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pMAL™ Protein Fusion and Purification System

#E8000S ..... $400 (USA)

Description

In the Protein Fusion and Purification System, the cloned gene is inserted into a pMAL vector down-stream from the *malE* gene, which encodes maltose-binding protein (MBP). This results in the expression of an MBP-fusion protein (1,2,3). The technique uses the strong P_{tac} promoter and the translation initiation signals of MBP to express large amounts of the fusion protein. The fusion protein is then purified by a one-step affinity purification for MBP (4).

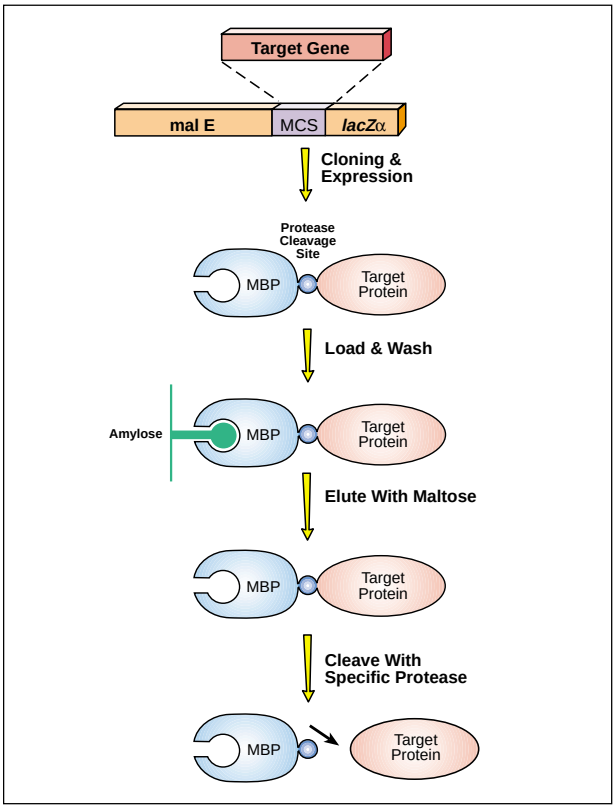
The System Includes

- pMAL-c2X: 10 µg
- pMAL-p2X: 10 µg
- Amylose Resin: 15 ml
  (binding capacity ~40 mg)
- Factor Xa: 50 µg
- anti-MBP antiserum: 25 µl
  (for Western blot analysis)
- MBP2*: 10 µg
  (marker for SDS-polyacrylamide gels)
- MBP2-paramyosin-ΔSal: 100 µg
  (control for Factor Xa cleavage)
- *E. coli* Host
- A Comprehensive Instruction Manual

Method Overview

The system uses the pMAL vectors which are designed so that insertion interrupts a *lacZ*α gene allowing a blue-to-white screen for inserts on X-gal (5). pMAL-c2 series has an exact deletion of the *malE* signal sequence, resulting in cytoplasmic expression of the fusion protein. pMAL-p2 series contains the normal *malE* signal sequence, which directs the fusion protein through the cytoplasmic membrane. pMAL-p2 fusion proteins capable of being exported can be purified from the periplasm. Between the *malE* sequence and the polylinker there is a spacer sequence coding for 10 asparagine residues. This spacer insulates MBP from the protein of interest, increasing the chances that a particular fusion will bind tightly to the amylose resin. The vectors also include a sequence coding for the recognition site of a specific protease. This allows the protein of interest to be cleaved from MBP after purification, without adding any vector-derived residues to the protein (6). For this purpose, the polylinker includes a restriction site superimposed on the sequence coding for the site of the specific protease. This is where the gene of interest is inserted. An *Eco*R I site in the same reading frame as that of λgt11 and a number of other useful sites are present directly downstream. The vectors also include the M13 origin of DNA replication which allows the production of single-stranded DNA for sequencing and mutagenesis by infecting with M13KO7 helper phage (NEB #N0315S).

*Patent #5,643,758



Expected Results

Expression from the pMAL vectors yields up to 100 mg fusion protein from a liter of culture. While no expression system works with every cloned gene, the pMAL Protein Fusion and Purification System gives substantial yields of protein in about 75% of the cases tested. A chapter in *Current Protocols in Molecular Biology* (3) provides an in-depth analysis of the use of the pMAL vectors. The System's instruction manual is available separately upon request.

References

1. Guan, C. et al. (1987) *Gene* 67, 21–30.
2. Maina, C.V. et al. (1988) *Gene* 74, 365–373.
3. Riggs, P., in Ausebel, F.M. et al. (eds), *Current Protocols in Molecular Biology* Greene Associates/Wiley Interscience, New York pp. 16.6.1–16.6.10.
4. Kellerman, O.K. and Ferenci, T. (1982) *Methods in Enzymol.* 90, 459–463.
5. Yanisch–Perron, C. et al. (1985) *Gene* 33, 103–119.
6. LaVallie, E., in Ausebel, F.M. et al. (eds), *Current Protocols in Molecular Biology* Greene Associates/Wiley Interscience, New York pp. 16.4.1–16.4.17.

