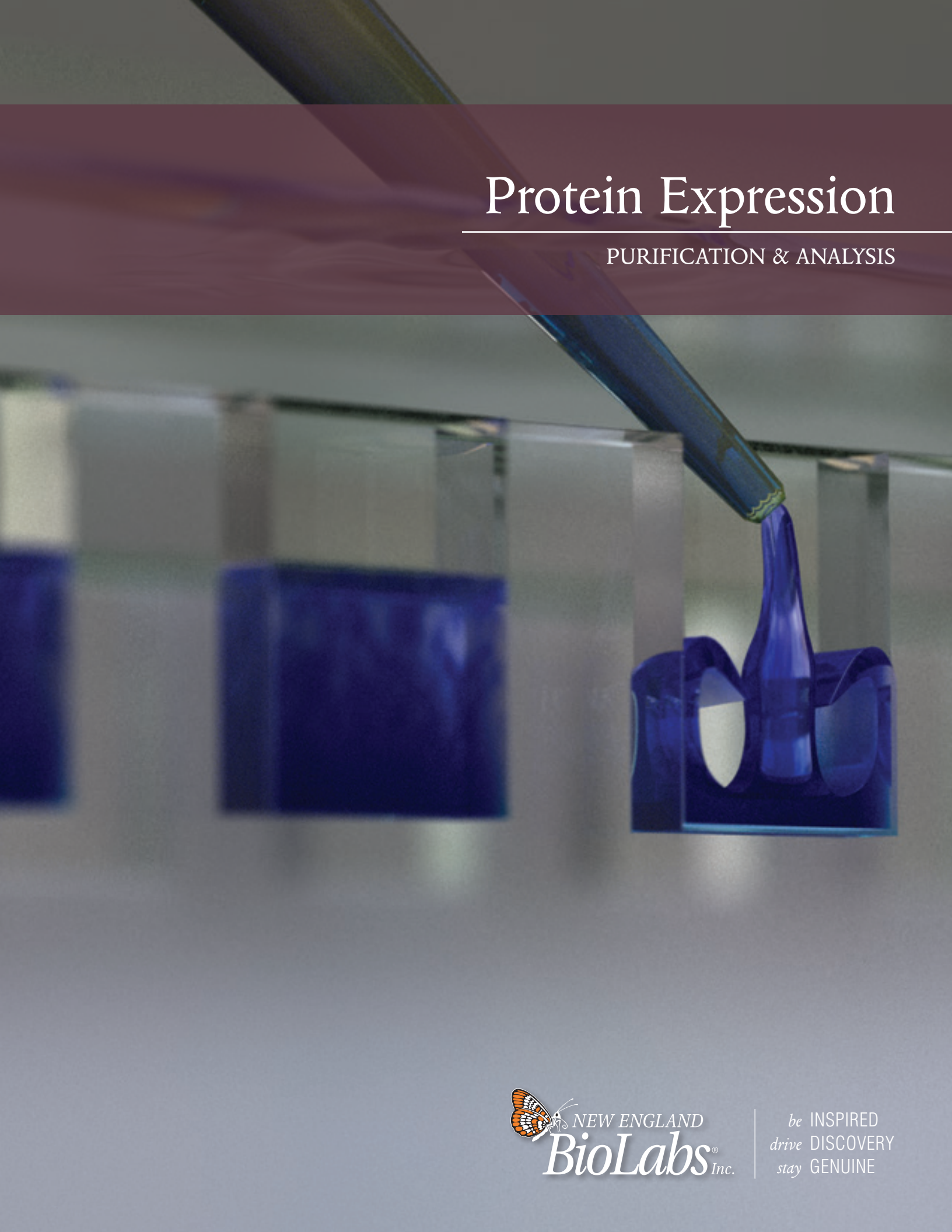


Protein Expression

PURIFICATION & ANALYSIS

NEW ENGLAND BioLabs® Inc.

be INSPIRED
drive DISCOVERY
stay GENUINE

Protein Expression & Purification

Protein expression can be a very complex, multi-factorial process. Each protein requires a specific intracellular environment to correctly and efficiently 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 successful production of all recombinant proteins. Therefore, it is critical to have a broad range of expression tools to ensure the successful expression of your target protein.

