Jul 01, 2020

TEV Protease

New England Biolabs[1]

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1 Works for me This protocol is published without a DOI.

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ABSTRACT

TEV Protease is a highly specific cysteine protease that recognizes the amino-acid sequence Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser) and cleaves between the Gln and Gly/Ser residues.

- Removal of affinity purification tags such as maltose-binding protein (MBP) or poly-histidine from fusion proteins
- Optimal activity and stability for up to 24 months
- Active in a wide range of buffers; optimal activity between pH 6.0 and 9.0.
- High substrate specificity with no non-specific proteolysis

TEV Protease (NEB# P8112) is recommended for cleavage of a His-tag following purification with NEBExpress® Ni-NTA Magnetic Beads, Ni Spin Columns or Ni Resin. First, the expression vector must be designed to contain a TEV site between the His-tag and the protein.

EXTERNAL LINK

https://www.neb.com/protocols/2018/11/06/his-tag-removal-from-protein-using-tev-protease

PROTOCOL CITATION

New England Biolabs 2020. TEV Protease. **protocols.io**
https://protocols.io/view/tev-protease-bfatjien

EXTERNAL LINK

https://www.neb.com/protocols/2018/11/06/his-tag-removal-from-protein-using-tev-protease

KEYWORDS

his-tag removal, TEV , TEV protease, P8112

CREATED

Apr 19, 2020

LAST MODIFIED

Jul 01, 2020

OWNERSHIP HISTORY

Apr 19, 2020 Anita Broellochs protocols.io

Jul 01, 2020 New England Biolabs Tech Support New England Biolabs

PROTOCOL INTEGER ID

35891

GUIDELINES

Notes:

1. If the fusion protein sample contains >2 M urea, >0.5 M Guanidine hydrochloride, >50 mM imidazole, pH values below 6 or above 9, or cysteine protease inhibitors then it will be necessary to dialyze the fusion protein into TEV protease reaction buffer before TEV Protease cleavage.
2. TEV protease is inhibited by reaction buffers containing >40% Glycerol.
3. Inhibition occurs in the presence of ≥ 5 mM Zn^{2+}, ≥ 1 mM $Cu^{2+}$and ≥ 10 mM Co^{2+}.
4. Compatible with 10mM $MgSO_4$, $MnCl_2$and $CaCl_2$and up to 100mM EDTA.
5. Compatible with the following protease inhibitors: aprotinin, benzamidine, leupeptin, pepstatin, PMSF.
6. Optimal activity achieved in ≤0.2M NaCl; however, the enzyme retains some activity in up to 2M NaCl.
7. Some substrates may require extended incubation periods (up to three days at either 4°C or 30°C) to achieve complete cleavage. The addition of more TEV Protease after 24 hours may also help achieve complete cleavage of some substrates.

MATERIALS

NAME	CATALOG #	VENDOR
TEV protease	P8112S	New England Biolabs

SAFETY WARNINGS

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

BEFORE STARTING

Optimal incubation times and enzyme concentrations must be determined empirically for a particular substrate. Reactions may be scaled-up linearly to accommodate larger sample amounts and reaction volumes. Typical reaction conditions are as follows:

Typical Reaction Conditions for TEV Protease

1 Dialyze the protein against [M]**20 Milimolar (mM) Tris-HCl** , pH**7.5** .

2

Determine the protein concentration.

3

Combine **15 µg of protein and H20** (if necessary) to make a **45 µl** total reaction volume.

4

Add **5 µl TEV Protease Reaction Buffer (10X)** to make a **50 µl** total reaction volume.

5

Add **1 µl TEV Protease** .

6

Incubate at **30 °C** for **01:00:00** or at **4 °C** **Overnight** .