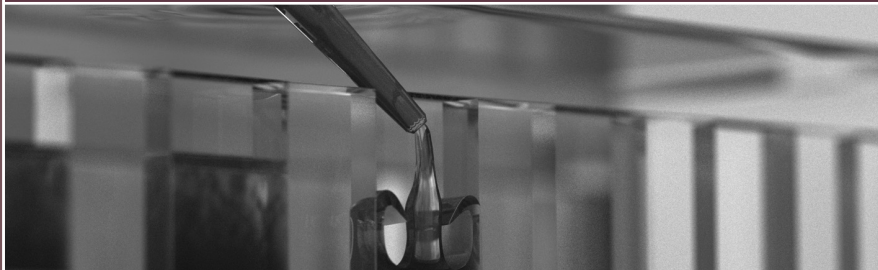


# PROTEIN EXPRESSION & PURIFICATION



CLONING & MAPPING

DNA AMPLIFICATION & PCR

RNA ANALYSIS

**PROTEIN EXPRESSION & ANALYSIS**

GENE EXPRESSION & CELLULAR ANALYSIS

# 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.

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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 <sub>tac</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 higher expression and tight control
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
	pMAL™ Protein Fusion and Purification System	Fusion to MBP allows for purification on amylose resin
	<i>K. lactis</i> Protein Expression Kit	Vectors 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, simplifying purification
TOXIC GENES	<i>K. lactis</i> Protein Expression Kit	Utilizes a <i>LAC4</i> promoter that has been modified to lack background expression in <i>E. coli</i>
	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
PROTEIN LABELING OR LIGATION	IMPACT™ Kit	Elutes proteins with reactive ends (N-terminal cysteine and/or C-terminal thioester)
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

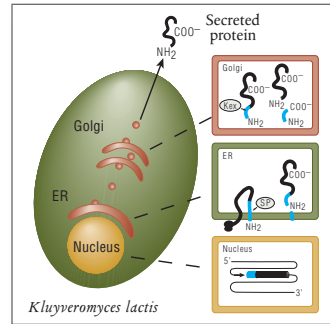
\* Kapust and Waugh (1999) *Protein Science*, 8, 1668–1674.

## *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  $\alpha$ -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.

### Advantages:

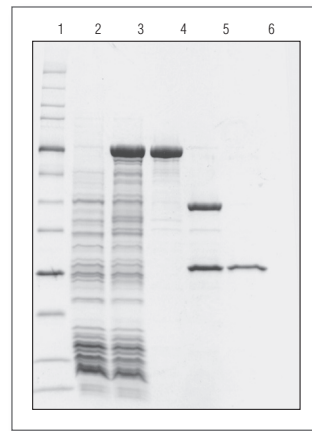
- High yield protein expression
- Rapid high cell density growth
- Methanol-free growth media
- Vector integration enhances strain stability
- Multiple protein expression

## 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 purification of MBP-paramyosin  $\Delta$ Sal stained with Coomassie Blue.

### Advantages:

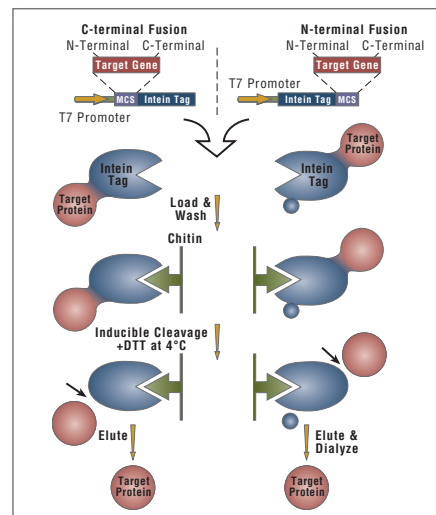
- Reliable *E. coli* expression: substantial yields (up to 100 mg/L) in more than 75% of the cases tested
- Expression in either the cytoplasm or periplasm: periplasmic expression enhances folding of proteins with disulfide bonds

