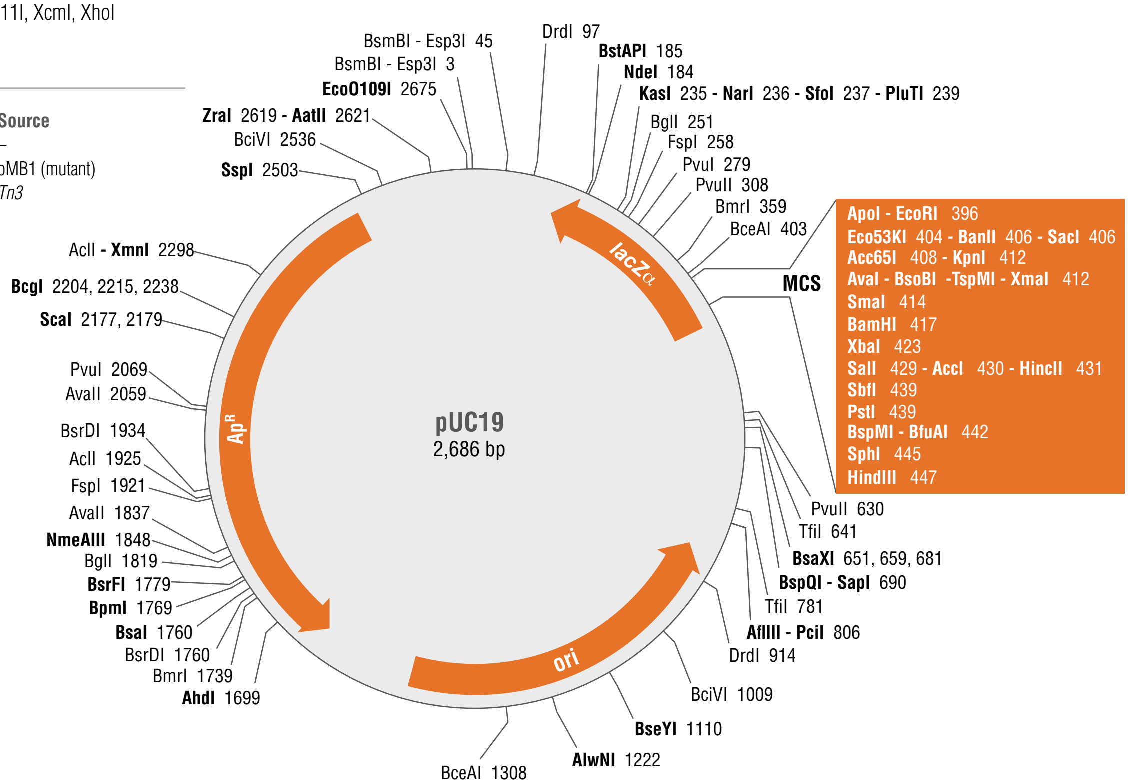
pUC18 is identical to pUC19 except that the MCS region (nt 397-454) is inverted.

pNEB193 is also identical to pUC19 except for the addition of several restriction endonuclease sites to the MCS. Its total length is 2713 bp.

Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. **Coordinates indicate position of cutsite on the top strand.**

Open reading frame (ORF) coordinates are in the form "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (Ap[R]) gene coordinates include the signal sequence.



pUC19 MCS

```
                SacI        SmaI         XbaI          SbfI
         EcoRI        KpnI        BamHI          SalI  PstI   SphI   HindIII
agtgAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGcgtaatcatggtcat
       .         .         .         .         .         .         .
      400       410       420       430       440       450       460
...S  N  S  S  P  V  R  P  D  E  L  T  S  R  C  A  H  L  S  P  T  I  M  T  M
                                   <------- lacZα translational start
```

pNEB193 MCS

```
                SacI        SmaI     BssHII          PacI                             SbfI
         EcoRI        KpnI        AscI        BamHI          XbaI  SalI    PmeI   PstI   SphI   HindIII
agtgAATTCGAGCTCGGTACCCGGGGGCGCGCCGGATCCTTAATTAAGTCTAGAGTCGACTGTTTAAACCTGCAGGCATGCAAGCTTGGcgtaatcatggtcat
       .         .         .         .         .         .         .         .         .         .
      400       410       420       430       440       450       460       470       480       490
...S  N  S  S  P  V  R  P  R  A  P  D  K  I  L  D  L  T  S  Q  K  F  R  C  A  H  L  S  P  T  I  M  T  M
                                                                     <------- lacZα translational start
```

References

(1) Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene*, 33, 103–119.

