Cleavage of the Fusion Protein (TEV Protease)

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1 Works for me
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ABSTRACT

TEV Protease, also known as Tobacco Etch Virus (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. It is often used for the removal of affinity purification tags such as maltose-binding protein (MBP) or poly-histidine from fusion proteins. TEV Protease has a 7xHis-tag for easy removal from a reaction using nickel affinity resins and has been engineered to improve thermal stability and decrease autolysis.

GUIDELINES

Affinity tags, such as Maltose Binding Protein (MBP) or polyhistidine (His), are essential tools for the production of recombinant proteins. MBP is known to significantly enhance the solubility of many proteins, resulting in higher yield. Whereas, His-tagging is widely employed for the purification of recombinant target proteins via immobilized metal affinity chromatography (IMAC). The His-tag advantages include small tag size and high affinity and specificity of poly-His tag binding to divalent metals at neutral pH. Despite these important advantages, it is often preferred to remove the affinity tag following purification in order to isolate the target protein. Although chemical and enzymatic methods can be used, proteolytic enzymes, such as TEV Protease (NEB #P8112) and Factor Xa (NEB #P8010) are the preferred method for cleavage of fusion proteins at designed cleavage sites.

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

MBP and the target protein are fused by a polylinker containing a TEV protease recognition site for easy removal of the MBP-tag. One unit of TEV Protease will cleave approximately 2 µg of fusion protein. Cleavage should be carried out in 1X TEV Protease Reaction Buffer or in Amylose column elution buffer supplemented with DTT to a final concentration of 1 mM. Depending on the particular fusion protein, the amount of TEV Protease can be adjusted to get an acceptable rate of cleavage.

1 If necessary, concentrate the fusion protein to **at least** [M]**0.5 milligram per milliliter (mg/mL)** .

2 Perform a pilot experiment with a small portion of your protein. For example:

2.1 Combine **15 µg fusion protein** and H2O to make a **total reaction volume of 45 µl**.

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

2.3 Add **1 µl TEV Protease** .

2.4 In a separate tube, combine **5 µg fusion protein** ,
5 µl TEV Protease Reaction Buffer (10X) and H2O to a volume of **50 µl. Do not add TEV Protease (control sample).**

2.5 Incubate reaction and control sample for **01:00:00** , **03:00:00** , and **08:00:00** at **30 °C** (an additional reaction can be made and incubated for 24 hours at 4°C).

2.6 Take **10 µl of reaction(s)** at the indicated times above and add
5 µl SDS-PAGE Sample Buffer (3X) . Take **10 µl control sample** and add
5 µl SDS-PAGE Sample Buffer (3X) after 8 hours (or longest incubation time).

2.7 Incubate the SDS-PAGE samples for **00:03:00** - **00:05:00** at **70 °C** - **100 °C** .

2.8 Analyze them by SDS-PAGE.

3 Scale up the pilot experiment linearly for the amount of the fusion protein to be cleaved. Save at least a small sample of the uncut fusion as a negative control.

4 Check for complete cleavage by SDS-PAGE.

5 TEV Protease and the cleaved MBP contain polyhistidine tags at their N-termini. They can be removed from the cleavage reaction by immobilized metal affinity chromatography, such as Nickel or Cobalt resin, thereby isolating the target protein in the flow through.

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