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TEV Protease (TEVp)

Cat. Number: E-9001, E-9005

This product is a recombinant version of the catalytic domain of the nuclear inclusion protein of Tobacco Etch Virus. The enzyme contains a histidine tag at the N-terminus and has a molecular weight 28.5 kDa. The TEV protease cleaves proteins at a specific site consisting of seven amino acid residues: Glu-Asn-Leu-Tyr-Phe-Glu-X (E-N-L-Y-F-Q-X). The seventh amino acid residue can be one of six: serine (S), glycine (G), alanine (A), methionine (M), cysteine (C), or histidine (H) [1]. Cleavage occurs between the glutamine and X amino acid residues (Gln-X).

TEV protease is inactivated by heating at 65°C for 10-15 minutes.

TEV protease is inhibited by the presence of 40% glycerol, 5 mM Zn^{2+} , 1 mM Cu^{2+} , 10 mM Co^{2+} , 200 mM NaCl, 2 M urea, 500 mM guanidine hydrochloride, and 50 mM imidazole in the reaction mixture.

TEV protease remains active:

- in the presence of 10 mM MgSO4, MnCl2, and CaCl2, and 100 mM EDTA;
- in the presence of protease inhibitors such as aprotinin, benzamidine, pepstatin, and phenylmethylsulfonyl fluoride (PMSF);
- at pH 6.0 9.0;
- at temperatures ranging from 4°C to 37°C.

Application

TEV protease is used to cleave recombinant fusion polypeptides that have a protease recognition site between the leader fragment and the target protein. The presence of a histidine tag on TEV protease allows for purification of the target protein from the enzyme using metal-chelate affinity chromatography.

Source

TEV protease is isolated from an *E. coli* strain containing a plasmid with a cloned Tobacco Etch Virus enzyme gene.

Activity Units

One unit of enzyme activity cleaves 2 μg of chimeric recombinant protein (~145 kDa, MBP-Bst) up to 90% in a total reaction volume of 10 μ l in 1 hour at 30°C in standard reaction buffer. The composition of the standard reaction buffer is 50 mM Tris-HCl (pH 7.5 at 25°C), 0.5 mM EDTA, and 1 mM DTT (1 mL of 10x buffer is supplied with the enzyme).

Enzyme concentration and packaging: 5 000 u/ml.

Cat. No.	Product Name	Quantity	Volume
E-12005	TEV Protease	1000 U	200 μΙ
E-12050		5000 U	1000 μΙ

Storage Buffer

50 mM Tris-HCl (pH 7.5 at 25°C), 250 mM NaCl, 1 mM EDTA, 1 mM TCEP, and 50% glycerol.

Quality Control

Each batch of enzyme is tested for enzyme activity, electrophoretic purity in SDS-PAGE, and nonspecific proteolytic activity.

Reaction conditions

The reaction buffer consists of 50 mM Tris-HCl (pH 7.5 at 25°C), 0.5 mM EDTA, and 1 mM DTT (1 mL of 10x buffer is supplied with the enzyme). The optimal reaction temperature is 30°C. Reaction time and substrate-to-enzyme ratio are determined empirically and may vary depending on the nature of the substrate. Reactions can be carried out at 4°C for long periods (16-24 hours).

Storage and transportation conditions

Store at -20°C.

Transportation at temperatures not exceeding +8°C is allowed for up to one day.

References

Kapust, R. B., Tözsér, J., Copeland, T. D., & Waugh, D. S. (2002). The P1' specificity of tobacco etch virus protease. Biochemical and biophysical research communications, 294(5), 949-955. https://doi.org/10.1016/S0006-291X(02)00574-0