



Now includes Protein Extraction/  
Lysis products

# Protein Expression

PURIFICATION & ANALYSIS



NEW ENGLAND  
**BioLabs**<sup>®</sup> 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 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](http://www.neb.com/ProteinExpression)



Visit [www.neb.com](http://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 <sub>lac</sub> promoter
	<i>K. lactis</i> Protein Expression Kit	Uses the strong <i>LAC4</i> promoter; multiple integrations of plasmid results in higher yield
	IMPACT™ Kit	Uses the T7 promoter for high level regulated expression
	NEBExpress Cell-free <i>E. coli</i> 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 <i>E. coli</i> Protein Synthesis System	Quickly generates analytical amounts of protein from DNA plasmids or linear templates; amenable to scale-up
	PURExpress® <i>In Vitro</i> Protein Synthesis Kits	Quickly generates analytical amounts of protein from DNA plasmids or linear templates
Co-expression of multiple proteins	<i>K. lactis</i> Protein Expression Kit	Easily co-express 2–4 proteins
	PURExpress <i>In Vitro</i> 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 <i>E. coli</i> *
	<i>K. lactis</i> Protein Expression Kit	Utilizes <i>K. lactis</i> 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
	<i>K. lactis</i> Protein Expression Kit	Vectors are sold separately that generate fusions to MBP allowing for purification on amylose resin
	NEBExpress Cell-free <i>E. coli</i> Protein Synthesis System	Compatible with all types of affinity purification; target protein can be synthesized from any T7 promoter vector
Post-translational modification	<i>K. lactis</i> Protein Expression Kit	Secretion of both N- and O- glycosylated proteins
Secreted expression	<i>K. lactis</i> Protein Expression Kit	Eliminates cell lysis step, simplifying purification
Toxic proteins	<i>K. lactis</i> 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 <i>In Vitro</i> Protein Synthesis Kits	Cell-free environment not affected by “toxicity” of expressed protein
	NEBExpress Cell-free <i>E. coli</i> 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 <i>In Vitro</i> 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.

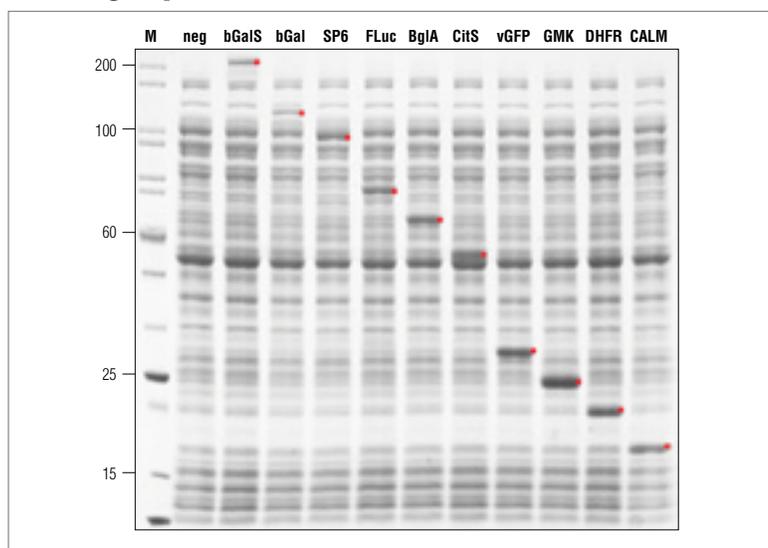
# NEBExpress® Cell-free *E. coli* Protein Synthesis System

