Spring 2008, vol. 3.1

NEB expressions

a scientific update from New England Biolabs

Welcome to the spring edition of NEB expressions. This issue introduces the PURExpress™ In Vitro Protein Synthesis Kit, a rapid method for gene expression analysis. This novel reconstituted system offers several advantages over commercially available extract-based in vitro transcription/translation systems, and is ideal for high throughput technologies. The advantages of in vitro transcription/translation are discussed in the feature article. This issue also highlights our protein markers and ladders available to support your expression analysis work.

As always, we invite your feedback on our products, services and corporate philosophy.

inside:

NEW ENGLAND BioLabs® Inc.
leader in enzyme technology

The Next Generation of Cell-free Protein Synthesis

in vitro transcription/translation using the PURE approach

Yu Zheng, Ph.D and Shaorong Chong, Ph.D,
New England Biolabs, Inc.

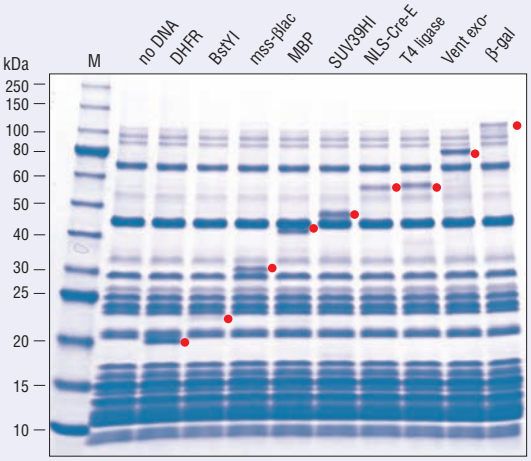
Background of cell-free protein synthesis

Since the early pioneering work of Nirenberg and Matthaei in 1961 (1), which demonstrated *in vitro* protein translation using cell extracts, cell-free protein synthesis has become an important tool for molecular biologists by playing a central role in a wide variety of applications (2). In the post-genomic era, cell-free protein synthesis has the potential to become one of the most important high throughput technologies for functional genomics and proteomics.

The biggest advantage, compared to protein production in living cells, is that cell-free protein synthesis is the quickest way to obtain an expressed phenotype (protein) from a genotype (gene). Starting with a PCR or plasmid template, *in vitro* protein synthesis and functional assays can be carried out in a few hours. Moreover, it is independent of host cells. Proteins which are toxic or prone to proteolytic degradation can be readily prepared *in vitro*.

(continued on page 2)

Protein expression using the PURExpress™ *In Vitro* Protein Synthesis Kit from NEB



25 µl reactions containing 250 ng template DNA 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. Note that expressed proteins can be reverse-purified using affinity chromatography (reagents not supplied). The red dot indicates the protein of interest. Marker M is the Protein Ladder (NEB #P7703). For more information on PURExpress see page 4.

Advantages

- Cleaner system eliminates sample degradation
- Requires the mixing of only two tubes followed by the addition of template DNA
- Obtain results in only a few hours

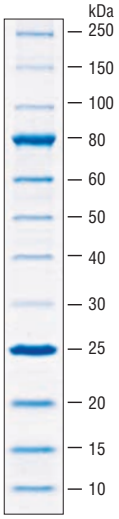
New Protein Ladder

achieve accuracy in your protein expression analysis

Advantages

- Suitable for analysis of a wide range of proteins
- Sharp, uniform bands
- Convenient band spacing for accurate molecular weight determination
- Easy-to-identify reference bands
- Value pricing

For more information about protein markers and ladders available at NEB, please turn to page 5.



The Protein Ladder (NEB #P7703) offers a more consistent banding pattern than other commercially available ladders, making it the ideal choice for accurate size determination. 10–20% SDS-PAGE

Special Offer

Through 6/30 receive a free Protein Ladder sample with any purchase by including NEB #P7703G with your order. Limit one sample per order. Valid in the U.S. only.

The Next Generation of Cell-free Protein Synthesis

(continued from page 1)

Commercially available cell-free protein synthesis systems are typically derived from cell extracts of *Escherichia coli* S30, rabbit reticulocytes or wheat germ. The drawback of extract-based systems is that they often contain nonspecific nucleases and proteases that adversely affect protein synthesis. In addition, the cell extract is like a "black box" in which numerous uncharacterized activities may modify or interfere with subsequent downstream assays.