(2) Lin-Chao, S., Chen, W.-T. and Wong, T.-T. (1992) *Mol. Microbiol.*, 6, 3385–3393

(3) Miki, T. et al. (1987) *Protein Eng.*, 1, 327–332.

The Genetic Code

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V	
	Ala	Arg	Asn	Asp	Cys	Gln	Glu	Gly	His	Ile	Leu	Lys	Met	Phe	Pro	Ser	Thr	Trp	Tyr	Val	
5´	GCA	CGA	AAC	GAC	UGC	CAA	GAA	GGA	CAC	AUA	CUA	AAA	AUG	UUC	CCA	UCA	ACA	UGG	UAC	GUA	3´
	C	C	U	U	U	G	G	C	U	C	C	G		U	C	C	C		U	C	
	G	G						G		U	G	A			G	G	G			G	
	U	U						U			U				U	U	U			U	
		OR									OR					OR					
		AGA									UUA					AGC					
		G									G					U					

Second Position

First Position (5´end)	U		C		A		G		Third Position (3´ end)
U	UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys	U
	UUC		UCC		UAC		UGC		C
	UUA	Leu	UCA		UAA	Stop	UGA	Stop	A
	UUG		UCG		UAG	Stop	UGG	Trp	G
C	CUU	Leu	CCU	Pro	CAU	His	CGU	Arg	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	Gln	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	Lys	AGA	Arg	A
	AUG	Met	ACG		AAG		AGG		G
G	GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	Glu	GGA		A
	GUG		GCG		GAG		GGG		G

Termination Signals
UAA (Ochre)
UAG (Amber)
UGA (Opal)

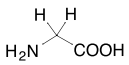
Single Letter Code

A	=	adenosine
C	=	cytidine
G	=	guanosine
T	=	thymidine
U	=	uridine
B	=	C or G or T
D	=	A or G or T
H	=	A or C or T
K	=	G or T
M	=	A or C
N	=	A or C or G or T
R	=	A or G
S	=	C or G
V	=	A or C or G
W	=	A or T
Y	=	C or T

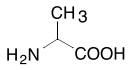
Amino Acid Structures

Each amino acid is accompanied by its three- and one-letter code, residue molecular weight (actual molecular weight minus water) and side-chain pK_a where appropriate.

