LD Biopharma, Inc.
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- PRODUCT DATA SHEET -

Name of Product: EN-TEV Protease
Catalog Number: RM5-0021
Manufacturer: LD Biopharma, Inc.

Introduction

TEV protease recognizes a linear epitope of the general form E-Xaa-Xaa-Y -Xaa-Q-(G/S), with cleavage occurring between Q and G or Q and S. The most commonly used sequence is ENLYFQ / G. A more stable mutant (S219N) of TEV protease (EN-TEV) was expressed and purified from E.coli with an N-terminal poly histidine tag was described by the Doudna laboratory [Lucast *et al.*, 2001]. The protease is used to cleave affinity tag from fusion protein. The optimal temperature for cleavage is 30 °C. EN-TEV could be easily removed from the cleavage reaction by affinity chromatography using the polyhistidine tag at N-terminal of the EN-TEV enzyme.

Gene Symbol:	TEV
Accession Number:	ABF71454.1
Species:	Tobacco Etch Virus (TEV)
Size:	50 µg / Vial
Composition:	1.0 mg/ml, sterile-filtered, in 50 mM pH 8.0 Tris-HCl Buffer, with 0.5mM EDTA and1mM DTT.
Storage:	In Liquid. Keep at -20°C for long term storage. Product is stable at 4 °C for 2-3 weeks.

Key Reference

Kapust, R. B., Tözsér, J., Fox, J. D., Anderson, D. E., Cherry, S., Copeland, T. D., and Waugh, D. S. *Tobacco etch virus protease: Mechanism of autolysis and rational design of stable mutants with wild-type catalytic proficiency*. Prot. Eng. **14**: 993-1000 (2001).

Typical Reaction Condition

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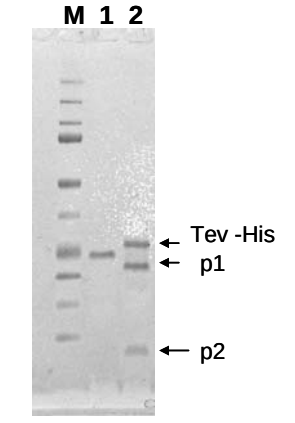
The "standard" reaction buffer for TEV protease is 50 mM Tris-HCl (pH 8.0), 0.5 mM EDTA and 1mM DTT. The duration of the cleavage reaction is typically overnight, although lots of cleavage will happen in the first few hours and prolonged incubation times may not lead to proportional increases in cleavage. TEV protease is maximally active at 34 °C, but we recommend performing the digest at room temperature (20 °C) or 4 °C. TEV protease is only three-fold less active at 4 °C than at 20 °C (Nallamsetty *et al*., manuscript in preparation).

Typically, a good rule of thumb for initial test of digestion is 1 ug TEV enzyme per 50 – 100 ug target protein ratio. Perform a small-scale reaction first, if possible, to gauge the efficiency of processing.

Quality Control

1. Purity: > 98% by SDS-PAGE.
2. Activity: Completely digestion 50ug of T7 Tag-HBxAg protein using 1ug TEV enzyme (in 1: 50 ratio) in either 3 hours at 37 °C or 12 hours at 4 °C.



4 -12 % SDS-PAGE

M: Protein Marker

Lane 1. N-terminal tag HBxAg-11R protein before TEV enzyme digestion.

Lane 2: N-terminal tag HBxAg-11R protein digested using 1:50 dilution (1ug TEV/ 50ug target protein at 4°C for overnight reaction) in buffer 20 mM Tris-HCl, pH 8.0, 100 mM KCl.

Recombinant Protein Sequence

GHHHHHHHGESLFKGPRDYNPISSTICHLTNESDGHTTSLYGIGFGPFIITNKHLFRRNNGTLL
VQSLHGVFKVKNTTTLQQHLIDGRDMIIIRMPKDFPPFPQKLKFREPQREERICLVTTNFQTKS
MSSMVSDTSCTFPSSDGIFWKHWIQTKDGQCGSPLVSTRDGFIVGIHSASNFTNTNNYFTSVPK
NFMELLTNQEAQQWVSGWRLNADSVLWGGHKVFMVKPEEPFQPVKEATQLMNRRRRR