Some of these limitations can be partially overcome, for instance, by using engineered strains or by adding various inhibitors. Nevertheless, the problems cannot be solved at the root level.

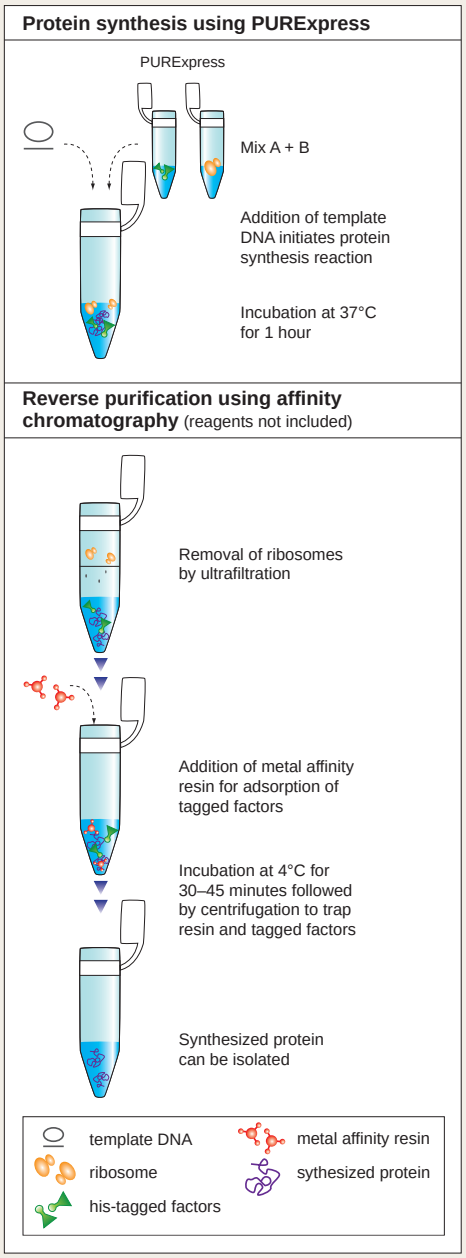


Figure 1: *Schematic diagram of protein synthesis and purification using PURExpress.*

The "PURE" approach

The first complete *in vitro* reconstitution of protein translation from *E. coli* was accomplished in 2001 in Dr. Takuya Ueda's lab at the University of Tokyo. This became known as the "PURE" system, which stands for "Protein synthesis Using Recombinant Elements"(3). This system was then commercialized as the **PURESYSTEM®** by the Post Genome Institute (PGI) (Tokyo, Japan). Except for the ribosomes and tRNAs, which are highly purified from *E. coli*, the PURE system reconstitutes the *E. coli* translation machinery with fully recombinant proteins.

PURExpress™ from NEB is based on the **PURESYSTEM®** from PGI, and improves on the original "Classic II" Kit by optimizing components to increase yield of protein synthesis. For more information please see page 4.

The PURE system includes:

- His-Tagged Translation Factors
  - Initiation Factors (IF1, IF2, IF3)
  - Elongation Factors (EF-Tu, EF-Ts, EF-G)
  - Release Factors (RF1, RF2, RF3)
  - Ribosome Recycling Factor
  - 20 Aminoacyl tRNA synthetases
  - Methionyl tRNA formyltransferase
- *E. coli* Ribosomes
- *E. coli* tRNAs
- Energy Regeneration System
- NTPs, Amino Acids, Salts, Buffer

In addition, recombinant T7 RNA polymerase is used to couple transcription to translation. The PURE system represents an important step towards a totally defined *in vitro* transcription/translation system, thus avoiding the "black box" nature of the cell extract-based systems.

Advantages and applications of the PURE system

The PURE system is more robust and convenient than most extract-based systems for many *in vitro* applications. The immediate advantage is the significantly reduced level of all contaminating activities. It can be used to express a wide range of protein targets and has the capacity for a yield of more than 100 µg/ml. The activity of the synthesized protein can often be directly assayed without purification due to the low background activity of the translation mixture. All recombinant protein factors inside the PURE system are His-tagged, in some cases allowing the synthesized protein to be "reverse-purified" (Figure 1,2)(3). The purity of this system allows it to withstand more than five freeze-thaw cycles without losing its efficiency, further extending its shelf life (Figure 3)(Cantor, E., unpublished observation).