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.

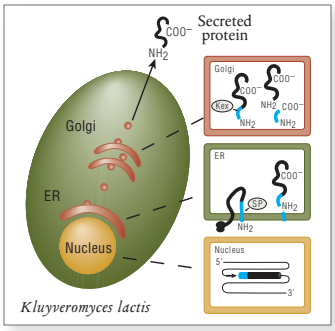
APPLICATION	KIT	ADVANTAGES
High yield expression	pMAL™ Protein Fusion and Purification System	Substantial yields (up to 100 mg/L) in more than 75% cases tested; uses the strong P_{tac} promoter
	K. lactis Protein Expression Kit	Uses the strong *LAC4* promoter; multiple integrations of plasmid results in higher yield
	IMPACT™ Kit	Uses the T7 promoter for high level regulated expression
Cell-free expression	PURExpress® *In Vitro* Protein Synthesis Kits	Quickly generates analytical amounts of protein
Co-expression of multiple proteins	*K. lactis* Protein Expression Kit	Easily co-express 2–4 proteins
	PURExpress *In Vitro* Protein Synthesis Kits	Bicistronic constructs or multiple plasmids with appropriate transcription and translation regulatory elements can be used
Enhanced solubility	pMAL Protein Fusion and Purification System	Fusion to MBP enhances solubility of proteins in *E.coli* *
	K. lactis Protein Expression Kit	Utilizes *K. lactis* eukaryotic folding pathway
Affinity tag chromatography	IMPACT Kit	Utilizes an intein-CBD tag on either the N- or C- terminus, offers single-step purification
	pMAL Protein Fusion and Purification System	Fusion to MBP allows for purification on amylose resin
	K. lactis Protein Expression Kit	Vectors are sold separately that generate fusions to MBP allowing for purification on amylose resin
Post-translational modification	*K. lactis* Protein Expression Kit	Secretion of both *N*- and *O*- glycosylated proteins
Periplasmic expression	pMAL Protein Fusion and Purification System	Periplasmic expression enhances folding of proteins with disulfide bonds
Secreted expression	*K. lactis* Protein Expression Kit	Eliminates cell lysis step, simplifying purification
Toxic proteins	*K. lactis* Protein Expression Kit	Secretion of protein from the cell
	IMPACT Kit	Can express the toxic gene in two pieces and ligate proteins together using intein-mediated protein ligation (IPL)
	pMAL Protein Fusion and Purification System	Can export toxic proteins into periplasmic space
	PURExpress *In Vitro* Protein Synthesis Kits	Cell-free environment not affected by "toxicity" of expressed protein
Protein labeling or ligation	IMPACT Kit	Generates proteins with reactive ends (N-terminal cysteine and/or C-terminal thioester) allowing for labeling or ligation of proteins or peptides
	PURExpress *In Vitro* Protein Synthesis Kits	Allows introduction of modified, unnatural, or labeled amino acids
No additional amino acid residues	IMPACT Kit	Start of native protein is fused adjacent to site of cleavage
	pMAL Protein Fusion and Purification System	Start of protein is fused adjacent to protease site

* Kapust and Waugh (1999) *Protein Science*, 8, 1668–1674.

K. lactis
Protein Expression Kit
Yeast

This kit provides a simple method to clone and express your gene of interest in the yeast *Kluyveromyces lactis*. This system offers many advantages over bacterial systems and eliminates the methanol containing medium and antibiotic requirements of *Pichia pastoris*. With easy-to-use protocols and highly competent *K. lactis* cells included, this system can take you from bench top to large scale production with ease.

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 where the Kex protease removes the α-MF domain. The protein of interest is then secreted from the cell.