Small

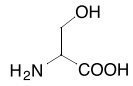


Glycine (Gly, G)
MW: 75.07

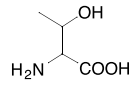


Alanine (Ala, A)
MW: 89.09

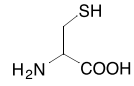
Nucleophilic



Serine (Ser, S)
MW: 105.09, pK_a ~16

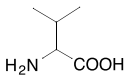


Threonine (Thr, T)
MW: 119.1, pK_a ~16

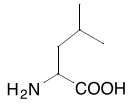


Cysteine (Cys, C)
MW: 121.2, pK_a = 8

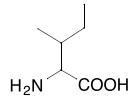
Hydrophobic



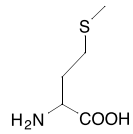
Valine (Val, V)
MW: 117.1



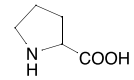
Leucine (Leu, L)
MW: 131.2



Isoleucine (Ile, I)
MW: 131.2

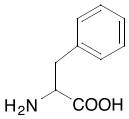


Methionine (Met, M)
MW: 149.2

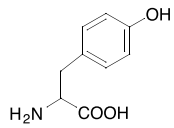


Proline (Pro, P)
MW: 115.1

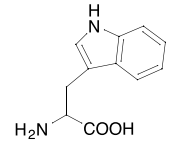
Aromatic



Phenylalanine (Phe, F)
MW: 165.2

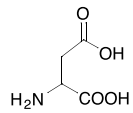


Tyrosine (Tyr, Y)
MW: 181.2, pK_a = 10.46

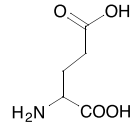


Tryptophan (Trp, W)
MW: 204.2

Acidic

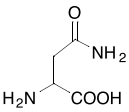


Aspartic Acid (Asp, D)
MW: 133.1, pK_a = 3.9

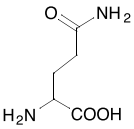


Glutamic Acid (Glu, E)
MW: 147.1, pK_a = 4.07

Amide

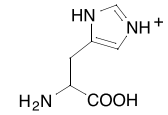


Asparagine (Asn, N)
MW: 132.1

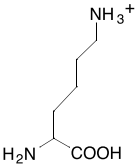


Glutamine (Gln, Q)
MW: 146.1

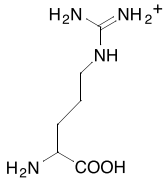
Basic



Histidine (His, H)
MW: 155.2, pK_a = 6.04



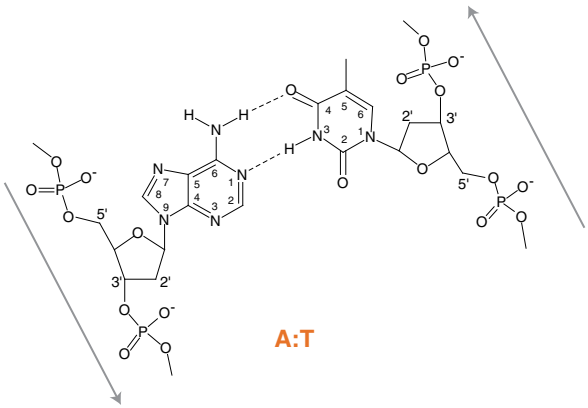
Lysine (Lys, K)
MW: 146.2, pK_a = 10.79

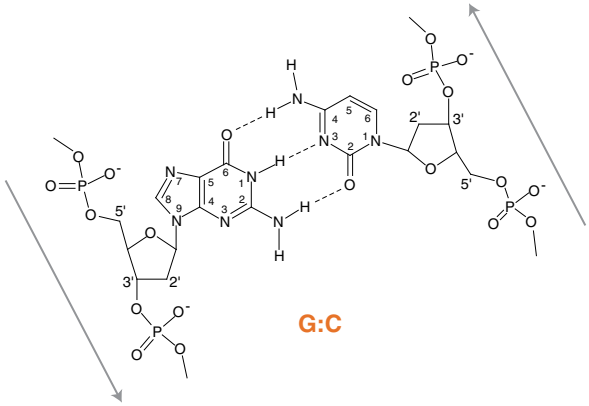


Arginine (Arg, R)
MW: 174.2, pK_a = 12.48

DNA Base Pairs

The structures of the adenosine:thymidine and guanosine:cytidine base pairs are shown in the context of the ribose phosphodiester backbones.
The numbering schemes of the ribose and nucleotide moieties are indicated. Arrows indicate the polarity of each strand from 5´ to 3´.





Nucleic Acid Data

Average weight of a DNA basepair (sodium salt) = 650 daltons
1.0 A_{260} unit ds DNA = 50 µg/ml = 0.15 mM (in nucleotides)
1.0 A_{260} unit ss DNA = 33 µg/ml = 0.10 mM (in nucleotides)
1.0 A_{260} unit ss RNA = 40 µg/ml = 0.11 mM (in nucleotides)

MW of a double-stranded DNA molecule = (# of base pairs) x (650 daltons/base pair)
Moles of ends of a double-stranded DNA molecule = 2 x (grams of DNA) / (MW in daltons)
Moles of ends generated by restriction endonuclease cleavage:
a) circular DNA molecule: 2 x (moles of DNA) x (number of sites)
b) linear DNA molecule: 2 x (moles of DNA) x (number of sites) + 2 x (moles of DNA)