The advantages of the PURE system have been demonstrated in various *in vitro* applications including:

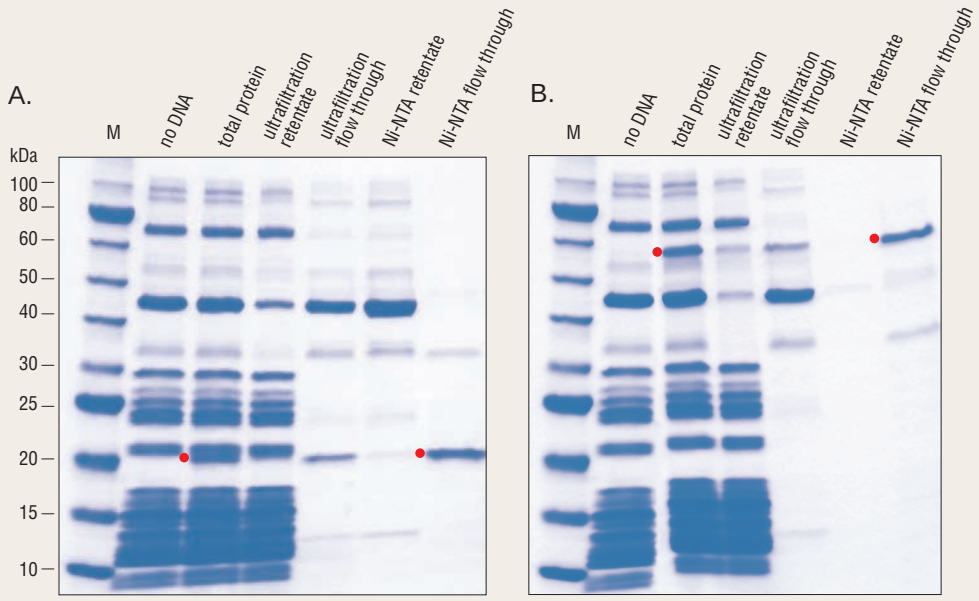


Figure 2: Expression and reverse purification of DHFR (A) and T4 DNA Ligase (B) using PURExpress. *125 µl reactions were carried out according to recommendations in accompanying manual. Samples were analyzed on a 10–20% Tris-glycine gel and stained with Coomassie Blue. Note that in both cases, the desired protein can be visualized in the total protein fraction. The red dot indicates the protein of interest. Marker M is the Protein Ladder (NEB #P7703).*

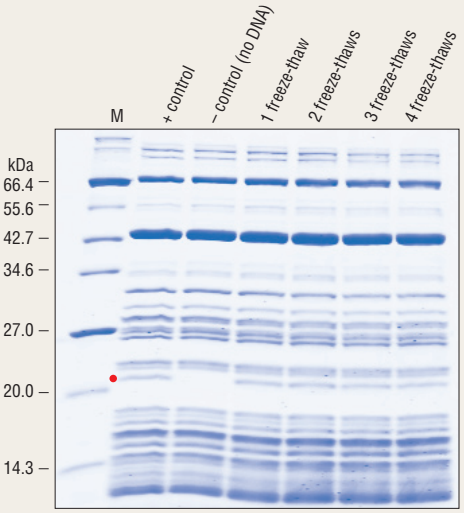


Figure 3. PURExpress retains activity after multiple freeze-thaw cycles. *Solutions A and B were subjected to four rounds of freeze-thaw treatment and then used to set up standard reactions according to manual recommendations. The red dot indicates the protein of interest. Marker M is the Protein Marker, Broad Range (NEB #P7702).*

(i) High throughput functional genomics and proteomics

The simple format of the PURE system allows it to be easily integrated into high throughput platforms for functional genomics and proteomics studies. The absence of any nuclease activities ensures the stability of linear DNA templates during protein synthesis. Individual DNA templates for *in vitro* expression can be generated by PCR, eliminating the time-consuming cloning process. This feature is particularly useful for high throughput screening at the whole genome scale, either for novel activities or for protein-protein interactions. For structural genomics projects, the PURE system can be an alternative route to acquire difficult protein targets which resist traditional cellular expression (4).