## 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:

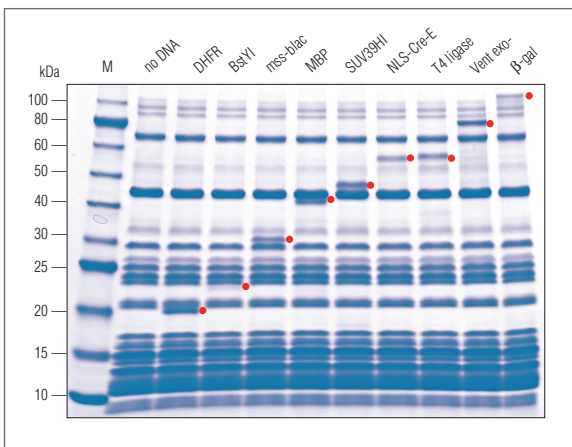
- Yields proteins with native sequence
- Desired protein is released without the use of separate, expensive proteases
- One-step purification
- Uses T7 promoter for higher levels of expression

## 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.

### 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).

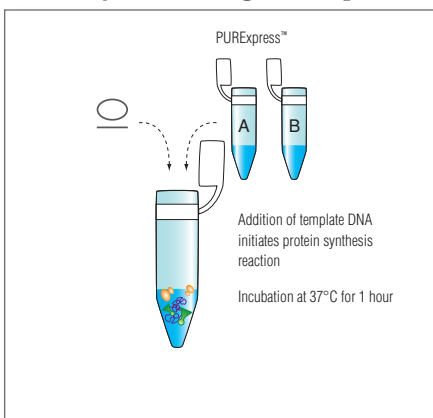
### Advantages:

- Cleaner system eliminates sample degradation
- Synthesized protein can often be visualized on a Coomassie stained gel
- Requires only the mixing of two tubes followed by the addition of template DNA
- Results available in a few hours
- Quickly generates analytical amounts of protein for further characterization

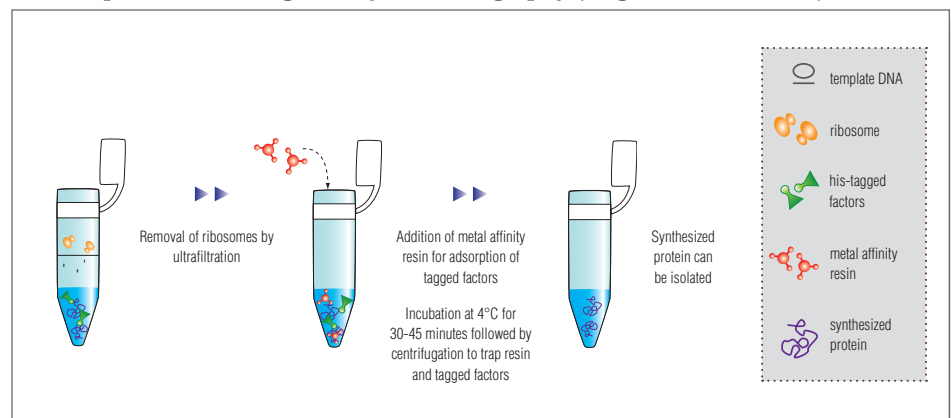
### Applications:

- Gene expression analysis
- Confirmation of open reading frames
- Examination of the effects of mutations on ORFs
- Generation of truncated proteins to identify active domains and functional residues
- Introduction of modified, unnatural or labeled amino acids
- Epitope mapping
- Expression of toxic proteins

### Protein synthesis using PURExpress™



### Reverse purification using affinity chromatography (reagents not included)



### Ordering Information

PRODUCT	CATALOG #	PRICE
<i>K. lactis</i> Protein Expression Kit	E1000S	\$595
pMAL™ Protein Fusion and Purification System	E8200S	\$450
IMPACT™ Kit	E6901S	\$259
PURExpress™ <i>In Vitro</i> Protein Synthesis Kit	E6800S	\$220

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.

