TEV Protease

Introduction

TEV protease is a highly specific cysteine protease with the highest catalytic efficiency. The recognition sequence is ENLYFQ▼S, but the amino acid at the P1' position can also be G, A, M, C, or H (1). TEV proteases are commonly used to remove affinity-purified tags from fusion proteins, such as Glutathione S-transferase (GST), His, or other tagged proteases for the removal of fusion proteins. TEV protease has a His-tag tag that can be easily removed from the reaction using nickel-affinity resin, so it is suitable for the digestion reaction of His-tagged fusion proteins, while the recombinant protein with His-tag that is not completely digested, the excised His-tag, and TEV Protease (His-tag) can all be removed by nickel column binding, and the final flow-through solution contains the desired target protein. In order to preserve the structure and biological activity of the protein of interest as much as possible, it is recommended to digest with TEV Protease overnight at 4°C. TEV Protease is active in the pH range of 6.0-9.0, while when pH is less than or equal to 5, it reduces or even loses enzyme activity.

Compositions and storage

Components \ Size	1000 U	5000 U	10000 U	Storage
TEV Protease	0.1 mL	0.5 mL	1 mL	-80°C
10X TEV Protease Reaction Buffer	1 mL	5X 1 mL	10X 1 mL	-80 °C
0.1 M DTT	0.5 mL	2.5 mL	5 mL	-80 °C
Shipping: Dry Ice	Shelf life: 2 years			

Note: It is recommended to aliquot upon first use to avoid repeated freeze-thaw cycles: Store aliquots at -20°C for a validity period of 6 months.

Protocol

1. For a particular protein, the optimal incubation time and enzyme concentration must be determined empirically. Reactions can be linearly scaled up to accommodate larger sample volumes and reaction volumes. Typical reaction conditions are as follows (operate on ice):

Reagent	Volume	Final content
TEV-Fusion protein	X µL	15 µg
10X TEV Protease Reaction Buffer	5 µL	1X
TEV Protease	1 µL	10 U
0.1 M DTT	0.5 µL	1 mM
Nuclease-Free Water	To 50 µL	-

2. Gently mix the reaction up and down with a pipette and then centrifuge quickly.

3. Incubate at 30°C for 2 h or overnight at 4°C.

*Note: The amount of TEV Protease, incubation temperature, and incubation time in the reaction can be optimally adjusted to achieve specific protein digestion by evaluating the digestion results after the initial incubation.

4. 10 μL of the sample was subjected to SDS-PAGE electrophoresis to determine whether the digestion was complete. If digestion is incomplete, the reaction time can be extended appropriately (the digestion efficiency of the protein can be determined by analyzing the amount of lysate formed after digestion and the amount of unlysed protein remaining after digestion).

Note

1. Enzyme activity unit (U) definition: After 2 h of reaction at 30°C, 1 U of TEV protease cleaved 2 μg of control substrate >95%.

2. Storage buffer: 50 mM Tris-HCl，1 mM EDTA，250 mM NaCl, 5 mM DTT，50% (v/v) glycerol，0.1% (w/v) Triton X-100, pH 7.5 @ 25°C。

3. 10X TEV Protease Reaction Buffer: 500 mM Tris-HCl，5 mM EDTA，pH 8.0 @ 25°C。

4. Quality assurance:

   - Purity：SDS-PAGE ≥95%。
   - Non-specific protease: 10 U of TEV protease and protein standard mixture were incubated at 37°C for 20 h with less than 10% protein degradation.

5. Inactivation conditions: 65°C, 20 min.

6. For research use only. Not to be used in clinical diagnostic or clinical trials.

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