(ii) Protein engineering

Directed evolution of proteins *in vitro* is a powerful tool for improving and creating biocatalysts. A number of *in vitro* evolution methodologies, such as mRNA display (5), ribosome display (6) and *in vitro* compartmentalization (7), depend on *in vitro* translation. For example, NEB first demonstrated that the PURE system is uniquely suited for the *in vitro* selection of restriction endonucleases using the *in vitro* compartmentalization method (8), as it is free of nonspecific nuclease activity. In this study, the PURE system and the DNA library were dispersed into more than 10^9 aqueous droplets in a water-in-oil emulsion. The droplet encapsulation provides a linkage between the phenotype (expressed protein) and the genotype (DNA), which sets the stage for the specific selection of restriction enzyme genes. Other researchers have reported that using the PURE system greatly improves the efficiency of ribosome display (9).

Systematically mutagenized protein-coding libraries can be used as well to test if a specific mutation(s) affects protein function. Again, only a few PCR steps are needed to obtain the mutant protein, providing a quick experimental verification of hypotheses.

(iii) Study of protein expression, translation and folding

The PURE system contains the minimal set of factors necessary for *in vitro* protein translation. It is largely free of chaperones and other cellular factors for post-translational modifications, thus providing a starting point to study the involvement of these factors in transcription/translation regulation and nascent chain folding (10). It can also be used to produce "clean" proteins which, if purified from traditional cellular hosts, may come with undesired modifications or bound co-factors. A number of research labs studying translation routinely use home-made reconstituted systems to study different aspects of translation.

(iv) Incorporation of unnatural amino acids

Another important advantage of the PURE system is the ability to control its composition. For example, omission of the release factor 1 (RF1) in the PURE system allows unnatural amino acids to be efficiently incorporated at specific amber codon sites via chemically mis-acylated suppressor tRNA (3,11). It was recently reported that the translation apparatus of *E. coli* can tolerate a wide range of amino acid derivatives, revealing even greater potential for the ribosomal synthesis of unnatural peptides using reconstituted systems (12).

The future of cell-free protein synthesis

Future generations of cell-free protein synthesis systems should contain defined components, providing a clean background and at the same time be able to produce correctly folded proteins of any type in high yields. The PURE system clearly represents a breakthrough towards this goal. Improved reconstituted systems will be customized for expression of complex proteins such as large membrane proteins, multi-subunit assemblies and specifically modified proteins etc. Customization will also permit diversity in the protein synthesis conditions, such as elevated or decreased temperature, ionic strength and redox environment etc.

Protein translation is one of the core processes in living organisms that serve as a "central node" to network other biological processes. There is no doubt that creative applications of the reconstituted systems will go beyond producing proteins and into such diverse fields as synthetic biology, systems biology and medical diagnostics.

References:

1. Nirenberg, M.W. and Matthaei, J.H. (1961) The dependence of cell-free protein synthesis in *E. coli* upon naturally occurring or synthetic polyribonucleotides. *Proc. Natl. Acad. Sci. USA*, 47, 1588–1602.
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10. Kaiser, C.M., et al. (2006) Real-time observation of trigger factor function on translating ribosomes. *Nature*, 444, 455–460.
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12. Hartman, M.C., et al. (2007) An expanded set of amino Acid analogs for the ribosomal translation of unnatural peptides. *PLoS ONE*, 2, e972.

New PURExpress™ *In Vitro* Protein Synthesis Kit

advanced PURE technology, only from NEB

A rapid method for gene expression analysis, the PURExpress™ *In Vitro* Protein Synthesis Kit is a novel cell-free transcription/translation system reconstituted from purified components necessary for *E. coli* translation. It is based on the PURE system technology originally developed by Dr. Takuya Ueda at the University of Tokyo and commercialized as the **PURESYSTEM®** by Post Genome Institute (PGI) (Tokyo, Japan). All of the activities in PURExpress have been expressed from recombinant sources, enabling the system to be free of contaminating exonucleases, RNases and proteases. This prevents degradation of template DNA 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 only a few hours, PURExpress saves valuable laboratory time and is ideal for high throughput technologies.