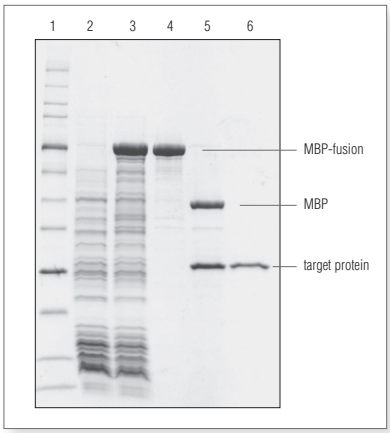
ADVANTAGES

- High yield protein expression
- Rapid high cell density growth
- Methanol-free growth media
- Glycerol-free formulation for optimal performance in HPLC and mass spec analysis
- Multiple protein expression

pMAL Protein Fusion & Purification System
E. coli

This system takes advantage of the strong P_{tac} promoter and the translation initiation signals of maltose binding protein (MBP) to enhance solubility and expression levels of a desired protein in *E. coli*. The resulting product is an MBP fusion protein, which is then purified by affinity chromatography.

Protein Expression using pMAL



SDS-polyacrylamide gel electrophoresis of fractions from the Amylose affinity purification of MBP-paramyosin ΔSal.

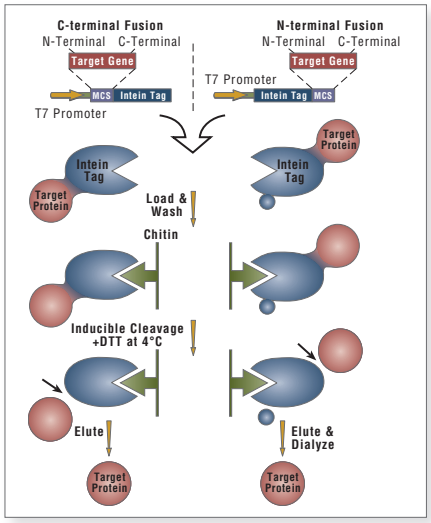
ADVANTAGES

- Reliable *E. coli* expression: substantial yields (up to 100 mg/L) in more than 75% of the cases tested
- Expression in either the cytoplasm or periplasm: periplasmic expression enhances folding of proteins with disulfide bonds

IMPACT Kit
E. coli

This *E. coli* expression system utilizes engineered protein splicing elements (inteins) fused to a chitin binding domain (CBD) as affinity tags. This allows the recombinant protein to be purified in a single chromatographic step. The target protein can be fused at the C- or N- terminus, maximizing the probability of successful expression and purification.

Schematic of the IMPACT-System



ADVANTAGES

- Yields proteins with native sequence
- Desired protein is released without the use of separate, expensive proteases
- One-step purification
- Uses T7 promoter for higher levels of expression

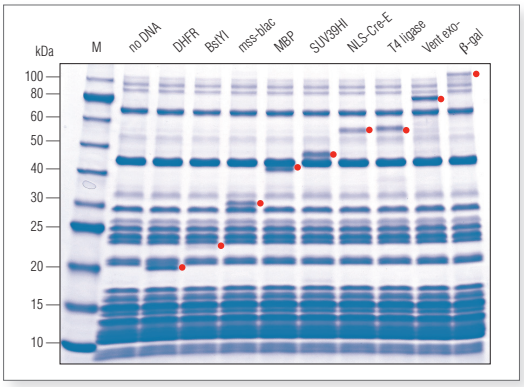
Visit www.neb.com for FAQs, protocols and citation lists.

PURExpress *In Vitro* Protein Synthesis Kit

Cell Free Expression

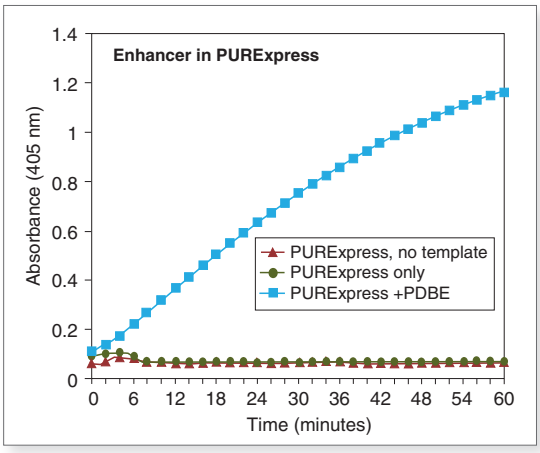
A rapid method for gene expression analysis, PURExpress is a novel cell-free transcription/translation system reconstituted from purified components necessary for *E. coli* translation. Express a wide range of proteins free of modification or degradation by simply mixing two tubes followed by the addition of template DNA. With results available in only a few hours, PURExpress saves valuable laboratory time and is ideal for high throughput technologies. Choose from several kits depending on your needs. The original PURExpress Kit contains all the components in two tubes. The PURExpress Δ Ribosome Kit allows users to add their own ribosomes when performing protein translation experiments. In the PURExpress Δ RF 123 Kit, the three release factors are supplied separately, allowing the user to perform a protein synthesis reaction/ribosome display experiment with/without release factors of their choice. The PURExpress Δ (aa, tRNA) Kit can be used to run a protein synthesis reaction by adding modified amino acids and tRNA mixtures to the reaction. The PURExpress Disulfide Bond Enhancer is also available to enhance correct disulfide bond formation of target proteins.