## Cell-Free Protein 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.

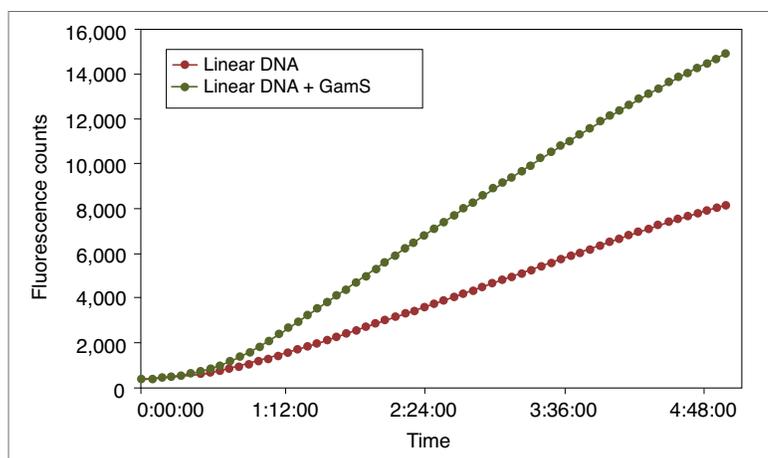
The NEBExpress Cell-free *E. coli* Protein Synthesis System contains all the components required for protein synthesis, and ready for you to add your target template DNA. This complete system includes a highly active cell extract from a genetically engineered strain, a reaction buffer, and an optimized 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  $\mu$ l 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  $\mu$ l reactions containing 100 ng linear template DNA, the components of the NEBExpress Cell-free *E. coli* Protein Synthesis System and 1.5  $\mu$ g 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
- Includes T7 RNA Polymerase and all components for coupled transcription/translation for your target template DNA
- 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

## 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	NEB #
NEBExpress Cell-free <i>E. coli</i> Protein Synthesis System (NEB #E5360S/L)	E5360S/L
NEBExpress GamS Nuclease Inhibitor	P0774S

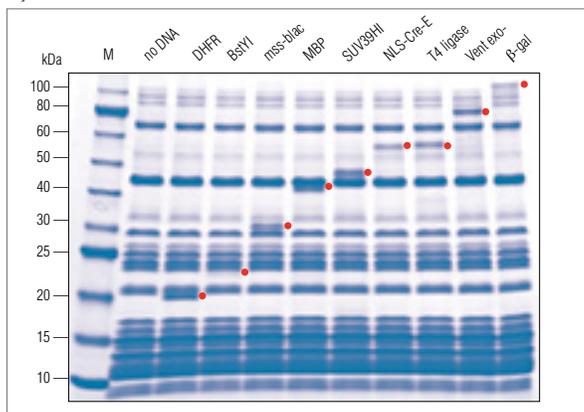
# PURExpress® *In Vitro* Protein Synthesis Kit

## Cell-Free Protein Expression

A rapid method for gene expression analysis, PURExpress is a 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 just two tubes, and then adding your template DNA. With results ready 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.

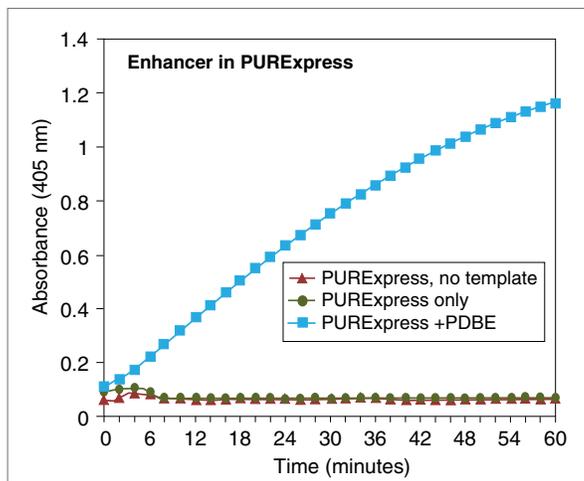
- The PURExpress *In Vitro* Protein Synthesis Kit is a comprehensive kit containing all components
- The PURExpress  $\Delta$  Ribosome Kit allows users to add their own ribosomes when performing protein translation experiments
- In the PURExpress  $\Delta$  RF 123 Kit, the three release factors are supplied separately, allowing the user to perform a protein synthesis reaction/ribosome display experiment with/without release factors of their choice
- The PURExpress  $\Delta$  (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  $\mu$ l 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
- Includes T7 RNA Polymerase and all components for coupled transcription/translation for your target template DNA

