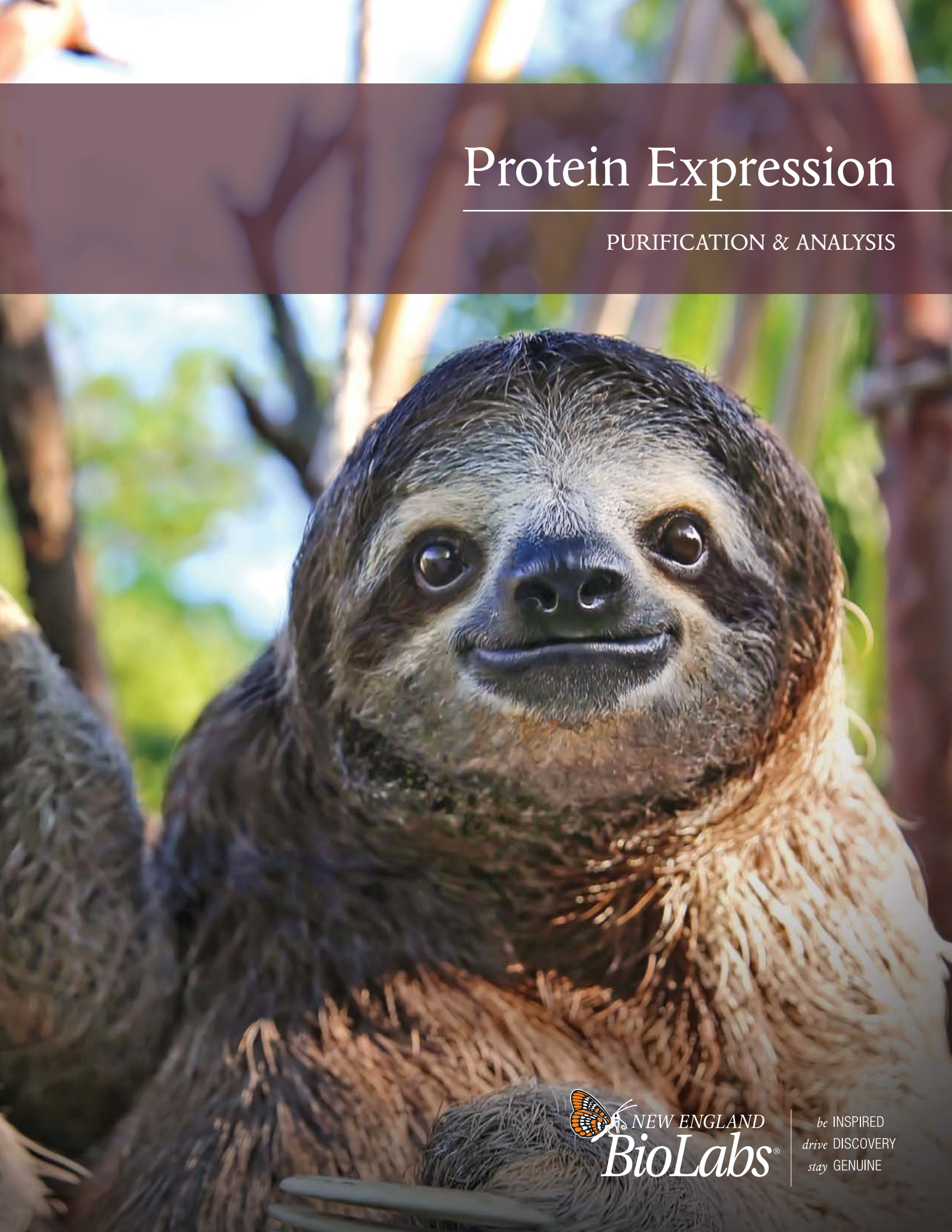
Protein Expression

PURIFICATION & ANALYSIS



NEW ENGLAND BioLabs®

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 the 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.

