Recombinant Viral TEV Protease

Catalog Number: 4469-TP

DESCRIPTION

Source *E. coli*-derived

Ala	TEV Protease Glu2039-Gln2279 (Ser2256Asn) Accession # NP_062908	Leu	6-His tag

N-terminus C-terminus

N-terminal Sequence Analysis Ala

Predicted Molecular Mass 28.5 kDa

SPECIFICATIONS

SDS-PAGE 25-30 kDa, reducing conditions

Activity Measured by its ability to cleave a fusion protein containing the recognition sequence Glu-Asn-Leu-Tyr-Phe-Gln, with the cleavage point after Gln.
TEV Protease cleaves ≥50% of the control substrate, as measured under the described conditions. It is recommended that the cleavage for each fusion protein be optimized by varying the amount of Recombinant Viral TEV Protease, reaction time, or incubation temperature.

Endotoxin Level <1.0 EU per 1 µg of the protein by the LAL method.

Purity >80%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation Supplied as a 0.2 µm filtered solution in Tris, NaCl, EDTA, DTT and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 0.5 mM EDTA, 1 mM DTT, pH 8.0
- Recombinant Viral TEV Protease (rvTEV) (Catalog # 4469-TP)
- Substrate: Any fusion protein containing the recognition sequence ENLYFQ. The cleavage site is after glutamine (Q).
- SDS-PAGE followed by protein staining

Assay

1. Dilute rvTEV Protease to 0.02 mg/mL in Assay Buffer.
2. Dilute Substrate to 0.5 mg/mL in Assay Buffer.
3. Form reaction mixture by combining 20 µL of diluted Substrate and 20 µL Assay Buffer. Also prepare two additional mixtures, to be used as controls, containing 20 µL of diluted Substrate and 30 µL of Assay Buffer.
4. Incubate all three mixtures (temperature equilibration), as well as the diluted rvTEV Protease, for 15 minutes at room temperature.
5. Add 10 µL diluted rvTEV Protease to the reaction mixture, excluding the controls, for a final volume of 50 µL in each reaction.
6. Incubate the reaction containing the diluted rvTEV Protease as well as one of the controls for 1 hour at room temperature. Keep the other control on ice.
7. Stop the reactions by mixing equal volumes of reaction mixture (including controls) and 2X reducing SDS-PAGE sample buffer together. Heat for 5 minutes at 100 °C.
8. Analyze the cleavage by SDS-PAGE followed by protein staining.

Final Assay Conditions Per Reaction:

- rvTEV Protease: 0.2 µg
- Substrate: 10 µg

PREPARATION AND STORAGE

Shipping The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

BACKGROUND

TEV Protease is the 241 amino acid (aa), 27 kDa catalytic domain of the nuclear inclusion a (NIa) protein encoded by the potyvirus, tobacco etch virus (TEV). It may be used in biotechnology to cleave affinity tags from recombinant proteins, either co-translationally or *in vitro* following purification. Its high specificity and activity at a wide range of pH and ionic strength make TEV Protease more versatile than many other proteases used for the same purpose. Unlike factor Xa, enteropeptidase or thrombin, TEV Protease has not been found to cleave at unintended sites, even when present at a high concentration. TEV Protease is a 3C-type protease that cleaves substrates with a consensus sequence of ENLYFQG. Cleavage occurs between Q and G. Since the final aa remains on the cleaved protein where it could potentially affect structure or function, substitution of a variety of aa have been tested. In order of efficiency, S, A, M, Y, D, N, E, K or L may be effectively used in place of G. Several of the remaining aa may also vary, giving a final consensus sequence of ExxYF(M)Q(E)/G(S, A or others) where aa in parenthesis are alternatives and x is any aa. The autocatalytic site of NIa at S2256 has been mutated to an N for improved stability of the protease.

References:

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