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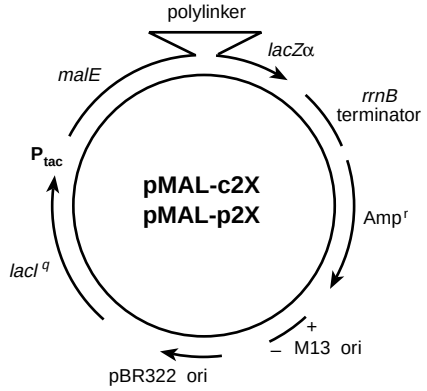
System Components & Companion Products

Vectors

The pMAL-c2 series of vectors have an exact deletion of the *malE* signal sequence, resulting in cytoplasmic expression of the fusion protein. The pMAL-p2 series of vectors contain the normal *malE* signal sequence, which directs the fusion protein through the cytoplasmic membrane. All of the vectors include a sequence coding for the recognition site of a specific protease (Factor Xa [X], Enterokinase [E] or Genenase™ I [G]) which allows the protein of interest to be cleaved from MBP after purification.



pMAL™ Vectors

pMAL-c2X
#N8076S 10 µg .....$75 (USA)

pMAL-p2X
#N8077S 10 µg .....$75 (USA)

Note: pMAL-c2X and pMAL-p2X are supplied with the pMAL Protein Fusion and Purification System (NEB #E8000S).

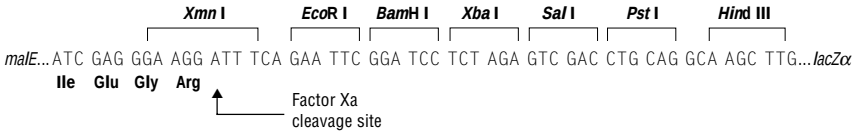
pMAL-c2E *(not supplied with the system)*
#N8066S 10 µg .....$75 (USA)

pMAL-p2E *(not supplied with the system)*
#N8067S 10 µg .....$75 (USA)

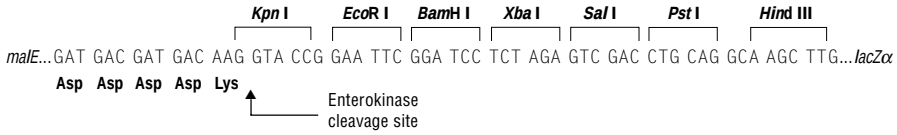
pMAL-c2G *(not supplied with the system)*
#N8068S 10 µg .....$75 (USA)

pMAL-p2G *(not supplied with the system)*
#N8069S 10 µg .....$75 (USA)

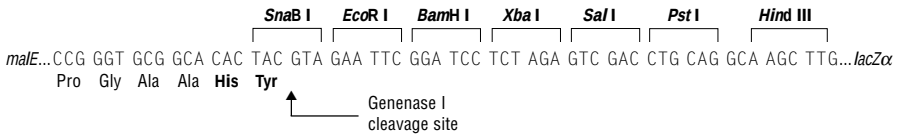
pMAL™-c2X, -p2X polylinker:



pMAL-c2E, pMAL-p2E Polylinker



pMAL-c2G, pMAL-p2G Polylinker



System Components & Companion Products (*continued)*

Amylose resin

#E8021S	15 ml	..... $100 (USA)
#E8021L	100 ml	..... $300 (USA)

Factor Xa

#P8010S	50 µg	.....$50 (USA)
#P8010L	250 µg	..... $200 (USA)

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 conformation of the protein substrate (1,2,3). A number of the secondary sites have been sequenced that show cleavage following gly-arg. Acetylation of the γ amino group of lysine has been shown to block nonspecific cleavage at lysine residues (4). 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 (Hall, I., Riggs, P., unpublished observations).

Source: Factor Xa protease is purified from bovine plasma.

Unit Definition: 1 µg of Factor Xa will cleave 50 µg of test substrate to 95% completion in 6 hours or less.

Unit Assay Conditions: 20 mM Tris-HCl, 100 mM NaCl, 2 mM $CaCl_2$ (pH 8.0), 50 µg of an MBP fusion protein test substrate and enzyme. Incubate at 23°C.

References:

1. Nagai, K. et al., (1985) *PNAS USA* 82, 7252–7255.
2. Quinlan, R.A. et al., (1989) *J. Cell Sci.* 93, 71–83.
3. Eaton, D. et al., (1986) *Biochem.* 25, 505–512.
4. Wearne, S.J., (1990) *FEBS Lett.* 263, 23–26.

Enterokinase *(not supplied with the system)*

#P8070S	5 units	. . . . . .$80 (USA)
#P8070L	25 units	. . . . . .$320 (USA)

Enterokinase is a specific protease that cleaves after the lysine in its preferred cleavage site Asp-Asp-Asp-Asp-Lys. It will sometimes cleave at other basic residues, depending on the conformation of the protein substrate.