1 µg of 1000 bp DNA = 1.52 pmol = 9.1 x 1011 molecules
1 µg of pUC18/19 DNA (2686 bp) = 0.57 pmol = 3.4 x 1011 molecules
1 µg of pBR322 DNA (4361 bp) = 0.35 pmol = 2.1 x 1011 molecules
1 µg of M13mp18/19 DNA (7249 bp) = 0.21 pmol = 1.3 x 1011 molecules
1 µg of λ DNA (48502 bp) = 0.03 pmol = 1.8 x 1010 molecules

1 pmol of 1000 bp DNA = 0.66 µg
1 pmol of pUC18/19 DNA (2686 bp) = 1.77 µg
1 pmol of pBR322 DNA (4361 bp) = 2.88 µg
1 pmol of M13mp18/19 DNA (7249 bp) = 4.78 µg
1 pmol of λ DNA (48502 bp) = 32.01 µg

1.0 kb DNA = coding capacity for 333 amino acids ≈ 37,000 dalton protein
10,000 dalton protein ≈ 270 bp DNA
50,000 dalton protein ≈ 1.35 kb DNA

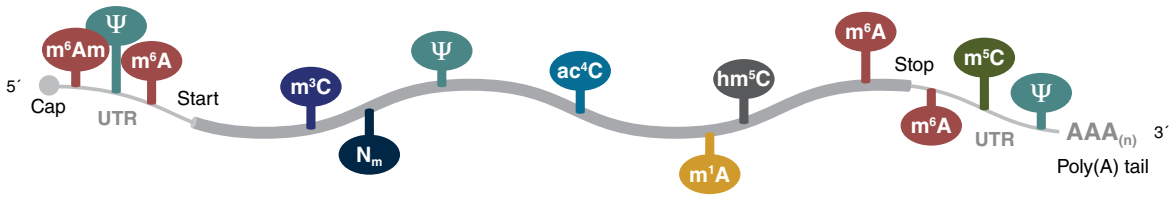
Isotope Data

Isotope	Particle Emitted	Half Life
^{14}C	β	5,730 years
^{3}H	β	12.3 years
^{125}I	γ	60 days
^{32}P	β	14.3 days
^{33}P	β	25 days
^{35}S	β	87.4 days

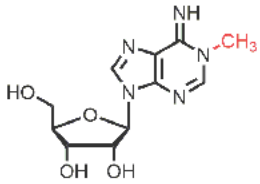
1 Ci = 1,000 mCi
1 mCi = 1,000 µCi
1 µCi = 2.2 x 10^6 disintegrations/minute
1 Becquerel = 1 disintegration/second
1 µCi = 3.7 x 10^4 Becquerels
1 Becquerel = 2.7 x 10^{-5} µCi

Messenger RNA Modifications

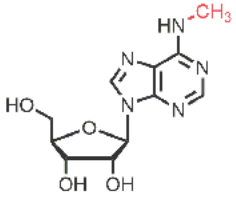
In nature, ribonucleic acid undergoes extensive chemical modification that can result in altered function or stability. The figure below shows examples of base and ribose modifications commonly found in native mRNAs



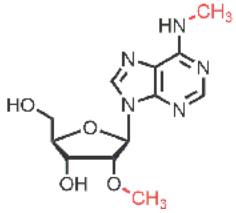
Nucleoside name
(Short name)
[New nomenclature]



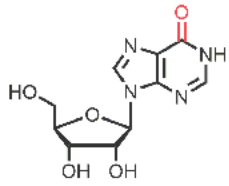
1-methyladenosine
(m^1A) [1A]



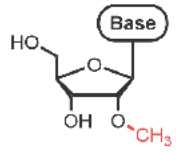
***N*^{6}-methyladenosine**
(m^6A) [6A]