Currently, most commercially available *in vitro* transcription/translation systems are derived from S30, rabbit reticulocyte or wheat germ extracts. PURExpress offers several advantages over the more complex extract-based systems:

- Lack of endogenous nuclease or protease activity eliminates sample degradation
- Suitable for circular or linear template*
- Synthesized protein can often be visualized on a Coomassie stained gel, simplifying analysis
- Easy-to-use protocol requires the mixing of two tubes, followed by the addition of template DNA
- Protein expression is complete in approximately one hour
- Purified components offer tighter control of translational, secretory and folding machinery
- Transcription/translation components can often be removed by affinity chromatography, simplifying purification†
- Resistant to multiple freeze/thaw cycles

Special Offer

Through 6/30, receive PURExpress for only $55 by completing a short survey (75% off retail price, no additional discounts apply, valid in the U.S. only). Visit www.neb.com/surveys/PURE/

Kit Includes:

Solution A (Yellow): 20 amino acids, buffer salts, dNTP's, creatine phosphate

Solution B (Red): 20 tRNA synthetases, 3 initiation factors, 3 elongation factors, 3 release factors, ribosome release factor, T7 RNA polymerase, energy regeneration enzymes

Control (DHFR) template and Universal Primer

PURExpress *In Vitro* Protein Synthesis Kit
#E6800S 10 reactions $220

* PCR templates should be free of non-specific amplification products that can interfere with transcription and/or translation.
† Reverse purification may not work for all proteins. Proteins less than 60 kDa are more readily purified than proteins near the MW cutoff for the spin column membrane.

PURExpress™ In Vitro Protein Synthesis Kit from NEB. *This easy-to-use system requires the mixing of only two tubes followed by the addition of template DNA.*

Advantages of *in vitro* protein synthesis:

- Significantly faster than protein expression in living cells
- Synthesis and assays can be carried out in a few hours
- Independent of host cells
- Ability to express toxic proteins

Applications of *in vitro* transcription/translation kits:

- Confirmation of open reading frames
- Examination of the effects of mutations on ORF's
- Generation of truncated proteins to identify active domains and functional residues
- Generation of analytical amounts of proteins for further characterization
- Introduction of modified, unnatural or labeled amino acids
- Epitope mapping
- Expression of proteins that are toxic in conventional expression systems

Specialized applications of PURExpress:

- Ribosome display
- Translation/protein folding studies
- *in vitro* compartmentalization

A more in depth discussion of these applications is found on pages 1–3.

Protein Markers and Ladders

quality, consistency and value

For protein expression analysis, NEB offers a selection of highly pure protein markers and ladders. Our protein markers are available unstained (13 bands, 2.3 to 212 kDa) and prestained (8 bands, 6.5 to 175 kDa). For easy identification, we also offer a prestained marker containing two colored bands. The newest addition to this product line is the Protein Ladder, an unstained ladder that resolves into 12 sharp, evenly spaced bands in the range of 10–250 kDa when analyzed by SDS-PAGE.

Special Offer

Through 6/30 receive a free Protein Ladder sample with any purchase by including NEB #P7703G with your order. Offer valid in the U.S. only. Limit one sample per order.

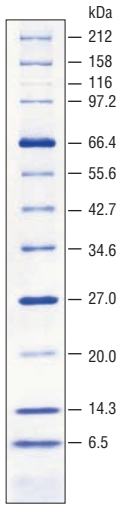
Advantages

- Suitable for a wide range of expressed proteins
- Uniform band intensities and convenient band spacing
- Easy-to-identify reference bands
- Protein Ladder is ideal for accurate molecular weight determination
- ColorPlus Prestained Protein Marker contains two colored bands for unambiguous detection

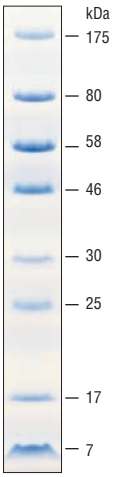
Protein Ladder/Marker Selection Chart	Catalog #	Range	Small Pack # of mini-gel lanes	Small Pack Price ($)	Small Pack Price per lane ($)	Large Pack # of mini-gel lanes	Large Pack Price ($)	Large Pack Price per lane ($)
Protein Marker, Broad Range	P7702S/L	2–212 kDa	150	66	0.44	750	264	0.35
Prestained Protein Marker, Broad Range	P7708S/L	7–175 kDa	175	95	0.54	875	380	0.43
ColorPlus Prestained Protein Marker, Broad Range	P7709S/L	7–175 kDa	175	105	0.60	875	420	0.48
New Protein Ladder	P7703S	10–250 kDa	100	70	0.70	N/A	N/A	N/A

Note: Prestained and ColorPlus Protein Markers are available in an extra-small size (V) that contains enough reagent for 83 mini-gel lanes.