Our NEBExpress™ portfolio of products includes solutions for expression and purification of a wide range of proteins, 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. For the full list of products available, visit **www.neb.com/ProteinExpression**

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

APPLICATION	KIT	ADVANTAGES
High yield expression	NEBExpress™ MBP 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
	NEBExpress Cell-free *E. coli* Protein Synthesis System	Uses a highly active cell extract and T7 RNA Polymerase promoter to routinely achieve yields of 0.5 mg/ml.
Cell-free expression	NEBExpress Cell-free *E. coli* Protein Synthesis System	Quickly generates analytical amounts of protein from DNA plasmids or linear templates; amenable to scale-up
	PURExpress® *In Vitro* Protein Synthesis Kits	Quickly generates analytical amounts of protein from DNA plasmids or linear templates
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	NEBExpress MBP 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
	NEBExpress MBP 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
	NEBExpress Cell-free *E. coli* Protein Synthesis System	Compatible with all types of affinity purification; target protein can be synthesized from any T7 promoter vector
Post-translational modification	*K. lactis* Protein Expression Kit	Secretion of both *N*- and *O*- glycosylated proteins
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)
	PURExpress *In Vitro* Protein Synthesis Kits	Cell-free environment not affected by "toxicity" of expressed protein
	NEBExpress Cell-free *E. coli* Protein Synthesis System	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
	NEBExpress MBP Protein Fusion and Purification System	Start of protein is fused adjacent to protease site

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

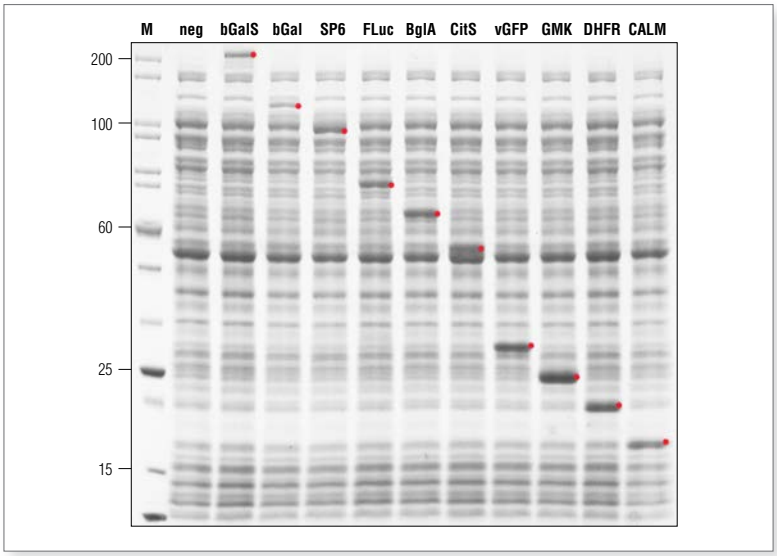
NEBExpress Cell-free *E. coli* Protein Synthesis System

Cell Free Expression

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.

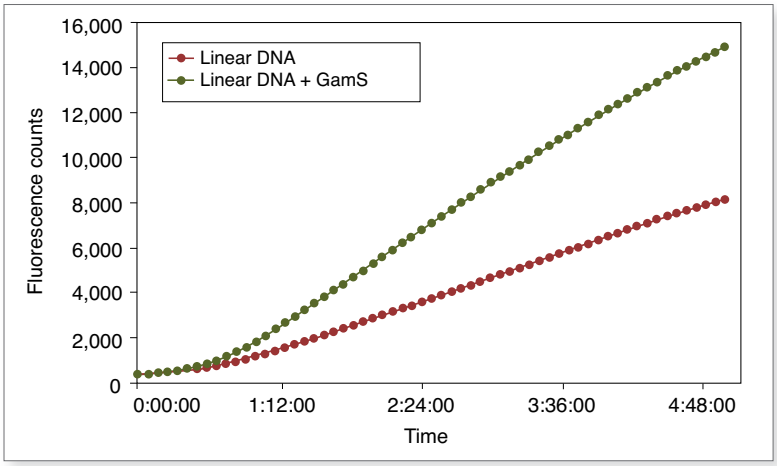
The NEBExpress Cell-free *E. coli* Protein Synthesis System contains all the components required for protein synthesis, except for the target template DNA. It is a combination of a highly active cell extract from a genetically engineered strain, a reaction buffer, and an optimized T7 RNA Polymerase, which together yield robust expression of a wide variety of protein targets ranging from 17 to 230 kDa.

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.