## 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 #
PURExpress <i>In Vitro</i> Protein Synthesis Kit	E6800S/L
PURExpress $\Delta$ Ribosome Kit	E3313S
PURExpress $\Delta$ (aa, tRNA) Kit	E6840S
PURExpress $\Delta$ RF123 Kit	E6850S
PURExpress Disulfide Bond Enhancer	E6820S
<i>E. coli</i> Ribosome	P0763S

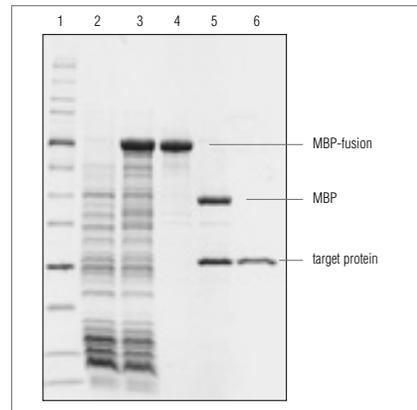
For additional information, companion products and kit components sold separately, please visit [www.neb.com](http://www.neb.com). Licensing information for these products can be found on our website.

## 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  $\Delta$ Sal.

## 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

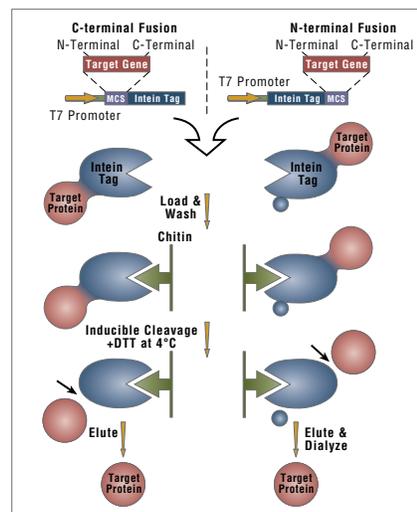
PRODUCT	NEB #
NEBExpress MBP Fusion and Purification System	E8201S

## 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	NEB #
IMPACT Kit	E6901S

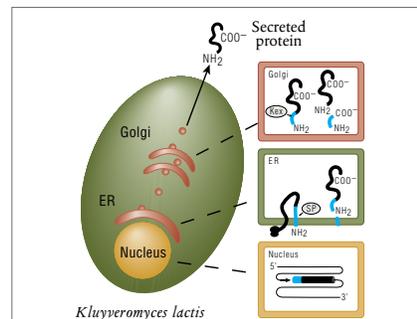
## *K. lactis*

## 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  $\alpha$ -MF domain (blue) and a desired protein (black) is expressed. A signal peptide in the  $\alpha$ -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  $\alpha$ -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	NEB #
<i>K.Lactis</i> Protein Expression Kit	E1000S