# 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. NEB Express and T7 Express are offered with varying levels of control. Several strains are available with the added control of IPTG-induced expression of non-T7 plasmids by *lacI<sup>q</sup>*. Only NEB offers exceptional control of expression from 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	CATALOG #	SIZE	PRICE
<ul style="list-style-type: none"> <li>• Versatile expression strain</li> <li>• Protease deficient</li> </ul>	NEB Express Competent <i>E. coli</i>	#C2523H/I	20 x 0.05 ml/6 x 0.2 ml	\$166/\$130
<ul style="list-style-type: none"> <li>• Control of IPTG induced expression</li> <li>• Protease deficient</li> </ul>	NEB Express <i>I<sup>q</sup></i> Competent <i>E. coli</i>	#C3037H/I	20 x 0.05 ml/6 x 0.2 ml	\$166/\$130
<ul style="list-style-type: none"> <li>• Most popular T7 expression strain</li> <li>• Protease deficient</li> </ul>	T7 Express Competent <i>E. coli</i>	#C2566H/I	20 x 0.05 ml/6 x 0.2 ml	\$166/\$130
<ul style="list-style-type: none"> <li>• T7 expression</li> <li>• Protease deficient</li> <li>• Reduced basal expression</li> </ul>	T7 Express <i>I<sup>q</sup></i> Competent <i>E. coli</i>	#C3016H/I	20 x 0.05 ml/6 x 0.2 ml	\$166/\$130
<ul style="list-style-type: none"> <li>• T7 expression</li> <li>• Protease deficient</li> <li>• Better reduction of basal expression</li> </ul>	T7 Express <i>lysY</i> Competent <i>E. coli</i>	#C3010H/I	20 x 0.05 ml/6 x 0.2 ml	\$166/\$130
<ul style="list-style-type: none"> <li>• T7 expression</li> <li>• Protease deficient</li> <li>• Highest level of expression control</li> </ul>	T7 Express <i>lysY/I<sup>q</sup></i> Competent <i>E. coli</i>	#C3013H/I	20 x 0.05 ml/6 x 0.2 ml	\$166/\$130
<ul style="list-style-type: none"> <li>• T7 expression</li> <li>• Protease deficient</li> <li>• Crystallography</li> <li>• SeMet labeling</li> </ul>	T7 Express Crystal Competent <i>E. coli</i>	#C3022H/I	20 x 0.05 ml/6 x 0.2 ml	\$206/\$160
<ul style="list-style-type: none"> <li>• Protease deficient/B strain</li> <li>• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm</li> </ul>	SHuffle Express Competent <i>E. coli</i>	#C3028H	6 x 0.05 ml	\$60
<ul style="list-style-type: none"> <li>• T7 expression</li> <li>• Protease deficient/B strain</li> <li>• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm</li> </ul>	SHuffle T7 Express Competent <i>E. coli</i>	#C3029H	6 x 0.05 ml	\$60
<ul style="list-style-type: none"> <li>• T7 expression</li> <li>• Protease deficient/B strain</li> <li>• Tight control expression of toxic proteins</li> <li>• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm</li> </ul>	SHuffle T7 Express <i>lysY</i> Competent <i>E. coli</i>	#C3030H	6 x 0.05 ml	\$60
<ul style="list-style-type: none"> <li>• K12 strain</li> <li>• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm</li> </ul>	SHuffle Competent <i>E. coli</i>	#C3025H	6 x 0.05 ml	\$60
<ul style="list-style-type: none"> <li>• T7 expression/K12 strain</li> <li>• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm</li> </ul>	SHuffle T7 Competent <i>E. coli</i>	#C3026H	6 x 0.05 ml	\$60
<ul style="list-style-type: none"> <li>• T7 expression/K12 strain</li> <li>• Tight control/expression of toxic proteins</li> <li>• Enhanced capacity to correctly fold proteins with multiple disulfide bonds in the cytoplasm</li> </ul>	SHuffle T7 <i>lysY</i> Competent <i>E. coli</i>	#C3027H	6 x 0.05 ml	\$60