FEATURES

- 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

APPLICATIONS

- Quickly generate analytical amounts of protein for further characterization
- High throughput screening and liquid handling
- Epitope mapping and protein folding
- Expression of toxic proteins

Ordering Information

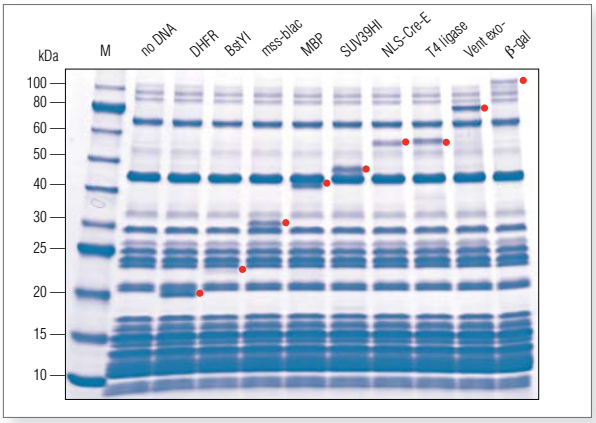
PRODUCT	SIZE
NEBExpress Cell-free *E. coli* Protein Synthesis System (NEB #E5360S/L)	10/100 rxns
NEBExpress GamS Nuclease Inhibitor (NEB #P0774S)	75 µg

PURExpress® *In Vitro* Protein Synthesis Kit

Cell Free Expression

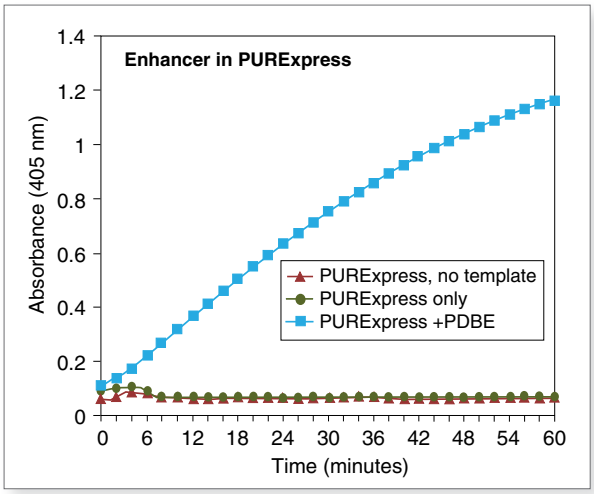
A rapid method for gene expression analysis, PURExpress is a novel cell-free transcription/translation system reconstituted from purified components necessary for *E. coli* translation. Express a wide range of proteins free of modification or degradation by simply mixing two tubes followed by the addition 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.

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.

FEATURES

- Suitable for circular or linear DNA template
- Visualize synthesized protein directly on a Coomassie stained gel
- Protein expression in approximately 2 hours
- Templates can be plasmid DNA linear DNA or mRNA
- 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	SIZE
PURExpress *In Vitro* Protein Synthesis Kit (NEB #E6800S/L)	10/100 rxns
PURExpress Δ Ribosome Kit (NEB #E3313S)	10 rxns
PURExpress Δ (aa, tRNA) Kit (NEB #E6840S)	10 rxns
PURExpress Δ RF123 Kit (NEB #E6850S)	10 rxns
PURExpress Disulfide Bond Enhancer (NEB #E6820S)	50 rxns
E. coli Ribosome (NEB #P0763S)	1 mg

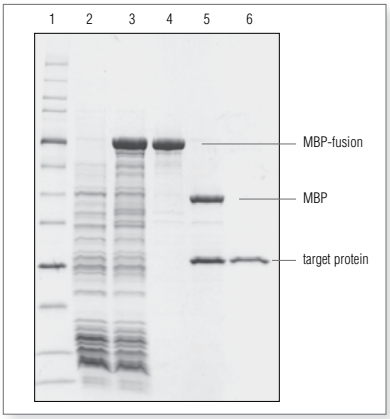
For additional information, companion products and kit components sold separately, please visit www.neb.com. Licensing information for these products can be found on our website.

NEBExpress MBP Fusion and 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 the NEBExpress MBP Fusion and Purification System



SDS-polyacrylamide gel electrophoresis of fractions from the Amylose affinity purification of MBP6-TEV-paramyosin ΔSal.