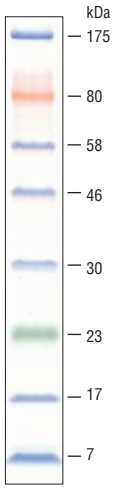
Protein Ladders & Markers



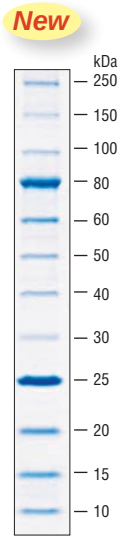
Protein Marker, Broad Range (2–212 kDa)*
10–20% SDS-PAGE
NEB #P7702



Prestained Protein Marker, Broad Range (7–175 kDa)
10–20% SDS-PAGE
NEB #P7708

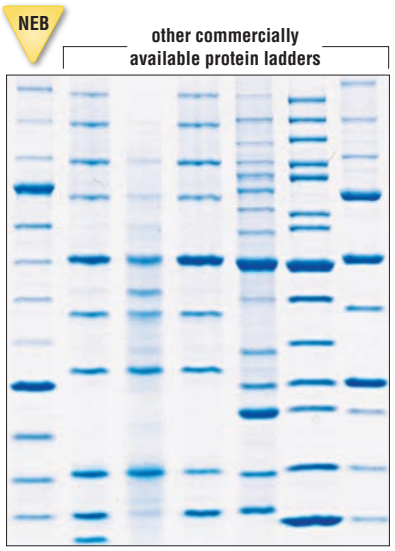


ColorPlus Prestained Protein Marker, Broad Range (7–175 kDa)
10–20% SDS-PAGE
NEB #P7709



Protein Ladder, (10–250 kDa)
10–20% SDS-PAGE
NEB #P7703

*Note that 2.3 and 3.4 kDA bands run at the dye front.



Choose the NEB Protein Ladder for sharp, evenly spaced bands, convenient band spacing, accurate molecular weight determination and value pricing.

> technical tip

Using NEB Protein Markers and Ladders

Thawing

- Markers and Ladders should be thawed on ice

Loading a sample

- Vortex gently to ensure the solution is homogenous
- Aliquot the following amount into a separate microcentifuge tube:

Marker/Ladder	mini gel	full-size gel
Protein Marker, Broad Range	7 µl	15 µl
Protein Ladder	5 µl	10 µl
Prestained Protein Marker	6 µl	12 µl (blotting)
	15 µl	30 µl (visualizing)
ColorPlus Prestained Protein Marker	6 µl	12 µl (blotting)
	15 µl	30 µl (visualizing)

- Heat sample at 95–100°C for 3–5 minutes
- After a quick microcentrifuge spin, load directly onto a gel
- To ensure uniform mobility, load an equal volume of 1X SDS loading buffer into unused wells

Storage

- Recommended storage for all protein markers and ladders is -20°C

Analysis

- The Protein Ladder (NEB #P7703) and the Protein Marker, Broad Range (NEB #P7702) give their true molecular weight values when run on a gel. For accurate size determination, the Protein Ladder is recommended.
- "Apparent" molecular weights are assigned to the Prestained Protein Markers (NEB #P7708, #P7709). These markers are not recommended for size determination. When a protein is covalently bound to a charge-carrying dye molecule, the protein's overall charge can be affected and alter its mobility within the gel. In addition, prestained protein markers will resolve differently on varying gel types. For more information, visit www.neb.com.

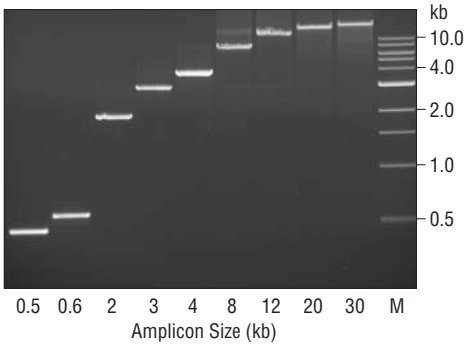
New LongAmp *Taq* DNA Polymerase

optimized for larger PCR products

A unique blend of *Taq* and Deep Vent$_R$® DNA Polymerases, LongAmp *Taq* DNA Polymerase enables amplification of larger PCR products with a fidelity 6X higher than *Taq* DNA Polymerase alone.