For additional information, companion products and kit components sold separately, please visit [www.neb.com](http://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
NEBExpress Competent <i>E. coli</i> *	<ul style="list-style-type: none"> <li>• Versatile non-T7 expression strain</li> <li>• Protease deficient</li> </ul>	C2523H/I	20 x 0.05 ml/6 x 0.2 ml
NEBExpress I <sup>q</sup> Competent <i>E. coli</i>	<ul style="list-style-type: none"> <li>• Control of IPTG induced expression from <math>P_{lac}</math>, <math>P_{lac}</math> and <math>P_{trc}</math></li> <li>• Protease deficient</li> </ul>	C3037I	6 x 0.2 ml
T7 Express Competent <i>E. coli</i>	<ul style="list-style-type: none"> <li>• Most popular T7 expression strain</li> <li>• Protease deficient</li> </ul>	C2566H/I	20 x 0.05 ml/6 x 0.2 ml
T7 Express <i>lysY</i> Competent <i>E. coli</i>	<ul style="list-style-type: none"> <li>• T7 expression</li> <li>• Protease deficient</li> <li>• Better reduction of basal expression</li> </ul>	C3010I	6 x 0.2 ml
T7 Express <i>lysY/I<sup>q</sup></i> Competent <i>E. coli</i>	<ul style="list-style-type: none"> <li>• T7 expression</li> <li>• Protease deficient</li> <li>• Highest level of expression control</li> </ul>	C3013I	6 x 0.2 ml
SHuffle Express Competent <i>E. coli</i>	<ul style="list-style-type: none"> <li>• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm</li> <li>• Protease deficient/B strain</li> </ul>	C3028J	12 x 0.05 ml
SHuffle T7 Express Competent <i>E. coli</i>	<ul style="list-style-type: none"> <li>• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm</li> <li>• T7 expression</li> <li>• Protease deficient/B strain</li> </ul>	C3029J	12 x 0.05 ml
SHuffle T7 Express <i>lysY</i> Competent <i>E. coli</i>	<ul style="list-style-type: none"> <li>• T7 expression</li> <li>• Protease deficient/B strain</li> <li>• Tightly controlled expression of toxic proteins</li> <li>• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm</li> </ul>	C3030J	12 x 0.05 ml
SHuffle T7 Competent <i>E. coli</i>	<ul style="list-style-type: none"> <li>• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm</li> <li>• T7 expression/K12 strain</li> </ul>	C3026J	12 x 0.05 ml
BL21 Competent <i>E. coli</i>	<ul style="list-style-type: none"> <li>• Routine expression for non-T7 Vectors</li> <li>• Protease deficient</li> </ul>	C2530H	20 x 0.05 ml
BL21(DE3) Competent <i>E. coli</i>	<ul style="list-style-type: none"> <li>• Routine T7 Expression</li> <li>• Protease deficient</li> </ul>	C2527H/I	20 x 0.05 ml/6 x 0.2 ml
Lemo21(DE3) Competent <i>E. coli</i>	<ul style="list-style-type: none"> <li>• Tunable T7 Expression for difficult targets</li> <li>• Protease deficient</li> </ul>	C2528J	12 x 0.05 ml
NiCo21(DE3) Competent <i>E. coli</i>	<ul style="list-style-type: none"> <li>• Expression and purification of His-tagged proteins</li> <li>• Protease deficient</li> </ul>	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

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
<b>NEBExpress Ni-NTA Magnetic Beads</b> (NEB #S1423)	● (His-tag)			●		●				
<b>NEBExpress Ni Spin Columns</b> (NEB #S1427)	● (His-tag)			●		●				
<b>NEBExpress Ni Resin</b> (NEB #S1428)	● (His-tag)	●	●			●				
<b>Amylose Resin</b> (NEB #E8021)	● (MBP)	●				●				
<b>Amylose Resin High Flow</b> (NEB #E8022)	● (MBP)	●	●			●				
<b>Amylose Magnetic Beads</b> (NEB #E8035)	● (MBP)			●		●				
<b>Anti-MBP Magnetic Beads</b> (NEB #E8037)	● (MBP)			●		●				
<b>Chitin Resin</b> (NEB #S6651)	● (intein-CBD tag)	●				●				
<b>Chitin Magnetic Beads</b> (NEB #E8036)	● (intein-CBD tag)			●		●				
<b>Oligo d(T)<sub>25</sub> Magnetic Beads</b> (NEB #S1419)				●			●	●		
<b>Streptavidin Magnetic Beads</b> (NEB #S1420)				●	●	● (biotinylated bait)	● (biotinylated bait)			
<b>Hydrophilic Streptavidin Magnetic Beads</b> (NEB #S1421)				●	●	● (biotinylated bait)	● (biotinylated bait)			
<b>Protein A Magnetic Beads</b> (NEB #S1425)				●					●	
<b>Protein G Magnetic Beads</b> (NEB #S1430)				●					●	
<b>Magnetic mRNA Isolation Kit</b> (NEB #S1550)				●				●		

