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Protocol status: Working
We use this protocol and it's working

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Separating the Protein of Interest from MBP after TEV Protease Cleavage (NEB #E8201)

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ABSTRACT

- Both the MBP-tag and TEV Protease are polyhistidine-tagged for easy removal from the TEV Protease reaction. Loading the digest onto NEBExpress Ni Resin ([NEB #S1428](#)) sequesters both the MBP-tag and TEV Protease, thereby isolating the protein of interest in the column flow through.
- The binding capacity of NEBExpress Ni Resin is ≥ 10 mg/ml. The binding capacity will vary depending on the target protein, binding conditions and the accessibility of the His-tag.
- It is recommended to estimate the amount of the His-tagged MBP released by TEV Protease cleavage by first analyzing a sample by SDS-PAGE and comparing its band size and intensity to that of the provided MBP6 protein control.

MATERIALS

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NEBExpress MBP Fusion and Purification System New England Biolabs Catalog #E8201

SAFETY WARNINGS

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

Keywords: Ni, resin, MBP, TEV

Please note that NEBExpress Ni Resin is chemically resistant to DTT. If you are using a different resin that is incompatible with DTT, you will need to buffer exchange or modify the TEV Protease reaction conditions accordingly.

Purchase or prepare the following buffer:

Binding Buffer: 1X TEV Protease Reaction Buffer (or similar):

50 mM Tris-HCl, 0.5 mM EDTA, 1 mM DTT, pH 7.5

Resin Preparation

- 1 Gently shake the Ni Resin bottle to completely resuspend the slurry and transfer desired amount to a closed column; allow resin to settle.
- 2 Open column and allow storage buffer to flow through.
- 3 Equilibrate the resin by adding 5 column volumes (CV) of Binding buffer.

Binding

- 4 Load the TEV Protease digestion directly onto the equilibrated column and allow it to flow through the resin slowly (flow rate < 1 ml/minute). This provides sufficient time for the MBP-tag, TEV Protease, and any remaining MBP-fusion protein to effectively bind to the Ni resin.
- 5 Collect the flow through containing the protein of interest in a sterile tube.
- 6 Wash the column with 1 CV of Binding Buffer to maximize the yield of the target protein; collect the flow through.

- 7 Dialyze or buffer exchange target protein into desired storage buffer.

Resin Cleaning

- 8 Add [M] 1 Molarity (M) NaOH to the column and allow a contact time of 01:00:00 to 02:00:00 .

- 9 Add [M] 1.5 Molarity (M) NaCl to the column and allow a contact time of 00:10:00 – 00:15:00 followed by 10 CV of water.

Storage

- 10 For long-term storage, NEBExpress Ni Resin should be stored in 20% ethanol at 2 – 8°C.