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NEBExpress MBP Fusion and Purification System (NEB #E8201)

New England Biolabs[1]

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1 Works for me dx.doi.org/10.17504/protocols.io.bfayjifw

New England Biolabs (NEB)
Tech. support phone: **+1(800)632-7799** email: **info@neb.com**

New England Biolabs
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ABSTRACT

The NEBExpress MBP Fusion and Purification System takes advantage of the strong Ptac 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.

- Reliable *E. coli* expression: substantial yields (up to 100 mg/L)
- Fusion to MBP has been shown to enhance the solubility of proteins expressed in *E. coli*[1]
- Two-step purification: amylose elution followed by TEV Protease cleavage and Ni resin isolation results in a highly pure tag-free target protein
- Gentle elution with maltose; no detergents or harsh denaturants required

EXTERNAL LINK

https://www.neb.com/protocols/2020/02/05/nebexpress-mbp-fusion-and-purification-system-quick-start-protocol-neb-e8201

GUIDELINES

In the NEBExpress®MBP Fusion and Purification System, the pMAL-c6T vector provides a method for expressing and purifying a protein produced from a cloned gene or open reading frame. The cloned gene is inserted downstream from and in frame with the *malE* gene of *E. coli*, which encodes maltose-binding protein (MBP); this construct results in the expression of an MBP fusion protein[2,3]. The pMAL-c6T vector expresses the N-terminal hexahistidine tagged *malE* gene (lacking its secretory signal sequence and engineered for tighter binding to amylose) followed by a multiple cloning site containing a TEV protease recognition sequence and stop codons in all three frames. The pMAL-c6T vector expresses the MBP fusion in the cytoplasm. The method uses the strong "tac" promoter and the *malE* translation initiation signals to yield high-level expression of the cloned sequences[4,5]. The fusion protein is then purified by a one-step purification method using amylose resin and MBP's affinity for maltose[6].

Following amylose purification, the target protein can be cleaved from the MBP-tag using TEV Protease, without adding any vector-derived residues to the protein. Both the MBP-tag and TEV Protease are polyhistidine-tagged for easy removal from the reaction. Loading the digest onto NEBExpress Ni Resin (NEB #S1428) sequesters both the MBP-tag and TEV Protease, thereby isolating the target protein in the column flow through. The target protein yield can be up to 100 mg/L, with typical yields in the range of 10–40 mg/L.

Figure 1: Schematic illustration of the NEBExpress MBP Fusion and Purification System

