

EZCut™ TEV Protease, Recombinant

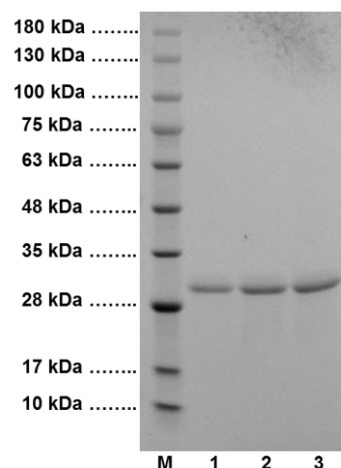
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Store at -80°C.

Cat. No.: 7847-1000	1000 U (100 µg)
Cat. No.: 7847-10000	10,000 U (1 mg)

ALTERNATE NAMES: Nuclear inclusion protein A, NIa protein**SOURCE:** *E. coli***FORM:** Liquid**FORMULATION:** 1 mg/ml solution in 0.1 M Tris-HCl, 0.5 M NaCl, 20% glycerol, 5 mM DTT and 0.5 mM EDTA, pH 8.0**PURITY:** ≥ 95% by SDS-PAGE**MOL. WT.:** 28.6 kDa (2038–2279 aa + C-terminal poly-his tag).

STORAGE CONDITIONS: Store at -80°C. Stable for at least 1 year as supplied. It may be further diluted to 0.1–0.5 mg/ml with 0.1 M Tris-HCl, 0.5 M NaCl, 20% glycerol, 5 mM DTT and 0.5 mM EDTA, pH 8.0 containing 5 mM DTT and 20% glycerol, and stored at -80°C in aliquots. Avoid repeated freezing and thawing cycles.

**SDS-PAGE (4–20%) of TEV Protease:**

M: Protein Marker

1: TEV Protease (3 µg)

2: TEV Protease (5 µg)

3: TEV Protease (8 µg)

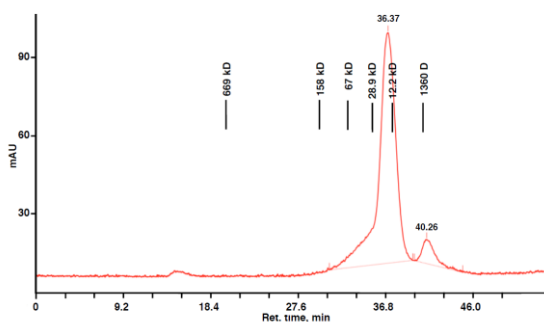
BACKGROUND: BioVision's EZCut™ TEV Protease is a cysteine protease that recognizes the cleavage site of Glu-Xaa- Xaa-Y- Xaa-Gln-(Gly/Ser) and cleaves between Gln and Gly/Ser. The optimal sequence is Glu-Asn-Leu-Tyr-Phe-Gln-Ser/Glycine (**ENLYFQSG**). It contains an enhanced form of a catalytic fragment of the NIa protein of Tobacco etch virus (TEV). TEV Protease is a restriction grade protease that has robust activity at 4°C with high specificity and great stability. The optimal temperature for cleavage with this enzyme is 34°C. The protease can be used for the removal of affinity tags from fusion proteins. It contains a C-terminal His tag and can be easily removed after cleavage reactions by passing the reaction through a Ni-chelating resin. BioVision's EZCut™ TEV Protease is an improved version of TEV protease that is highly site-specific, highly active, and significantly more stable than native TEV protease, resulting in enhanced long-term activity.

SPECIFIC ACTIVITY: The EZCut™ TEV Protease has an activity of ≥10,000 units/mg.**UNIT DEFINITION:** One unit is defined as the amount of EZCut™ TEV Protease required to cleave >90% of 10 µg a control protein substrate (containing an **ENLYFQSG** sequence at N-terminus) in 1 h at 37°C.

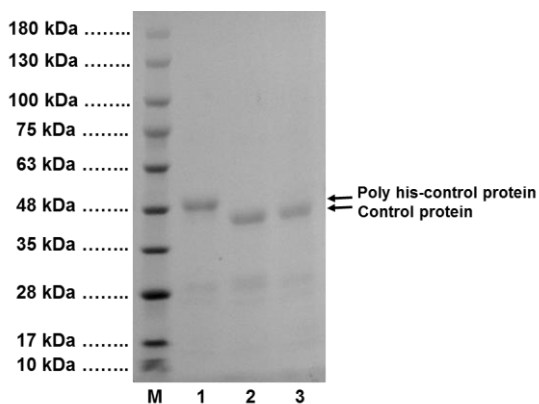
APPLICATIONS: Recombinant EZCut™ TEV Protease can be used to cleave solubility, secretion, detection, and purification affinity tags from recombinant proteins containing TEV protease-specific cleavage site resulting in the target protein with only one extra amino acid at N-terminus in comparison to other endopeptidases. The extra amino acid (Glycine/Serine) is a smaller amino acid and may not cause significant changes to protein structure.

CLEAVAGE PROTOCOL: In order to find the optimum cleavage conditions for a target fusion protein, it is recommended to run preliminary cleavage reactions at a small scale. Successful cleavage with TEV protease is dependent upon proper folding of the fusion protein that enables access of the TEV recognition sequence by the enzyme. The target fusion protein (1 mg/ml) containing TEV protease cleavage site should be purified to homogeneity. Recommended cleavage buffer is 50 mM Tris, buffer, 0.1 M NaCl, pH 8.0.

1. To 10 µl of target protein in an Eppendorf tube add 0.05–0.5 µg (0.5–5 U) of TEV protease.
2. Add 10 µl of freshly prepared 50 mM Tris, buffer, 0.1 M NaCl, pH 8.0 containing 5 mM DTT. Mix well by pipetting up and down; incubate at 37°C for 1 h. Analyze by SDS-PAGE.
3. Once optimum cleavage conditions are obtained, the reaction can be scaled up to cleave the entire amount of the target fusion protein. After completion of cleavage reaction, TEV can be removed by passing the entire mixture through Ni-chelating resin. Collect the flow through and wash with the resin with 50 mM Tris, buffer, 0.1 M NaCl, pH 8.0. Combine the washes with the flow through to obtain cleaved protein product.
4. Optimum temperature for TEV protease cleavage is 34°C. However, the reaction can also be carried out at 37°C or 30°C also. If the target protein is not stable at these temperatures, the cleavage reaction can also be performed at room temperature or 4°C. In such cases, the amount of TEV protease should be optimized for different target proteins.



SEC analysis of TEV protease (after removal of DTT, EDTA and glycerol) using a Superdex 200 HR 10/30 column at 0.5 ml/min in 50 mM Tris and 0.25 M NaCl pH 7.5.



Cleavage of a poly-his tagged control protein (10 µg) by TEV protease for 1 h at 37°C:

M: Protein Marker

1: 10 µg Poly his-control protein

2: 10 µg Poly his-control protein + 10 U TEV Protease

3: 10 µg Poly his-control protein + 1 U TEV Protease

RELATED PRODUCTS:

- TurboTEV Protease, Recombinant (Cat. No. 9205-1)
- TurboNuclease, Recombinant (Cat. No. 9207-50KU)
- Turbo3C (HRV3C) Protease, Recombinant (Cat. No. 9206-1)

FOR RESEARCH USE ONLY! Not to be used on humans.