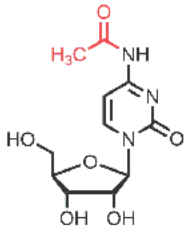
***N*^{6},2´-*O*-dimethyladenosine**
(m^6Am) [06A]



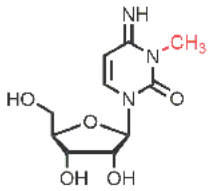
Inosine
(I) [9A]



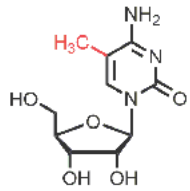
2´-*O*-methylnucleoside
(Nm) [ON]



***N*^{4}-acetylcytidine**
(ac^4C) [42C]

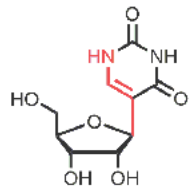


3-methylcytidine
(m^3C) [3C]

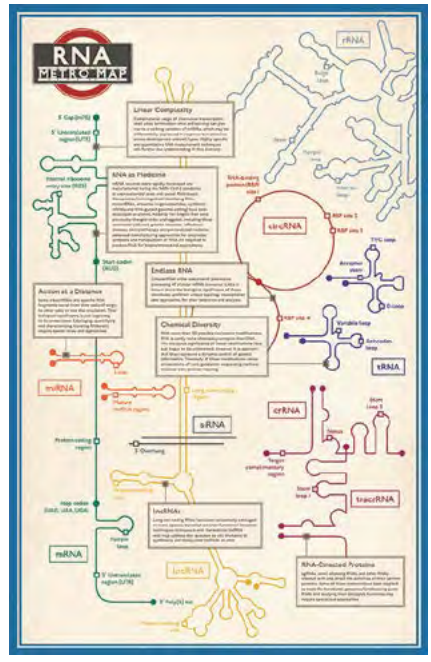


5-methylcytidine
(m^5C) [5C]

5-hydroxymethylcytidine
(hm^5C) [51C]



Pseudouridine
(Ψ) [9U]



Visit **NEBrna.com** to download our RNA Metro Map, and learn more about the various RNA structures and recent applications.

APPENDIX

Acids and Bases

Compound	Formula	Molecular Weight	Specific Gravity	% by Weight	Conc. Reagent Molarity
Acetic acid, glacial	CH_3COOH	60.0	1.05	99.5	17.4
Formic acid	HCOOH	46.0	1.20	90	23.4
Hydrochloric acid	HCl	36.5	1.18	36	11.6
Nitric acid	HNO_3	63.0	1.42	71	16.0
Perchloric acid	$HClO_4$	100.5	1.67	70	11.6
Phosphoric acid	$H3PO_4$	98.0	1.70	85	18.1
Sulfuric acid	$H2SO_4$	98.1	1.84	96	18.0
Ammonium hydroxide	NH_4OH	35.0	0.90	28	14.8
Potassium hydroxide	KOH	56.1	1.52	50	13.5
Sodium hydroxide	NaOH	40.0	1.53	50	19.1
β-mercaptoethanol	$HSCH_2CH_2OH$	78.1	1.11	100	14.3

Protein Data

Bacterial Cells: *E. coli* or *Salmonella typhimurium*

Cell Data	per cell	per liter at 10^9 cells/ml
Wet weight	9.5×10^{-13} g	0.95 g
Dry weight	2.8×10^{-13} g	0.28 g
Total protein	1.55×10^{-13} g	0.15 g
Volume	1.15 μm^3 = 1 femtoliter	–

Protein conc. in the cell: 135 mg/ml

Theoretical maximum yield for a 1 liter culture (10^9 cells /ml) if protein of interest is:

- 0.1% of total protein: 150 µg/liter
- 2.0% of total protein: 3 mg/liter
- 50.0% of total protein: 75 mg/liter

Common Plasmid Gene Products

Gene	Gene Product # of Residues	Molecular Weight (daltons)
tet (pBR322)	401	43,267
amp (pBR322, bla)	286	31,515
kan (pACYC177, nptI)	264	29,047
cam (pACYC184, cat)	219	25,663
*lacZ*α (pUC19)	107	12,232
lacZ	1,023	116,351