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



Reactions were carried out according to manual recommendations. Red dot indicates protein of interest. Marker M is the Protein Ladder (NEB #P7703).

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.

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

APPLICATIONS

- 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)
- Ribosome structure and function studies (NEB #E3313, #P0763)
- Release factor function studies/ ribosome display (NEB #E6850)
- Epitope mapping

Ordering Information

PRODUCT	NEB #
K. lactis Protein Expression Kit	E1000S
pMAL Protein Fusion and Purification System	E8200S
IMPACT Kit	E6901S
PURExpress *In Vitro* Protein Synthesis Kit	E6800S/L
PURExpress Δ Ribosome Kit	E3313S
PURExpress Δ (aa, tRNA) Kit	E6840S
PURExpress Δ RF123 Kit	E6850S
PURExpress Disulfide Bond Enhancer	E6820S
E. coli Ribosome	P0763S

For additional information, companion products and kit components sold separately, please visit www.neb.com. Licensing information for these products can be found on our website.

Competent Cells for Protein Expression

NEB also 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.NEB Express 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.

ADVANTAGES

- T1 phage resistance (*fhuA2*)
- Convenient formats available
- Bulk sales capabilities with custom packaging formats
- Free of animal products
- Deficient in proteases Lon/OmpT
- Do not restrict methylated DNA

STRAIN	CHARACTERISTICS	NEB #	SIZE
NEB Express Competent *E. coli**	• Versatile non-T7 expression strain • Protease deficient	C2523H/I	20 x 0.05 ml/6 x 0.2 ml
NEB Express I^q Competent *E. coli*	• Control of IPTG induced expression from P_{lac}, P_{tac} and P_{trc} • Protease deficient	C3037I	6 x 0.2 ml
T7 Express Competent *E. coli*	• Most popular T7 expression strain • Protease deficient	C2566H/I	20 x 0.05 ml/6 x 0.2 ml
T7 Express *lysY* Competent *E. coli*	• T7 expression • Protease deficient • Better reduction of basal expression	C3010I	6 x 0.2 ml
T7 Express *lysY*/I^q Competent *E. coli*	• T7 expression • Protease deficient • Highest level of expression control	C3013I	6 x 0.2 ml
T7 Express Crystal Competent *E. coli*	• T7 expression • Protease deficient • SeMet labeling for protein crystallography	C3022I	6 x 0.2 ml
SHuffle Express Competent *E. coli*	• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • Protease deficient/B strain	C3028H	6 x 0.05 ml
SHuffle T7 Express Competent *E. coli*	• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression • Protease deficient/B strain	C3029H	6 x 0.05 ml
SHuffle T7 Express *lysY* Competent *E. coli*	• 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	C3030H	6 x 0.05 ml
SHuffle T7 Competent *E. coli*	• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression/K12 strain	C3026H	6 x 0.05 ml
BL21 Competent *E. coli*	• Routine expression for non-T7 Vectors • Protease deficient	C2530H	20 x 0.05 ml
BL21(DE3) Competent *E. coli*	• Routine T7 Expression • Protease deficient	C2527H/I	20 x 0.05 ml/6 x 0.2 ml
Lemo21(DE3) Competent *E. coli*	• Tunable T7 Expression for difficult targets • Protease deficient	C2528H	6 x 0.05 ml
NiCo21(DE3) Competent *E. coli*	• Expression and purification of His-tagged proteins • Protease deficient	C2529H	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.

