

pMAL-pIII Vector



1-800-632-7799
info@neb.com
www.neb.com



N8101S

10 µg Lot: **0011410** Exp: **10/16**
500 µg/ml Store at **-20°C**

Description: The pMAL-pIII Vector is a derivative of pMAL-p2 in which the leader sequence of maltose binding protein (MBP, *malE*) has been replaced with the M13 pIII leader sequence. KpnI/Acc65I and EagI sites have been introduced within the pIII leader to facilitate direct transfer of sequences selected from any of the Ph.D. phage display peptide libraries into an expression vector. The corresponding peptides are expressed as N-terminal MBP fusions. These fusions can then be easily purified from *E. coli* periplasmic space by osmotic shock followed by affinity chromatogra-

phy on amylose resin. Since the peptide is expressed at the N-terminus of MBP, Factor Xa cannot be used to cleave the peptide from MBP.

Source: Isolated from *E. coli* strain ER2272 by standard DNA purification procedure.

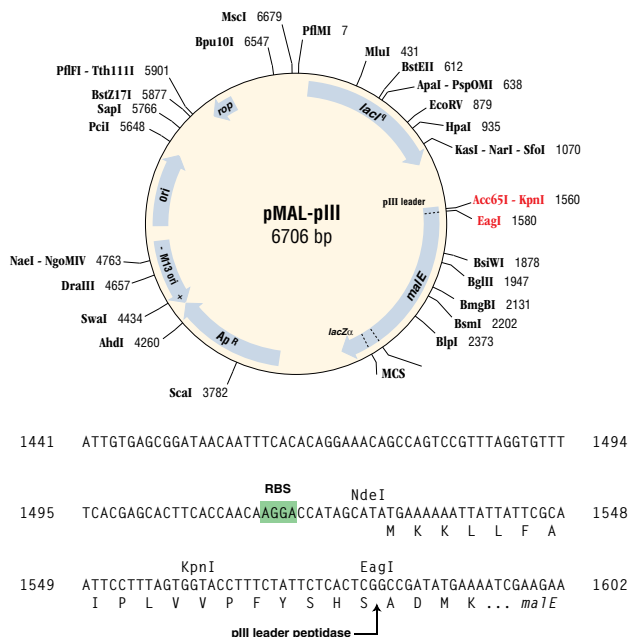
Supplied in: 10 mM Tris-HCl (pH 7.5 @ 25°C), 1 mM EDTA.

Advantages:

- Rapid characterization of selected sequences by ELISA
- Alternative to chemical peptide synthesis
- Monovalency of MBP fusion allows accurate K_D determination

Protocol:

1. Prepare double-stranded (RF) phage DNA from each selected Ph.D. clone using standard procedures, e.g. Sambrook, (3rd ed.), (pp 3.23–3.25). Alternatively, a cassette containing the insert sequence can be obtained by PCR of single stranded phage DNA with M13 extension primer (NEB #E8101) and -96gIII sequencing primer



pMAL-pIII Plasmid Map (top). Restriction sites used for subcloning fragments from PhD libraries into pMAL-pIII are shown in red. **pMAL-pIII cloning region (bottom).** Note PhD fragments are inserted into the M13KE pIII leader sequence, which is directly followed by the *malE* gene (see other side)

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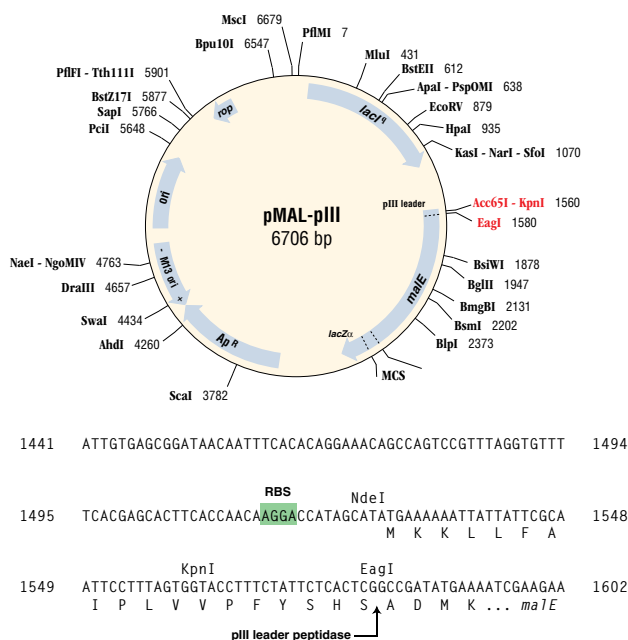
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(NEB #S1259).

2. Digest DNA with Acc65I and EagI in NEBuffer
3. Isolate small fragment(52-67 bp), containing selected peptide sequence and flanking leader sequence, by nondenaturing polyacrylamide or high-resolution agarose gel electrophoresis.
3. Ligate purified segment into Acc65I/EagI digested pMal-pIII vector.
4. Transform into suitable host, e.g. *E. coli* TB1 (NEB #E4122) or NEB Turbo Competent *E.coli* (NEB #C2984).
5. Carry out pilot scale expression experiments. Refer to pMal Protein Fusion and Purification System manual page 17. (<http://www.neb.com/nebecomm/ManualFiles/manualE8000.pdf>). To purify the fusion on amylose resin (NEB #E8021) based on the procedure on p.23 for a 1L culture:
 - a. Harvest cells by centrifugation at 4000 x *g* for 20 min. and discard the supernatant. Resuspend cells in 400 ml 30 mM Tris-HCl, 20% sucrose, pH 8.0 (80 ml for each gram of cells wet weight). Add EDTA to 1 mM and incubate for 5–10 min. at room temperature with shak-

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- b. Centrifuge at 8000 x *g* for 20 min. at 4°C. Remove all the supernatant. Resuspend the pellet in 400 ml of ice-cold 5 mM MgSO₄. Shake or stir for 10 min. in an ice bath.
- c. Centrifuge at 8000 x *g* for 20 min. at 4°C. The supernatant is the cold osmotic shock fluid.
- d. Add 8 ml 1 M Tris-HCl, pH 7.4 to osmotic shock fluid.
- e. Continue to amylose resin affinity purification (step 7, p. 24).

Usage Notes: NEB 10-beta Competent *E. coli* (High Efficiency) (NEB #C3019) is recommended for propagation and subcloning. NEB Express Competent *E. coli* (High Efficiency) (NEB #C2523) is recommended for expression using this vector.

References:

1. Zwick, M.B. et al (1998) *Anal. Biochem.*, 264, 87–97.

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2. Zagursky, R.J. et al (1984) *Gene*. 27, 193-91.

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Companion Products Sold Separately:

Amylose Resin	
#E8021S	15 ml
#E8021L	100 ml
Anti-MBP Monoclonal Antibody	
#E8032S	0.05 ml (1 mg/ml)
#E8032L	0.25 ml (1 mg/ml)

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#E8021S	15 ml
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Anti-MBP Monoclonal Antibody	
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