Nucleotide Physical Properties

Compound	Molecular Weight	λ max (pH 7.0)	Absorbance at λ max 1 M solution (pH 7.0)
ATP	507.2	259	15,400
CTP	483.2	271	9,000
GTP	523.2	253	13,700
UTP	484.2	262	10,000
dATP	491.2	259	15,200
dCTP	467.2	271	9,300
dGTP	507.2	253	13,700
dTTP	482.2	267	9,600

Tris Buffer: pH vs Temperature

pH of Tris Buffer (0.05 M)		
5°C	25°C	37°C
7.76	7.20	6.91
7.89	7.30	7.02
7.97	7.40	7.12
8.07	7.50	7.22
8.18	7.60	7.30
8.26	7.70	7.40
8.37	7.80	7.52
8.48	7.90	7.62
8.58	8.00	7.71
8.68	8.10	7.80
8.78	8.20	7.91
8.88	8.30	8.01
8.98	8.40	8.10
9.09	8.50	8.22
9.18	8.60	8.31
9.28	8.70	8.42

Agarose Gel Resolution

% Gel	Optimum Resolution for Linear DNA (kb)
0.5	30 to 1.0
0.7	12 to 0.8
1.0	10 to 0.5
1.2	7 to 0.4
1.5	3 to 0.2

# #

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For more than 45 years, the New England Biolabs catalog has been a resource for scientists worldwide. Each edition contains a collection of mini-reviews that address various scientific, environmental, or humanitarian topics. This catalog features a collection of mini-reviews that discuss anthropogenic climate change. As part of the effort to combat global warming, NEB will support the organization below that restores and protects a local mangrove ecosystem.

MIKOKO PAMOJA

Mikoko Pamoja is a non-profit organization based in the Gazi bay of Kwale County, Kenya. The project is a community-led effort to conserve and restore the local mangrove ecosystem. It protects 117 hectares of mangrove forests, which are crucial to mitigating climate change by sequestering carbon. Additionally, these mangroves provide essential protection against sea storms and ensure the security of thriving fisheries, improving the livelihood of two local villages.

The organization was registered as a user group under the Gazi-Gogoni Community Forest Association within the Buda Forest station in 2012. Since then, Mikoko Pamoja has been globally recognized and is celebrated as a model for successful community-based projects. In 2017, the organization was awarded the UN Equator Prize for its innovative nature-based solutions. The project has sequestered over 14,000 tonnes of CO_2 and raised over $120,000 for mangrove conservation and community benefit.

Mikoko Pamoja is the first community-type project in the world to restore and protect mangrove forests through 'blue carbon' credit sales in the voluntary market. The funds generated by these sales are used to support the local community, providing clean water sources, vital medical and school supplies, educational materials, and sanitation.

The organization is run by a 13-member committee and a Project Coordinator from the two villages in Gazi Bay, who work closely with environmental scientists to monitor mangrove growth, biodiversity, and other indicators of ecosystem health. By teaching sustainable development and responsible resource management, Mikoko Pamoja is inspiring individuals to make a positive impact on the environment, the community, and biodiversity.

Mikoko Pamoja takes a multi-faceted approach that promotes both environmental and social sustainability. The project respects traditional knowledge and cultural practices, ensuring the community's values are considered in decision-making. The success of Mikoko Pamoja is being replicated in nearby communities, bringing hope and a brighter future to people and nature alike.