Advantages

- Robust amplification of PCR products
- Amplification up to 40 kb from low complexity templates and up to 30 kb from difficult templates (e.g. human genomic DNA)
- Increased fidelity compared to *Taq* DNA Polymerase alone



Amplification of specific sequences from human genomic DNA using LongAmp Taq DNA Polymerase. *Amplicon sizes are indicated below the gel. Marker M is the NEB 1 kb DNA Ladder (NEB #N3232).*

LongAmp *Taq* DNA Polymerase
#M0323S 500 units.....$75
#M0323L 2,500 units.....$300

LongAmp *Taq* PCR Kit
#E5200S 100 rxns (50 µl).....$105

LongAmp *Taq* 2X Master Mix
#M0287S 100 rxns.....$135
#M0287L 500 rxns.....$540

New Phusion™ RT-PCR Kit

increased speed and fidelity

Experience the benefits of Phusion™ High-Fidelity DNA Polymerase in your two-step RT-PCR reactions. The Phusion™ RT-PCR Kit enables accurate cDNA amplification with high yields and short cycling times, and is ideal for producing cDNA to be used in cloning and gene expression studies.

Advantages

- Generate a broad range of RT-PCR products with high yield
- Amplify cDNA fragments with a fidelity 52X greater than *Taq*
- Increased reaction speed

Robustness of different RT-PCR Systems. *A typical RT-PCR experiment for amplifying short cDNA fragments (192-1113 bp). The Phusion RT-PCR system was compared to the RT-PCR systems from two major suppliers. Total RNA from human skeletal muscle was transcribed with random priming according to each supplier's recommendations. Amplification of cDNA was performed with a high-fidelity DNA polymerase recommended by the suppliers. Due to the various sizes of amplicons, a PCR protocol with the lowest annealing temperature and the longest extension time defined by amplicons was used. Compared to the Phusion RT-PCR Kit, competitors systems resulted in fewer amplicons with varying yields. M is a molecular size marker.*

Phusion RT-PCR Kit*
#F-546S 20 rxns (50 µl).....$95
#F-546L 100 rxns (50 µl).....$380

*Manufactured by Finnzymes Oy, distributed by New England Biolabs.

New Phire™ Hot Start DNA Polymerase

speed and specificity for high performance PCR

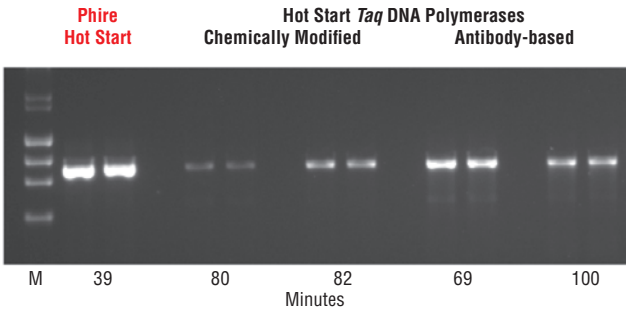
This novel PCR enzyme outperforms other *Taq*-based hot start polymerases. The polymerase's DNA-binding domain shortens extension times, improves yields and increases fidelity 2-fold compared to *Taq*. In addition, the Affibody®-based hot start technology allows complete reactivation in zero time at standard cycling temperatures. Phire™ Hot Start DNA Polymerase is an ideal solution for routine and high throughput PCR applications.

Special Offer

Through 5/30, request a FREE Phire sample by visiting www.neb.com/samples/Phire/ Offer valid in the U.S. only. Limit one per customer.

Advantages

- Activation step is eliminated
- Amplification up to three times faster than other hot start *Taq* polymerases
- Higher yields due to efficiency of polymerase
- Amplification of products significantly longer than other hot start *Taq* polymerases



Phire Hot Start DNA Polymerase outperforms both chemically modified and antibody based Hot Start Taq Polymerases. *A 1.0 kb fragment from the human glutathione peroxidase gene was amplified with five different hot start DNA polymerases according to suppliers' recommendations. After PCR, duplicates of each reaction were run on a 1% agarose gel. Total cycling times indicated. Marker M is a molecular size marker.*

Phire Hot Start DNA Polymerase*
#F-120S 220 rxns (50 µl) $110
#F-120L 1,000 rxns (50 µl) $440

*Manufactured by Finnzymes Oy, distributed by New England Biolabs.