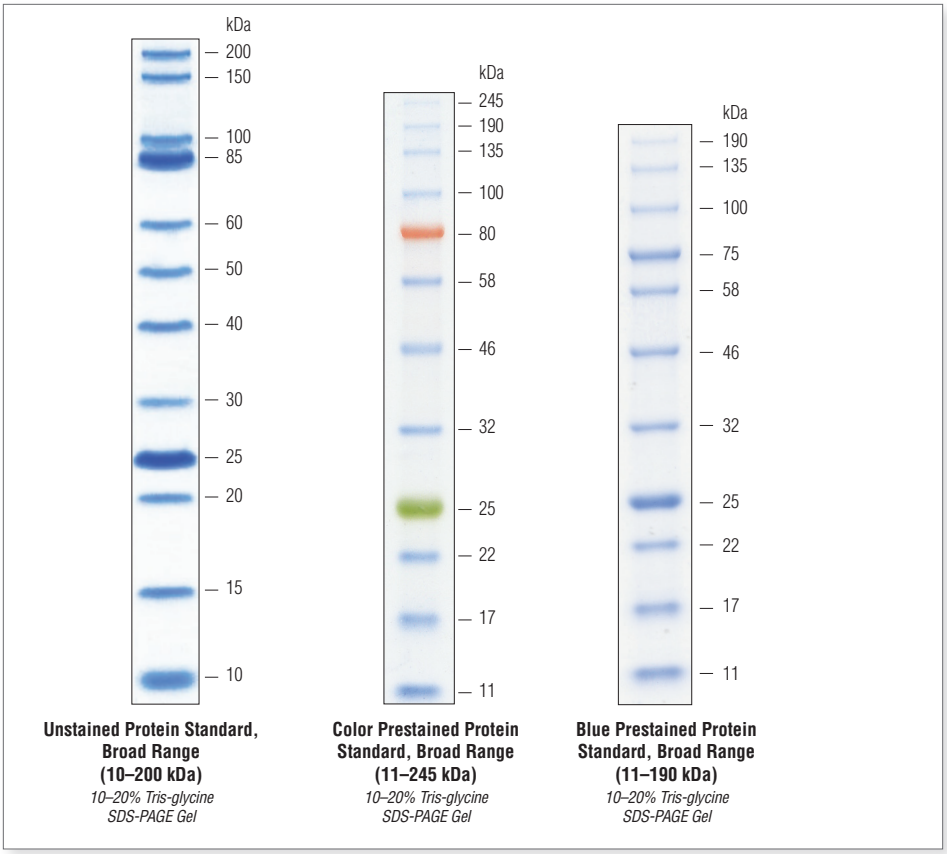
* NEB Express is the recommended strain for the pMAL Protein Fusion and Purification System.

Protein Standards

New England Biolabs offers a selection of highly pure protein standards. Sizes range from 10 to 250 kDa which is ideal for accurate molecular weight determination for a wide range of expressed proteins. We offer a blue prestained protein standard, as well as a colored prestained protein standard with multi-colored bands for easy identification, as well as an unstained protein standard. All three standards are provided pre-mixed with loading buffer and reducing agent.

ADVANTAGES

- Suitable for analysis of a wide range of expressed proteins
- Uniform band intensities and convenient band spacing
- Easy-to-identify reference bands



Unstained Protein Standard, Broad Range (10–200 kDa)
10–20% Tris-glycine SDS-PAGE Gel

Color Prestained Protein Standard, Broad Range (11–245 kDa)
10–20% Tris-glycine SDS-PAGE Gel

Blue Prestained Protein Standard, Broad Range (11–190 kDa)
10–20% Tris-glycine SDS-PAGE Gel

Ordering Information

PRODUCT	NEB #	SIZE
Unstained Protein Standard, Broad Range (10–200 kDa)	P7704S/L	150/750 gel lanes
Color Prestained Protein Standard, Broad Range (11–245 kDa)	P7712S/L	150/750 gel lanes
Blue Prestained Protein Standard, Broad Range (11–190 kDa)	P7706S/L	150/750 gel lanes

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