## Companion Products:

### Anti-MBP Monoclonal Antibody

Anti-MBP Monoclonal Antibody is a murine anti-maltose binding protein (MBP) antibody, isotype IgG2a. It is purified from tissue culture supernatant by protein A affinity chromatography.

### Anti-CBD Monoclonal Antibody

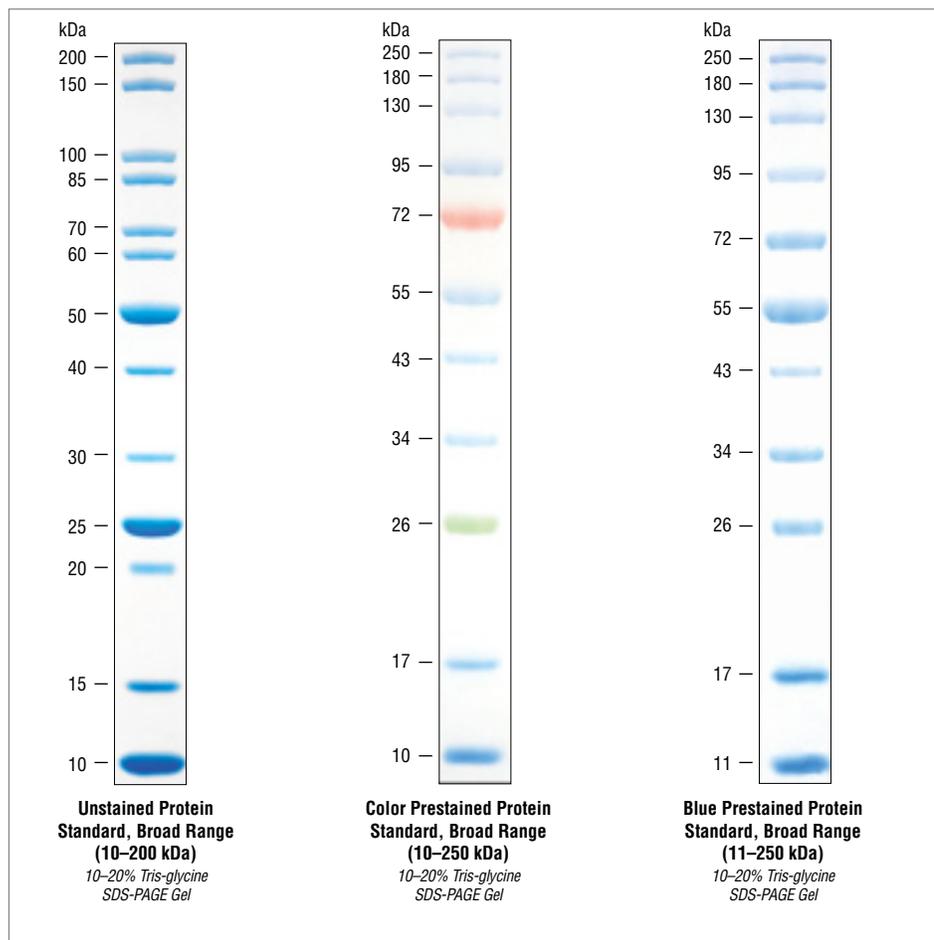
Anti-CBD Monoclonal Antibody is a murine anti-chitin binding domain (CBD) antibody, isotype IgG1. It has high purity and specificity for chitin binding domain tag, and is verified for use in both Western blotting and ELISA.