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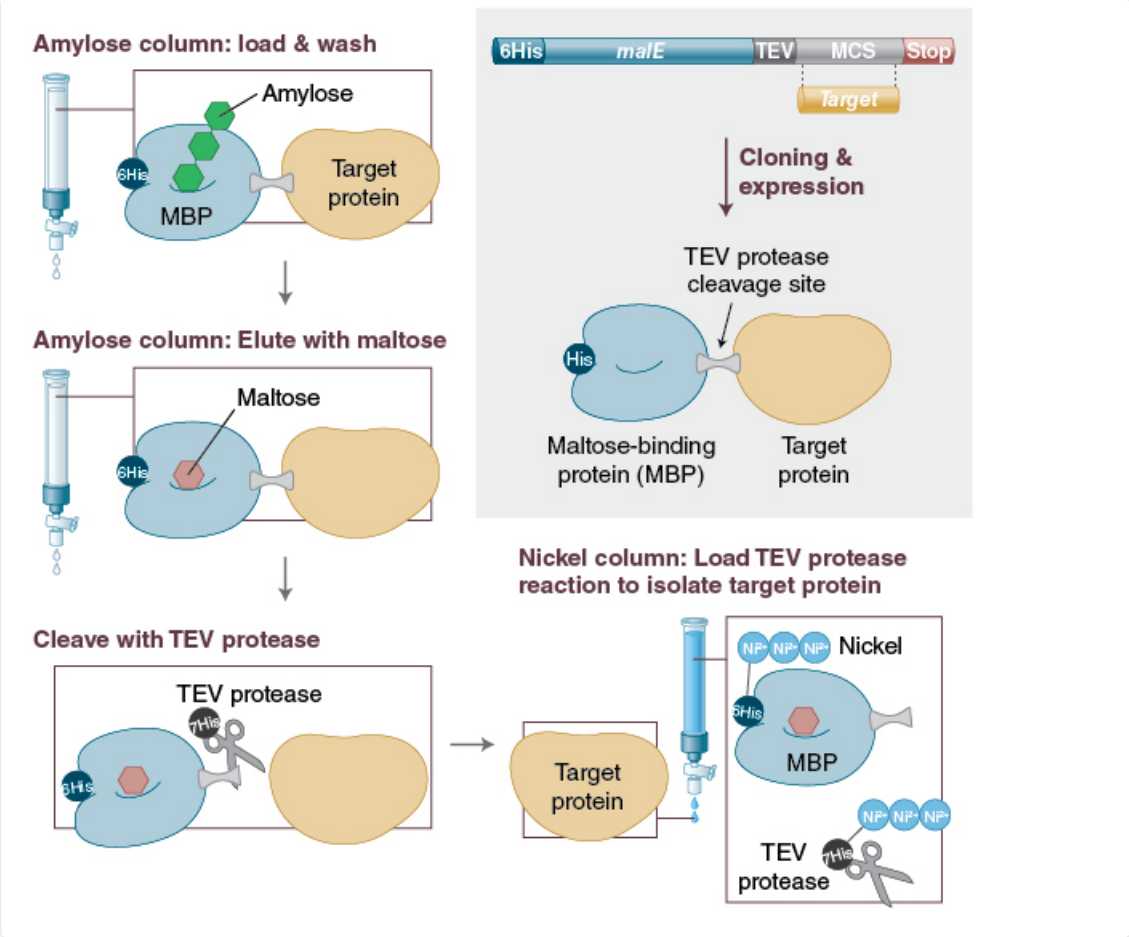
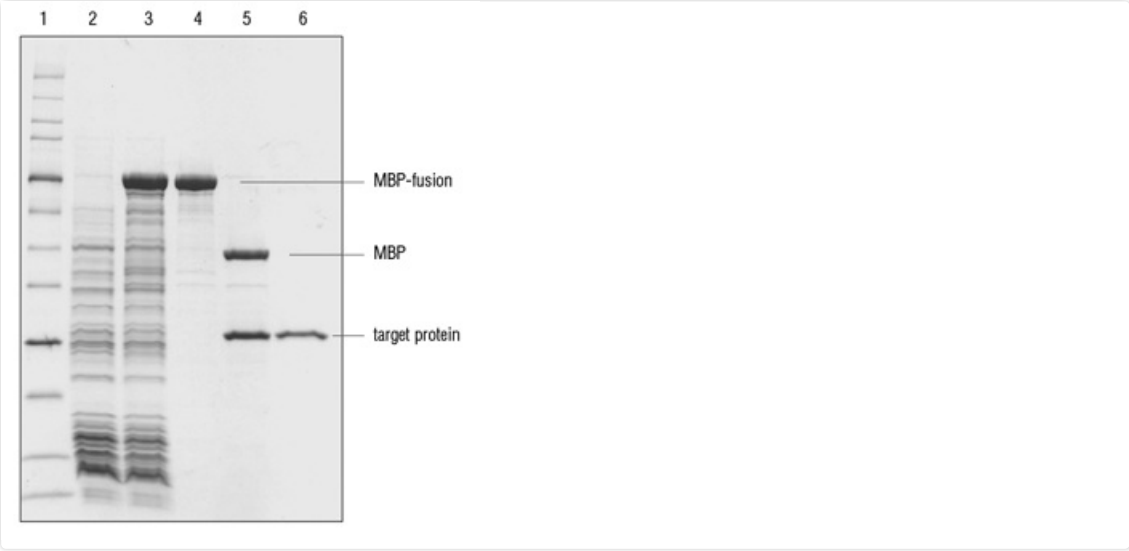


Figure 2. Protein Expression using the NEBExpress MBP Fusion and Purification System



SDS-polyacrylamide gel electrophoresis of fractions from the affinity purification of MBP6-TEV-Paramyosin ΔSal. Lane 1: Protein Standard. Lane 2: uninduced cells. Lane 3: induced cells. Lane 4: purified fusion protein eluted from amylose column with maltose. Lane 5: purified protein after TEV Protease cleavage. Lane 6: target protein isolated

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from NEBExpress Ni Resin flow through.

MATERIALS

NAME	CATALOG #	VENDOR
NEBExpress MBP Fusion and Purification System	E8201	New England Biolabs

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

1 Subclone the gene of interest into the pMAL-c6T vector.

2 Grow cells containing the pMAL-c6T fusion plasmid in LB containing ampicillin and 0.2% glucose to an A_{600} of ~0.5.

3 Induce by adding IPTG to a final concentration of **0.3 Milimolar (mM)** .

4 Grow for an additional:

02:00:00 at **37 °C** OR

04:00:00 at **30 °C** OR

06:00:00 – **08:00:00** at **Room temperature** OR

Overnight at **12 °C** to **16 °C** .

5 Harvest the cells and either freeze immediately or resuspend in 25 ml column buffer (CB) per liter of culture.

6 Lyse the cells by freeze-thaw followed by sonication.

7 Clarify the lysate by centrifugation at **20000 rpm 00:20:00** .

8 Dilute the supernatant (crude extract) by adding **125 ml cold CB** for every 25 ml crude extract.

9 Load the diluted crude extract on a 15 ml amylose column.

10 Wash the column with ≥ 12 column volumes of CB.

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11 Elute the fusion protein with CB containing [M]**10 Milimolar (mM) maltose** .

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