Source: Enterokinase is purified from *E. coli* containing a clone of the light chain of the bovine enterokinase gene, fused to a carrier protein (1,2). The fusion protein is cleaved and the enterokinase domain is purified away from the carrier.

Unit Definition: One unit is defined as the amount of enterokinase that will cleave 50 µg of a fusion protein that contains a fusion joint of asp-asp-asp-asp-lys to 95% completion in 8 hours at 23°C.

Unit Assay Conditions: 20 mM Tris-HCl, 50 mM NaCl, 2 mM $CaCl_2$ (pH 7.4 @ 25°C), 50 µg of an MBP fusion protein test substrate and enzyme in a 50 µl reaction. Incubate at 23°C.

References:

1. La Vallie, E.R. et al. (1993) *J. Biol. Chem.* 268, 23311–23317.
2. La Vallie, E.R. and Racie, L., unpublished observations.

Genenase™ I *(not supplied with the system)*

#P8075S	50 µg	.....$50 (USA)
#P8075L	250 µg	..... $200 (USA)

Genenase I is a variant of subtilisin BPN´ that has been engineered to have increased specificity by substituting amino acids in its active site (1,2). When designing fusion proteins for cleavage with Genenase I, we recommend the site Pro-Gly-Ala-Ala-His-Tyr. Genenase I will cleave at other histidine residues depending on the surrounding amino acids and the 3-dimensional conformation of the protein. Genenase I cleaves His-Tyr-Glu and His-Tyr-Asp slowly, but will not cleave His-Tyr-Pro or His-Tyr-Ile (2).

Source: *Bacillus amyloliquefaciens,* cloned in *Bacillus subtilis*.

Unit Definition: 0.5 µg of Genenase I will cleave 50 µg of test substrate to 95% completion in 8 hours or less at 23°C.

Unit Assay Conditions: 20 mM Tris-HCl (pH 8.0 @ 25°C), 200 mM NaCl, 50 µg of MBP fusion test substrate and enzyme. Incubate at 23°C.

References:

1. Carter, P. and Wells, J.A. (1987) *Science* 237, 394–399.
2. Carter, P. et al. (1989) *Proteins: Structure, Function, and Genetics* 6, 240–248.

Anti-MBP Anti Serum

#E8030S	0.2 ml	..... $100 (USA)

Rabbit serum prepared using purified maltose-binding protein; Suggested dilution for Western blotting or ELISA 1:10,000.

Anti-MBP Antibody *(not supplied with the system)*

#E8031S	0.08 ml	..... $200 (USA)

Anti-MBP Antibody is purified from serum by Protein A and maltose-binding protein affinity chromatography.

MBP2*

#E8044S	1.0 mg	.....$50 (USA)
#E8044L	5.0 mg	..... $200 (USA)

MBP2* is wild type MBP plus the amino acids encoded by the pMAL-c2 polylinker. It is purified from cells bearing a derivative of pMAL-c2 with a linker containing a stop codon inserted in the *Xmn* I site.

MBP2*-paramyosin ΔSal

#E8051S	100 µg	. . . . . .$50 (USA)

An MBP fusion protein used as a positive control for factor Xa cleavage.

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System Components & Companion Products (*continued)*

***E. coli* host TB1**
ara Δ(*lac proAB*) *rpsL* (ϕ80 *lacZΔM15*) *hsdR*
Available upon request at no charge with an order or for the cost of shipping if ordered separately (#E4122S).

***malE* primer** *(not supplied with the system)*
#S1237S 0.5 A_{260} units .....$95 (USA)

Used for sequencing downstream from the *malE* gene across the polylinker,

5´...dGGTCGTCAGACTGTCGATGAAGCC...3´

M13/pUC sequencing primer *(not supplied with the system)*
#S1224S 0.5 A_{260} units .....$95 (USA)

Used for sequencing upstream from the *lacZα* gene across the polylinker,

5´...dCGCCAGGGTTTTCCCAGTCACGAC...3´

M13KO7 Helper Phage *(not supplied with the system)*
#N0315S 1.8 ml .....$55 (USA)

Superinfection of cultures containing pMAL-2 clones with M13KO7 results in preferential packaging of single-stranded pMAL for mutagenesis and sequencing.

Notice to Buyer/User

The buyer/user has a non-exclusive license to use the pMAL vectors for research purposes only. A license to use the pMAL vectors for the commercial purposes is available from New England Biolabs, Inc. U.S. Patent #5,643,758.

Information presented herein is accurate and reliable to the best of our knowledge and belief, but is not guaranteed to be so. Nothing herein is to be construed as recommending any practice or any product in violation of any patent or in violation of any law or regulation. It is the user's responsibility to determine for himself or herself the suitability of any material and/or procedure for a specific purpose and to adopt such safety precautions as may be necessary.