## Ordering Information

PRODUCT	NEB #
Anti-MBP Monoclonal Antibody	E8032S/L
Anti-CBD Monoclonal Antibody	E8034S

# 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.



## 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

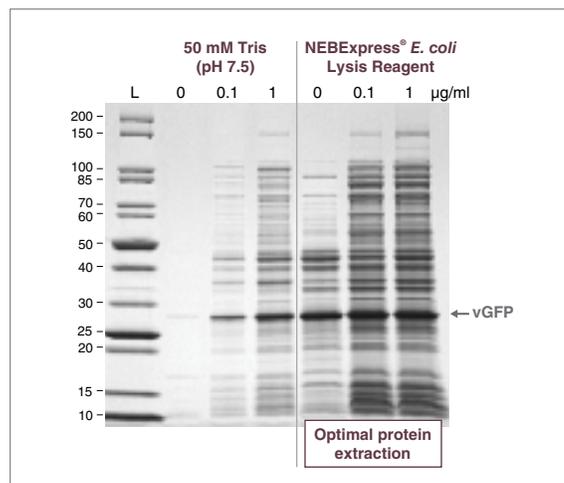
## Ordering Information

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

## NEBExpress T4 Lysozyme

NEBExpress T4 Lysozyme is a recombinant murein hydrolase that breaks down the bacterial cell wall by hydrolyzing the  $\beta$ -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 in 50 mM Tris-HCl pH 7.5 or in NEBExpress *E. coli* Lysis Reagent (NEB #P8116S): 4 UOD<sub>600</sub> 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 µg per 1 ml of cell suspension. The soluble proteins were harvested by centrifugation and analyzed on SDS-PAGE.

## Features

- 200-fold more active than chicken egg white lysozyme
- Recombinant and animal free
- 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.

## Ordering Information

PRODUCT	NEB #
NEBExpress T4 Lysozyme	P8115S/L

## NEBExpress *E. coli* Lysis Reagent

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.

## Features

- 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

## Ordering Information

PRODUCT	NEB #
NEBExpress <i>E. coli</i> Lysis Reagent	P8116S/L

## Protease Selection Chart

NEB offers several proteases that can be used for the removal of affinity tags and protein processing

PRODUCT NAME	RECOGNITION SITE	NEB #
TEV Protease	ENLYFQ <sup>▼</sup> (S/G/A/M/C/H)	P8112S
Factor Xa Protease	Ile-Glu/Asp-Gly-Arg <sup>▼</sup>	P8010S/L
Enterokinase, light chain	Asp-Asp-Asp-Asp-Lys <sup>▼</sup>	P8070S/L
Furin	Arg-X-X-Arg <sup>▼</sup>	P8077S/L

## 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/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.

### Features

- 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 Ni Spin Columns (NEB #S1427) or NEBExpress Ni-NTA Magnetic Beads (NEB #S1423)
- Engineered to prevent autolysis and improve stability
- Active in a wide range of buffers, with optimal activity between pH 6.0 and 8.0

### Ordering Information

PRODUCT	NEB #
TEV Protease	P8112S

## Factor Xa Protease

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.

### Features

- Allows for convenient removal of MBP by loading the MBP-fusion digest onto amylose resin and collecting protein of interest in the flow through

### Ordering Information

PRODUCT	NEB #
Factor Xa Protease	P8010S/L

## Enterokinase, light chain

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.

### Features

- Enterokinase, light chain can be removed from a reaction using Trypsin inhibitor-Agarose

### Ordering Information

PRODUCT	NEB #
Enterokinase, light chain	P8070S/L

## Furin

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-Arg. However, the enzyme prefers the site Arg-X-(Lys/Arg)-Arg. Furin is inhibited by EGTA,  $\alpha$ 1-Antitrypsin Portland and polyarginine compounds.

### Features

- An additional arginine at the P6 position appears to enhance cleavage

### Ordering Information

PRODUCT	NEB #
Furin	P8077S/L

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