

PROTEIN EXPRESSION & PURIFICATION

New England Biolabs is committed to providing advanced tools for protein expression and purification. As a leading supplier for reagents in the biotechnology field, we understand the importance of convenient and easy-to-use systems for high level expression and sample purification. We invite you to review our growing range of expression systems resulting from our experience in cloning, overexpression and purification.

There are many factors to consider when choosing the optimal system for protein expression and purification. Each kit offers different advantages which enable you to choose the system that best suits your protein purification needs. Various applications along with the recommended kits are highlighted below.

DNA CLONING

DNA AMPLIFICATION & PCR

EPIGENETICS

RNA ANALYSIS

SAMPLE PREP FOR NEXT GEN SEQUENCING

PROTEIN EXPRESSION & ANALYSIS

CELLULAR ANALYSIS

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
	<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
CELL-FREE EXPRESSION	PURExpress® <i>In Vitro</i> Protein Synthesis Kits	Quickly generates analytical amounts of protein
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	pMAL™ 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
	pMAL™ 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
POST-TRANSLATIONAL MODIFICATION	<i>K. lactis</i> 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	<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
	pMAL™ Protein Fusion and Purification System	Can export toxic proteins into periplasmic space
	PURExpress® <i>In Vitro</i> 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)
	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
	pMAL™ Protein Fusion and Purification System	Start of protein is fused adjacent to protease site

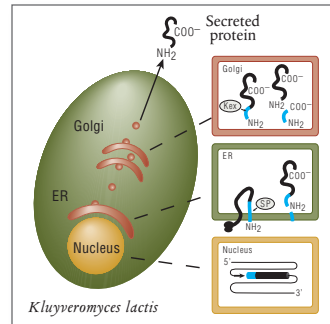
* Kapust and Waugh (1999) *Protein Science*, 8, 1668–1674.

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:

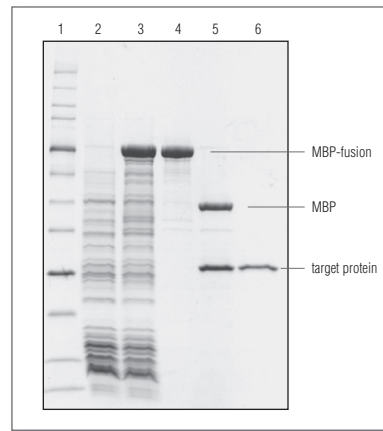
- High yield protein expression
- Rapid high cell density growth
- Methanol-free growth media
- Vector integration enhances strain stability
- Multiple protein expression

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 Δ Sal.

Advantages:

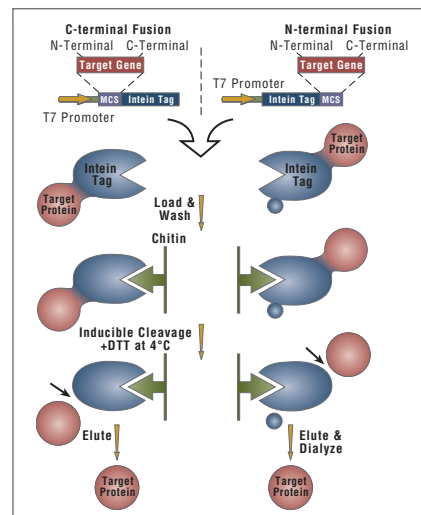
- Reliable *E. coli* expression: substantial yields (up to 100 mg/L) in more than 75% of the cases tested
- Expression in either the cytoplasm or periplasm: periplasmic expression enhances folding of proteins with disulfide bonds

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

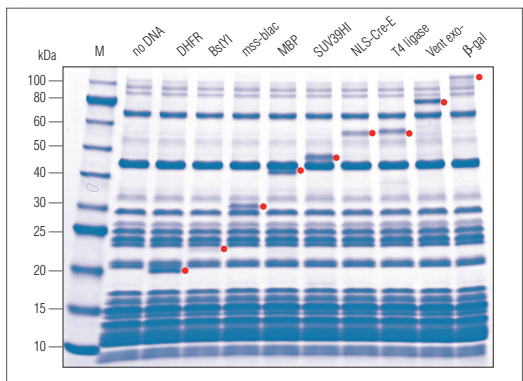
Visit www.neb.com for FAQs, protocols and citation lists.