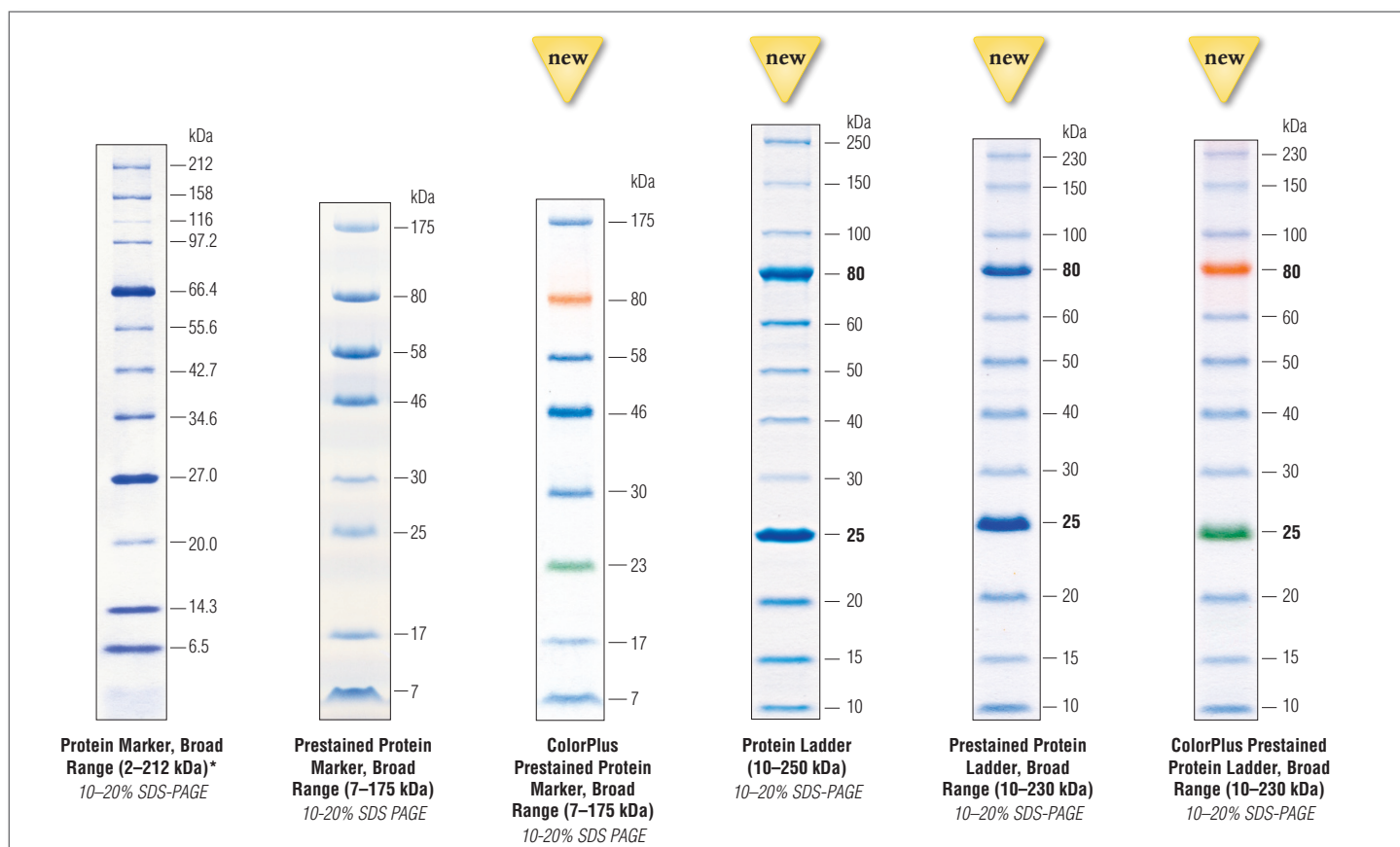
Note: Store Competent Cells at  $-80^{\circ}\text{C}$ . Once thawed, do not refreeze. Storage at  $-20^{\circ}\text{C}$  will result in a significant decrease in transformation efficiency. Cells lose efficiency whenever they are warmed above  $-80^{\circ}\text{C}$ , even if they do not thaw.

## 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 band run at the dye front

### Ordering Information

PRODUCT	CATALOG #	SIZE	PRICE
Protein Marker, Broad Range (2-212 kDa)	P7702S/L	150/750 mini-gel lanes	\$66/\$264
Prestained Protein Marker, Broad Range (7-175 kDa)	P7708V/S/L	83/175/875 mini-gel lanes	\$60/\$95/\$380
ColorPlus Prestained Protein Marker, Broad Range (7-175 kDa)	P7709V/S/L	83/175/875 mini-gel lanes	\$65/\$105/\$420
Protein Ladder (10-250 kDa)	P7703S	100 mini-gel lanes	\$70
Prestained Protein Ladder, Broad Range (10-230 kDa)	P7710S	100 mini-gel lanes	\$85
ColorPlus Prestained Protein Ladder, Broad Range (10-230 kDa)	P7711S	100 mini-gel lanes	\$95

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.



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