To learn more, please visit:
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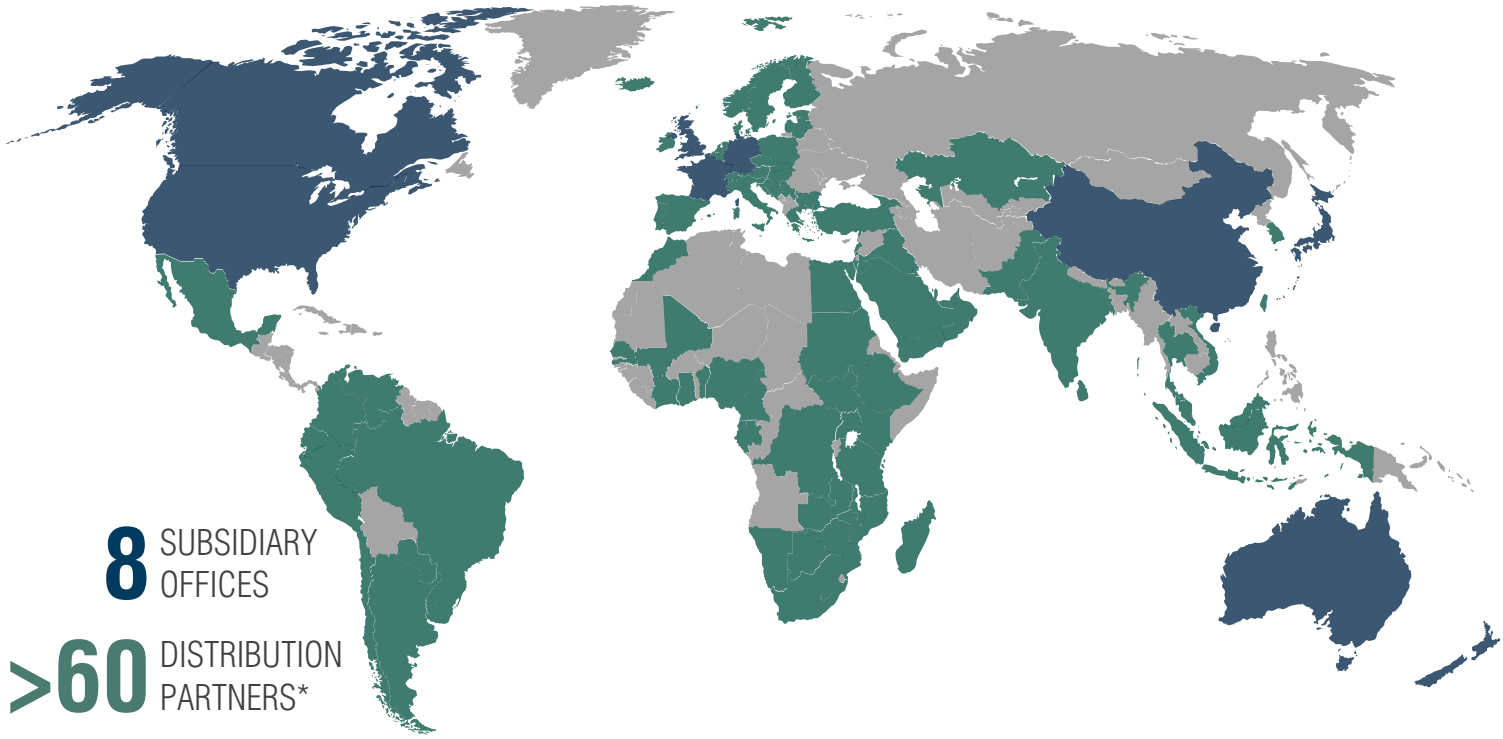

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Telephone +49 (0) 69/305 23140
Free Call 0800/246 5227 (Germany)
Free Call 00800/246 52277 (Austria)
Fax +49 (0) 69/305 23149
Free Fax 0800/246 5229 (Germany)
email: info.de@neb.com
www.neb-online.de

Japan
New England Biolabs Japan, Inc.
Telephone +81 (0) 3 4545 1422
Fax +81 (0) 3 5669 6194
email: cs.jp@neb.com
www.nebj.jp

Singapore
New England Biolabs Pte Ltd.
Telephone +65 638 59623
Fax +65 638 59617
email: sales.sg@neb.com
www.neb.sg

United Kingdom
New England Biolabs (UK), Ltd.
Telephone +44 (0) 1462 420616
Free Call 0800 318486
Fax +44 (0) 1462 421057
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