PURExpress® *In Vitro* Protein Synthesis Kit

Cell Free Expression

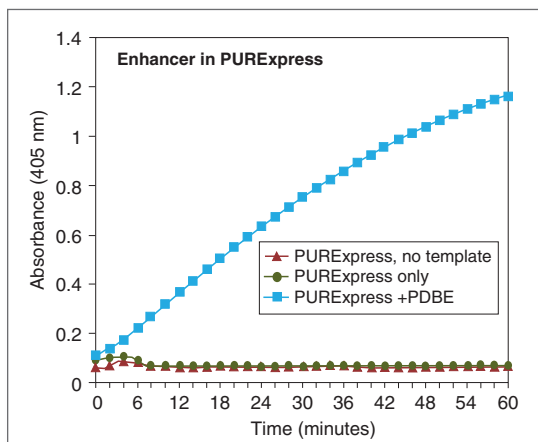
A rapid method for gene expression analysis, PURExpress® is a novel cell-free transcription/translation system reconstituted from purified components necessary for *E. coli* translation. Express a wide range of proteins free of modification or degradation by simply mixing two tubes followed by the addition 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 μ l 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 #
<i>K. lactis</i> Protein Expression Kit	E1000S
pMAL™ Protein Fusion and Purification System	E8200S
IMPACT™ Kit	E6901S
PURExpress® <i>In Vitro</i> Protein Synthesis Kit	E6800S
PURExpress® Δ Ribosome Kit	E3313S
PURExpress® Δ (aa, tRNA) Kit	E6840S
PURExpress® Δ 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. 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

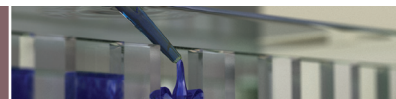
Expression Strains

CHARACTERISTICS	STRAIN	NEB #	SIZE
• Routine expression for non-T7 Vectors	BL21 Competent <i>E. coli</i>	C2530H	20 x 0.05 ml
• Routine T7 Expression	BL21(DE3) Competent <i>E. coli</i>	C2527H/1	20 x 0.05 ml/6 x 0.2 ml
• Tunable T7 Expression for difficult targets	Lemo21(DE3) Competent <i>E. coli</i>	C2528H	6 x 0.05 ml
• Expression and purification of His-tagged proteins	NiCo21(DE3) Competent <i>E. coli</i>	C2529H	20 x 0.05 ml
• Versatile non-T7 expression strain • Protease deficient	NEB Express Competent <i>E. coli</i> *	C2523H/1	20 x 0.05 ml/6 x 0.2 ml
• Control of IPTG induced expression from P _{lac} , P _{trc} and P _{trc} • Protease deficient	NEB Express I ^q Competent <i>E. coli</i>	C3037H/1	20 x 0.05 ml/6 x 0.2 ml
• Most popular T7 expression strain • Protease deficient	T7 Express Competent <i>E. coli</i>	C2566H/1	20 x 0.05 ml/6 x 0.2 ml
• T7 expression • Protease deficient • Reduced basal expression	T7 Express I ^q Competent <i>E. coli</i>	C3016H/1	20 x 0.05 ml/6 x 0.2 ml
• T7 expression • Protease deficient • Better reduction of basal expression	T7 Express <i>lysY</i> Competent <i>E. coli</i>	C3010H/1	20 x 0.05 ml/6 x 0.2 ml
• T7 expression • Protease deficient • Highest level of expression control	T7 Express <i>lysY/I^q</i> Competent <i>E. coli</i>	C3013H/1	20 x 0.05 ml/6 x 0.2 ml
• T7 expression • Protease deficient • SeMet labeling for protein crystallography	T7 Express Crystal Competent <i>E. coli</i>	C3022H/1	20 x 0.05 ml/6 x 0.2 ml
• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • Protease deficient/B strain	SHuffle® Express Competent <i>E. coli</i>	C3028H	6 x 0.05 ml
• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression • Protease deficient/B strain	SHuffle® T7 Express Competent <i>E. coli</i>	C3029H	6 x 0.05 ml
• 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	SHuffle® T7 Express <i>lysY</i> Competent <i>E. coli</i>	C3030H	6 x 0.05 ml
• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • K12 strain	SHuffle® Competent <i>E. coli</i>	C3025H	6 x 0.05 ml
• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression/K12 strain	SHuffle® T7 Competent <i>E. coli</i>	C3026H	6 x 0.05 ml
• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm • T7 expression/K12 strain • Tight control/expression of toxic proteins	SHuffle® T7 <i>lysY</i> Competent <i>E. coli</i>	C3027H	6 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.