FEATURES

- Reliable *E. coli* expression: substantial yields (up to 100 mg/L) in more than 75% of the cases tested
- Fusion to MBP significantly enhances proper folding of target proteins
- Two-step purification: amylose elution followed by TEV Protease (NEB #P8112) cleavage and Ni resin isolation, results in a highly pure tag-free protein

Ordering Information

PRODUCT	SIZE
NEBExpress MBP Fusion and Purification System (NEB #E8201S)	1 set

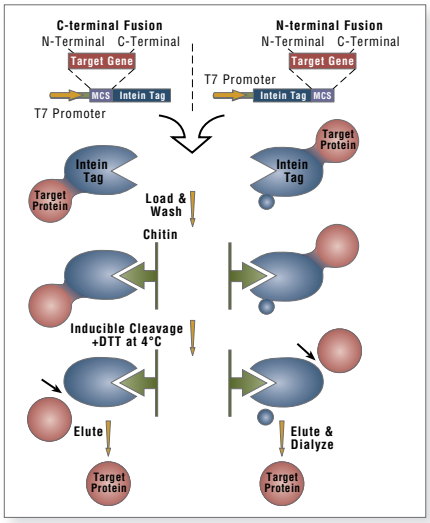
For additional information, companion products and kit components sold separately, please visit www.neb.com.

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



FEATURES

- 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

Ordering Information

PRODUCT	SIZE
IMPACT Kit (NEB #E6901S)	1 set

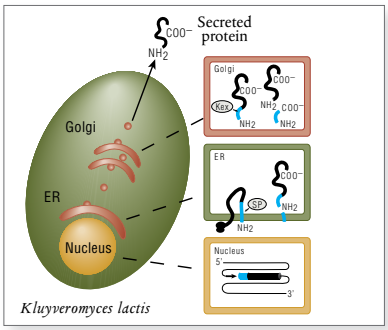
For additional information, companion products and kit components sold separately, please visit www.neb.com.

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.

FEATURES

- 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

Ordering Information

PRODUCT	SIZE
K. lactis Protein Expression Kit (NEB #E1000S)	1 set

For additional information, companion products and kit components sold separately, please visit www.neb.com.

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. 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.

FEATURES

- T1 phage resistance (*fhuA2*)
- Convenient formats available
- Bulk sales capabilities with custom packaging formats
- Free of animal products
- Deficient in proteases Lon/OmpT
- Does 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
SHuffle Express Competent *E. coli*	• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • Protease deficient/B strain	C3028J	12 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	C3029J	12 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	C3030J	12 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	C3026J	12 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	C2528J	12 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 NEBExpress MBP Fusion and Purification System.

Purification Beads, Columns and Resins

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.

Product Selection Chart

	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	IMMUNOPRECIPITATION	CELL SEPARATION/ CELL SORTING
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)				●					●	
Goat Anti-Mouse IgG Magnetic Beads (NEB #S1431)				●					● (Mouse IgGs)	●
Goat Anti-Rabbit IgG Magnetic Beads (NEB #S1432)				●					● (Rabbit IgGs)	●
Goat Anti-Rat IgG Magnetic Beads (NEB #S1433)				●					● (Rat IgGs)	●
Magnetic mRNA Isolation Kit (NEB #S1550)				●				●		

Companion Product:

TEV Protease

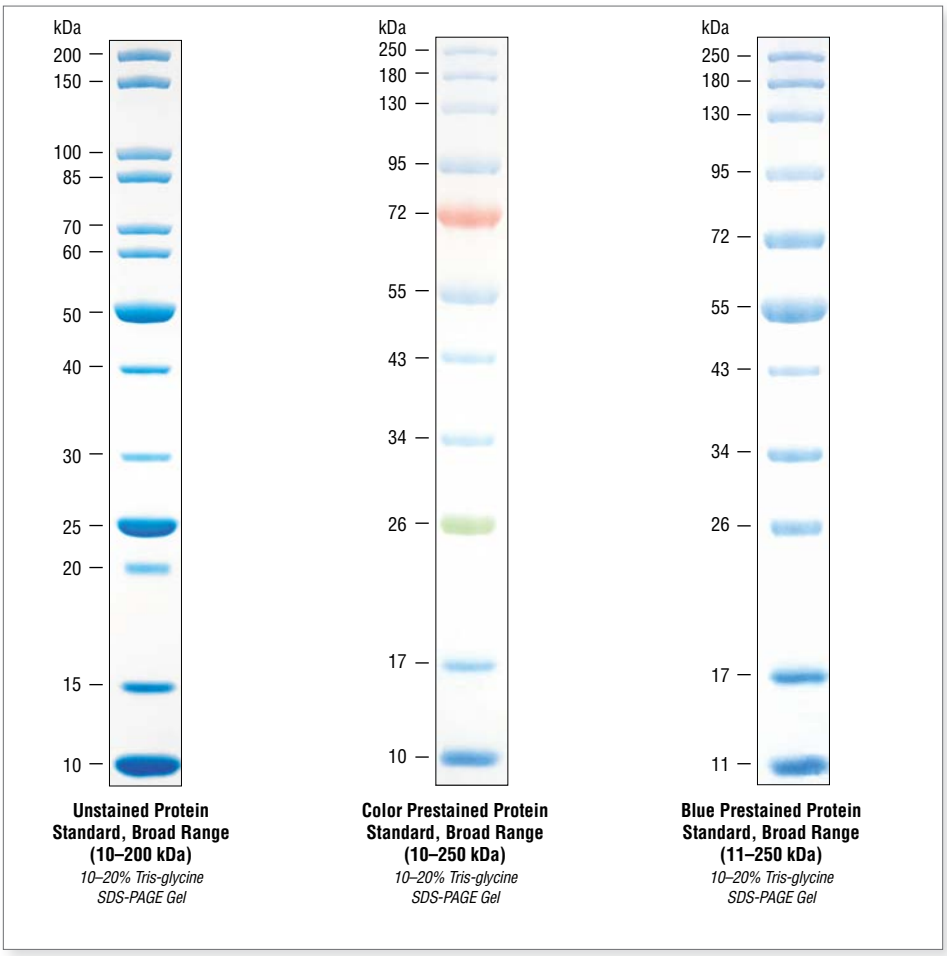
A highly-specific cysteine protease that is ideal for removal of affinity tags, such as maltose binding protein (MBP) or poly-histidine (His-tag) from fusion proteins.

Ordering Information

PRODUCT	SIZE
TEV Protease (NEB #P8112S)	1,000 units

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, and an unstained protein standard. All three standards are provided pre-mixed with loading buffer and reducing agent.



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

FEATURES

- Suitable for a wide range of expressed proteins
- Uniform band intensities
- Convenient band spacing
- Easy-to-identify reference bands
- The unstained protein standard allows accurate molecular weight determination when performing SDS-PAGE analysis
- Color protein standard contains two colored reference bands for unambiguous detection

Ordering Information

PRODUCT	NEB #	SIZE
Unstained Protein Standard, Broad Range (10–200 kDa)	P7717S/L	150/750 gel lanes
Color Prestained Protein Standard, Broad Range (10–250 kDa)	P7719S/L	150/750 gel lanes
Blue Prestained Protein Standard, Broad Range (11–250 kDa)	P7718S/L	150/750 gel lanes

One or more of these products are covered by patents, trademarks and/or copyrights owned or controlled by New England Biolabs, Inc. For more information, please email us at gbd@neb.com. The use of these products may require you to obtain additional third party intellectual property rights for certain applications.

Your purchase, acceptance, and/or 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 a duly authorized officer of NEB.

© Copyright 2019, New England Biolabs, Inc.; all rights reserved.

NEW ENGLAND BioLabs GmbH
www.neb-online.de
New England Biolabs GmbH, Brüningstr. 50, Geb. B852, 65926 Frankfurt/Main, Germany
Tel: +49/(0)69/305-23140 Toll Free: (Germany) 0800/246-5227 Toll Free: (Austria) 00800/246-52277 Fax: +49/(0)69/305-23149 e-mail: info.de@neb.com

NEW ENGLAND BioLabs
www.neb-online.fr
New England Biolabs France, Genopole Campus 1, Bât. 6, 5 rue Henri Desbruères, 91030 Evry cedex, France
Tel.: 0800 100 632 (Customer Service), Tel.: 0800 100 633 (Technical Service), FAX.: 0800 100 610, info.fr@neb.com

Distributors
For a complete list of all NEB subsidiaries and distributors, please go to:
www.neb.com/international-support

PEB – Version 10.0 – 11/19
x/f/yNEB100