pMAL-pIII Vector

NEW ENGLAND BioLabs Inc.
1-800-632-7799
info@neb.com
www.neb.com

N8101S 001121214121

N8101S

10 µg **Lot: 0011212** **Exp: 12/14**

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, *mal*E) 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 chromatography 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.

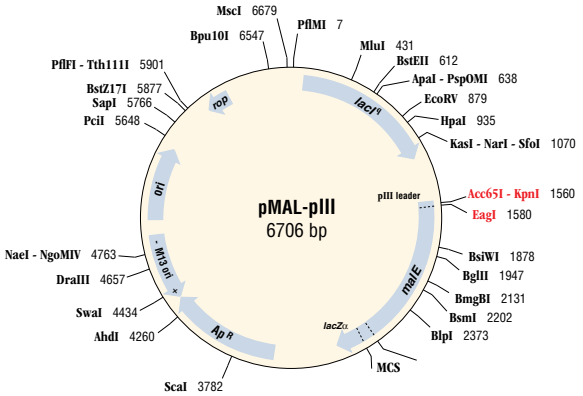
Supplied in: 10 mM Tris-HCl (pH 7.5 @ 25°C), 1 mM EDTA.

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




```
1441   ATTGTGAGCGGATAACAATTTCACACAGGAAACAGCCAGTCCGTTTAGGTGTTT   1494

                    RBS          NdeI
1495   TCACGAGCACTTCACCAACAAGGACCATAGCATATGAAAAAATTATTATTCGCA   1548
                                  M  K  K  L  L  F  A

                 KpnI                EagI
1549   ATTCCTTTAGTGGTACCTTTCTATTCTCACTCGGCCGATATGAAAATCGAAGAA   1602
       I  P  L  V  V  P  F  Y  S  H  S  A  D  M  K ... malE
                                      ↑
                                pIII leader peptidase
```

***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)

CERTIFICATE OF ANALYSIS

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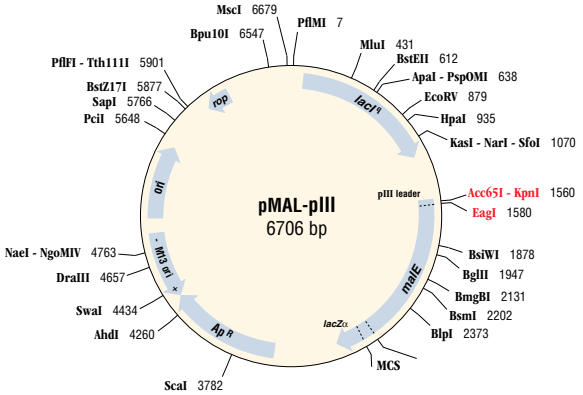


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CERTIFICATE OF ANALYSIS

(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 shaking or stirring.

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 $MgSO_4$. 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.
2. Zagursky, R.J. et al (1984) *Gene.* 27, 193-91.

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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Page 2 (N8101)