TEV Protease (with His-tag)

REF: EG24303S

Storage Condition

-20 ℃ , It is recommended to aliquot and store upon first use to avoid repeated freeze-thaw cycles.

Components

Component	Amount
TEV Protease (10 U/μl)	100 μl
10× TEV Buffer	1 ml

Description

TEV Protease (with His-tag) is derived from the tobacco etch virus and has been genetically engineered for recombinant expression in Escherichia coli. It retains the natural enzymatic activity of TEV protease while exhibiting improved tolerance to variations in pH and temperature. TEV Protease is a highly specific cysteine protease, widely utilized for the removal of affinity tags from fusion proteins. It precisely recognizes the seven-amino-acid sequence EXXYXQ↓(G/S) and cleaves between the glutamine and glycine/serine residues. The most commonly recognized sequence is Glu-Asn-Leu-Tyr-Phe-Gln↓Gly. The inclusion of a His-tag at the N-terminus of TEV Protease allows for its efficient removal via Ni-affinity chromatography following digestion, thereby simplifying the purification of the target protein.

Definition of Activity Unit

One unit of activity is defined as the amount of enzyme required to cleave >85% of 3 μg of substrate within 1 hour at 30℃ in 1× TEV Buffer.

Storage Buffer

50 mM Tris-HCl; 250 mM NaCl; 10 mM DTT; 1 mM EDTA; 50% Glycerol; pH 7.5 @ 25℃。

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Non-specific protease activity

A 50 μl reaction containing 3 μg of standard protein and 10 U of TEV Proteinase incubated for 1 hour at 30 ℃ results in no detectable degradation of the protein mixture as determined by SDS-PAGE.

Protocol

1. Prepare the following reaction system in an EP tube:

Reagent	Amount
Substrate	20 μg
10× TEV Buffer	5 μl
TEV Protease (10 U/μl)	1 μl
ddH_2O	Up to 50 μl

2. Incubate at 37℃ for 1 hour or Incubate at 4℃ overnight（about 16 hours）.

3. Add 16 μl of Protein Loading Buffer (SDS, 4×) (REF: EG21323S) to the above EP tube. Boil the sample for 10 minutes, and then take 10 μl for SDS-PAGE analysis.

4. After evaluating the initial results, you may optimize the cleavage reaction for your specific protein by optimizing the amount of TEV Protease, incubation temperature, or reaction time.

Notice

1. Compatible with the following protease inhibitors: aprotinin, benzamidine, leupeptin, pepstatin, PMSF.

2. Compatible with 400 mM imidazole, 2 M urea, 0.2 M NaCl, 10 mM $MgSO_4$, 10 mM $MnCl_2$, 10 mM $CaCl_2$, and up to 100 mM EDTA.

3. Inhibition occurs in the presence of ≥40% glycerol, ≥5 mM Zn^{2+}, ≥1 mM Cu^{2+}, and ≥10 mM Co^{2+}.

4. The optimal reaction conditions for TEV are pH 7.0 and 30℃. However, it remains active within the range of pH6~9 and 4~37 ℃. The reaction conditions can be selected according to the specific requirements of the target protein.

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