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TEV Protease, A grade

recombinant Tobacco Etch Virus Protease

Product	Cat#	Package size
recombinant Tobacco Etch Virus Protease	S5366.1000	1000 units
recombinant Tobacco Etch Virus Protease	S5366.1010	10000 units

Introduction:

TEV-protease is a highly site-specific cysteine protease that is found in the Tobacco Etch Virus (TEV). The Genaxon TEV protease comes with an N-terminal His-tag for simple removal from the cleavage reaction by immobilization on metal-affinity resins.

TEV protease recognizes a linear epitope of the general form E-Xaa-Xaa-Y-Xaa-Q-(S/G), with cleavage occuring between Q and S or Q and G. The optimum recognition site for this enzyme is the sequence Glu-Asn-Leu-Tyr-Phe-Gln-(Ser/Gly) (ENLYFQ(S/G)). The Genaxxon TEV protease contains a S219V mutation against autoproteolysis (self cleavage) and for increased catalytic activity and efficacy compared to the wild type enzyme. It is mainly used for removing affinity tags from purified peptides or proteins. Affinity tags are often applied to facilitate the expression and purification of recombinant proteins. Whereas many tagged proteins retain their structural integrity and biological activity, others clearly do not. Therefore, whenever possible, it is prudent to remove tags from recombinant proteins.

Coomassie SDS-PAGE

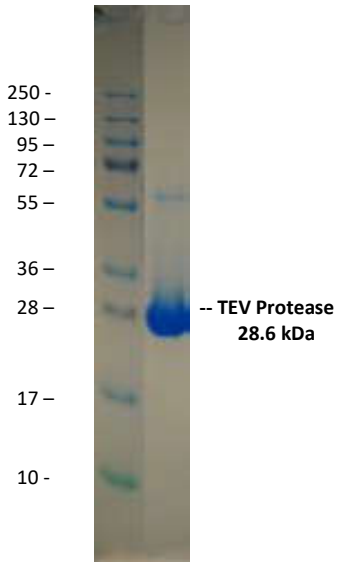

Fig. 1: Coomassie stain of assay grade TEV protease.
Analysis by SDS-PAGE under reducing conditions

Product description

TEV protease with an N-terminal His-tag for simple removal from the cleavage reaction by immobilization on metal-affinity resins. This TEV protease contains a S219V mutation against autoproteolysis (self cleavage) and for increased catalytic activity and efficacy compared to the wild type enzyme.

Purity: Greater than 95.0%, determined by reducing and non-reducing SDS-PAGE.

Source: *E.coli*

Activity: Specific activity: One unit of TEV Protease cleaves >85% of 3µg of control substrate in 1 hour at 30°C.

Protein Concentration: 7.8mg/mL (UV), ready for use.

Stability/Storage:
7.3mM TRIS/HCl; 36.6mM NaCl; 50% Glycerol;
0.366mM TCEP; pH8.5.
TEV Protease should be stored at -20°C.
Please prevent from repeated freeze-thaw cycles!

TEV quality test: A 6His tagged fusion protein (43.7 kDa) is incubated at 1mg/mL in a buffer of 50mM Tris/HCl, pH8.0, 0.5mM EDTA, 1mM DTT, at 30°C for 1 hour: without TEV and with TEV at a ratio of 1:400, 1:200, 1:150, 1:100, 1:75, 1:50 and 1:37.5 (w/w).

The cleaved product is 41.5 kDa. No non-specific cleavage has been observed.

The result shows that optimal results are achieved if TEV protease is used in a ration between 1:100 to 1:37.5 (w/w).

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Specificity

- Highly specific and active for its seven-amino acid sequence with minimal off-target effects.
- Activity more than 10,000 units per 1mg protein.
- The activity depends on the type of target protein.
- The optimal amount of enzyme should be tested for each target protein.
  For details see the “cleavage assay instructions” part of this manual (page 3).

Product Use Limitations

TEV protease is developed, designed, and sold for research purposes only. It is not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.
For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request (info@genaxxon.com).

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Cleavage Assay Instruction

1. Prepare the following assay buffer freshly prior usage: 50mM Tris, 0.5mM EDTA, 1mM DTT, pH8.0
2. Prepare a dilution of substrate protein (any fusion protein containing the recognition sequence ENLYFQ) in assay buffer to obtain a concentration of substrate of 1 mg/mL and a dilution of TEV protease to obtain a concentration of 0.04 mg/mL (or 1 unit/mL).
3. Pipette each reaction mixture in a microcentrifuge tube as indicated below (for control reactions prepare two additional mixtures containing 5µL of assay buffer replacing the TEV protease):

Reaction mixture:

Component	Volume	Final protein amount
Substrate (dil.)	20µL	20µg
TEV protease (dil.)	5µL	0.2µg
Assay buffer	175µL	---

4. Incubate the samples at 30°C or any other temperature below down to 4°C. Leave one of the two control reactions on ice or at +2°C to +8°C.
5. Remove 20µL aliquots from every reaction after 1, 2, 4, and 6 hours (and if necessary after over night incubation). Stop the reactions by mixing equal volumes of reaction mixture (including controls) and 2X reducing SDS-PAGE sample buffer and store at -20°C until ready to analyze.
6. Heat the samples for 5 minutes at 95 °C.

Analyze 10µL by SDSPAGE followed by protein staining.

Tips and Notes:

Cleavage conditions

Cleavage reactions with TEV protease are performed generally in 50mM Tris-HCl, pH8.0, 0.5mM EDTA, 1mM DTT for 1 hour at 30°C. Other conditions like lower temperatures and the addition of 1mM TCEP in place of DTT can be applied. If different conditions have to be used, the amount of TEV protease added, the incubation time and/or the incubation temperature have to be optimized.

The optimum salt concentration is 0mM but an increasing salt concentration up to 200mM NaCl will only result in a threefold decrease in activity.

How much TEV protease should be used?

As a rule of thumb 1µg TEV protease is used for 25 to 100µg substrate (minimal enzyme concentration: 1 unit/mL). More enzyme can be used if the cleavage sides are difficult to reach or if the substrate is aggregated. In such a case it might help to introduce a section of Glycine, Histidine or a Flag-Tag between the TEV cleavage side and the N-terminus of the protein.

Cleavage of the target protein directly on the column is not recommended

pH-Optimum: pH optimum of TEV protease is pH6.0 to pH8.5 but pH values between 4 and 9 can be applied.

Different buffer substances are tolerated by TEV protease. These are phosphate, MES or acetate.

Glycerol or Sorbitol can be added up 40% (w/v).

Detergents might have an influence on the activity of the protease.

Usage of DTT

If the target protein contains disulfide bridges, DTT should not be used in the reaction buffer. As an alternative 3mM lutathion/0.3mM oxidized Glutathion can be used.

If the target proteins contains a Zink-Finger, DTT can be substituted by β-Mercaptoethanol and EDTA by e.g. Citrate.

How to remove TEV protease after cleavage?

If the target protein contains no Poly-Histidin-Tag the Poly-Histidin-Tag of the Genaxxon TEV-Protease (DTT and EDTA should be removed before) can be used to remove TEV protease form the target protein by affinity chromatography.