May 18, 2020

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

New England Biolabs[1]

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

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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.

EXTERNAL LINK

https://www.neb.com/protocols/2020/02/06/separating-the-protein-of-interest-from-mbp-after-tev-protease-cleavage-neb-e8201

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.

BEFORE STARTING

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.

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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.

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