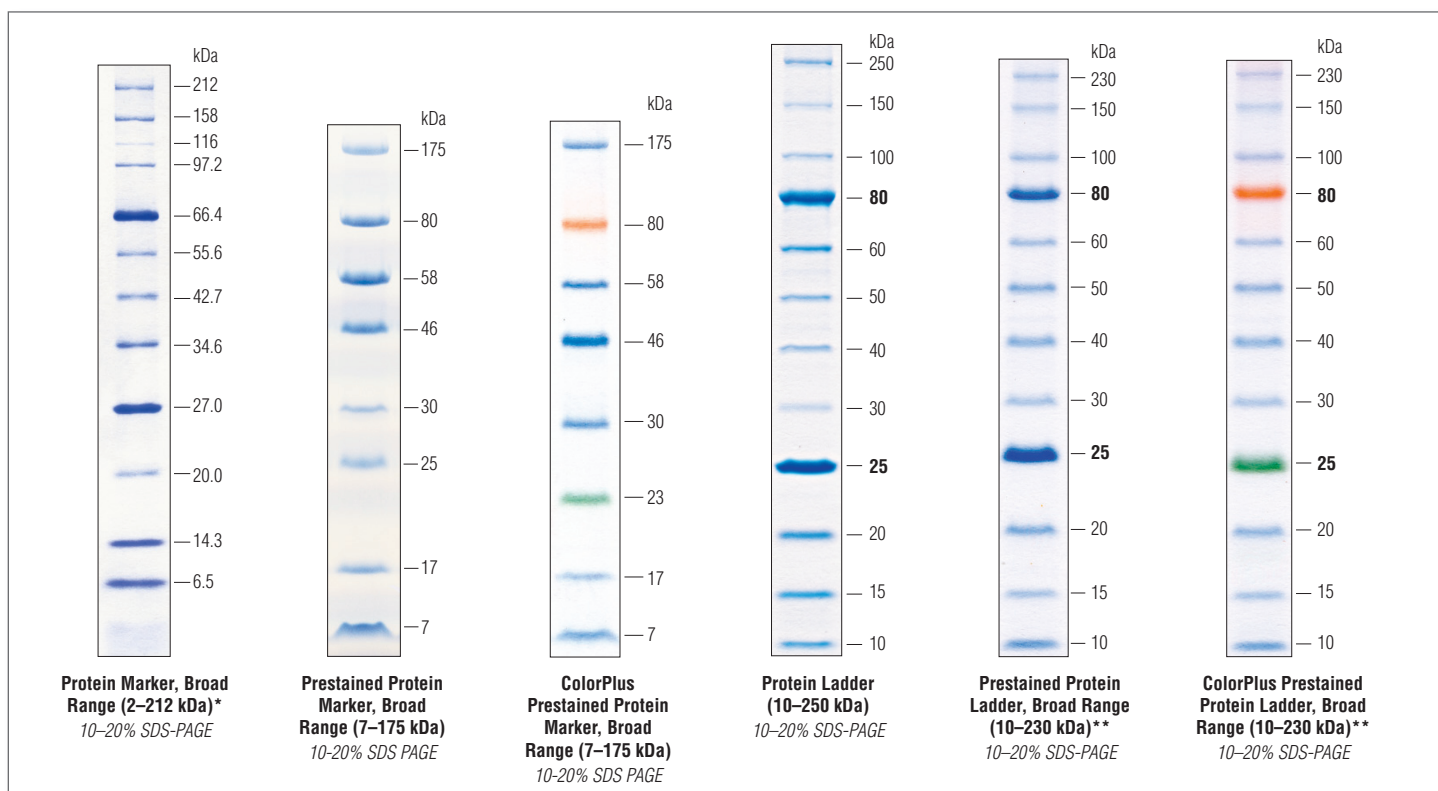


Protein Markers & Ladders

For protein expression analysis, NEB offers a selection of highly pure protein markers and ladders. Our protein markers are available unstained (2.3 to 212 kDa) and prestained (7 to 175 kDa). For easy identification, we also offer a ColorPlus prestained marker containing two colored bands. The newest addition 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. The Protein Ladder is also available in a prestained format or prestained with two colored bands for additional convenience.

Advantages:

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



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

** Optimized for Tris-Glycine gels.

Ordering Information

PRODUCT	NEB #	SIZE
Protein Marker, Broad Range (2–212 kDa)	P7702S/L	150/750 mini-gel lanes
Prestained Protein Marker, Broad Range (7–175 kDa)	P7708V/S/L	83/175/875 mini-gel lanes
ColorPlus Prestained Protein Marker, Broad Range (7–175 kDa)	P7709V/S/L	83/175/875 mini-gel lanes
Protein Ladder (10–250 kDa)	P7703S	100 mini-gel lanes
Prestained Protein Ladder, Broad Range (10–230 kDa)	P7710S	100 mini-gel lanes
ColorPlus Prestained Protein Ladder, Broad Range (10–230 kDa)	P7711S	100 mini-gel lanes

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.



www.neb.com

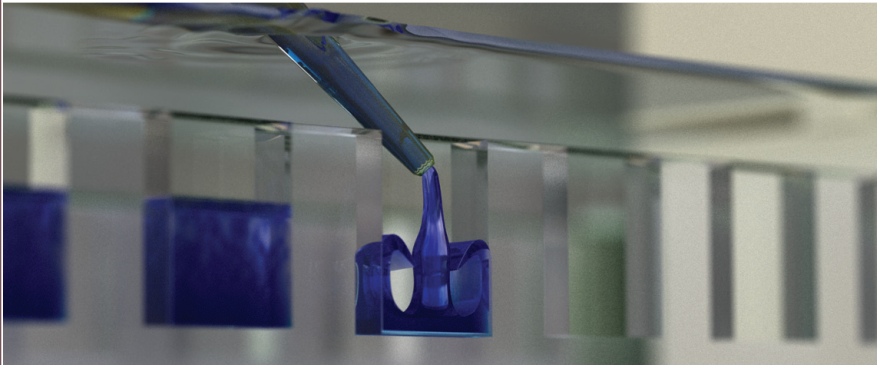
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PEB

Version 3.0

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