22nd Annual Molecular Biology Workshop

This intensive summer course held at Smith College in Northampton, MA, emphasizes hands-on molecular biology laboratory work and covers a wide variety of topics and techniques, including:

- Gene cloning
- Gene expression analysis
- PCR and qRT-PCR
- Genomics and bioinformatics
- DNA sequencing and fingerprinting
- RNAi and microarrays

This course is now offered in one- and two-week sessions. No previous experience in molecular biology is required or expected. For additional information, course dates and to fill out an application, visit the Summer Workshop website: http://www.science.smith.edu/neb

NEB FAQ Spotlight

shipping procedures

Q Why does New England Biolabs ship products using freezer packs instead of dry ice?

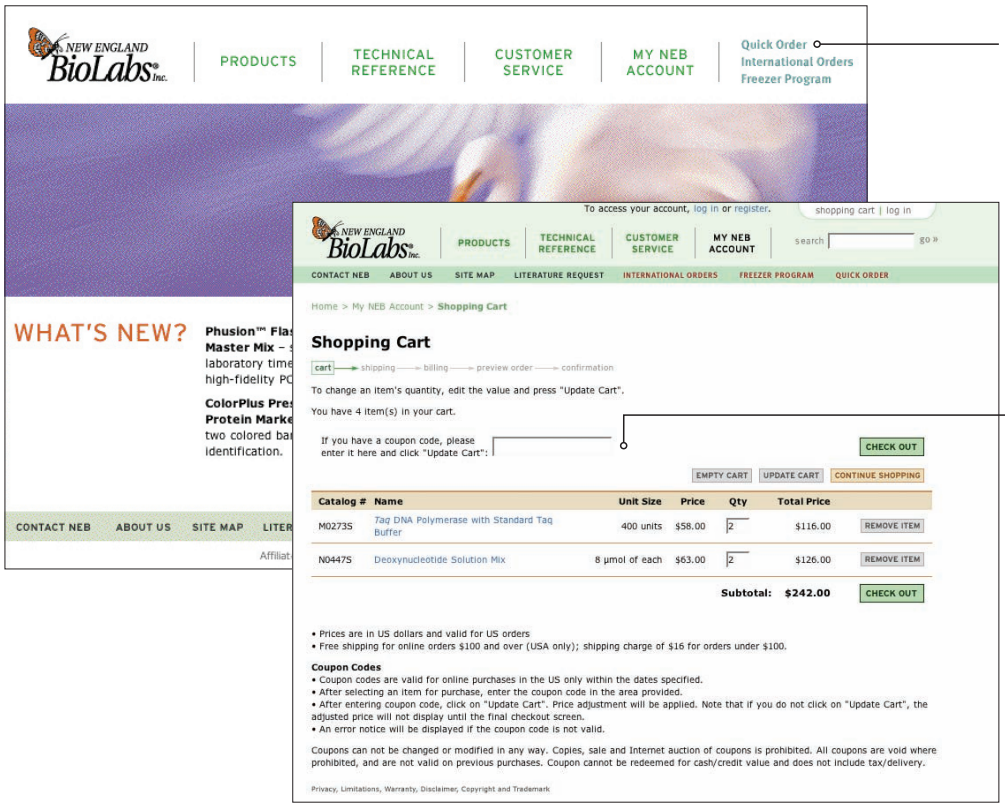
A NEB has always shipped many of its products, including restriction enzymes and modifying enzymes, with freezer packs. The freezer packs are designed to keep the contents of the shipping box below 4°C for three days. Since our products are shipped for overnight delivery, this is more than sufficient. Each product is carefully tested to be sure that this method of shipment is compatible with product stability; the more temperature sensitive products are shipped on dry ice. In addition, freezer packs are environmentally friendly in that they can be re-used.

Q Does NEB offer same day delivery?

A NEB offers same day delivery to most of the Boston and Cambridge area on orders placed before 10:00 AM (EST), and next business day delivery coast to coast if your order is received Monday through Friday by 8:00 PM (EST).

Online Ordering

For your convenience, orders can be placed anytime using our online ordering system. Simply click on the “Quick Order” link found on our homepage and enter the product number for the items you would like to purchase. Once the order is placed, customers can easily track delivery status and have access to order history. In addition, customers who place orders online are eligible for free shipping on orders over $100.



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