TEV Protease

1.2018

blirt

TEV Protease

Recombinant **TEV Protease** is a highly site-specific cysteine protease, which is found in the Tobacco Etch Virus. Due to its sequence specificity, the **TEV protease** is a very powerful reagent for the removal of fusion tags from recombinant proteins after protein purification. The enzyme has been genetically modified to increase its activity and resistance to autolysis. It consists of the 27 kDa catalytic domain with an N-terminal polyhistidine tag.

TEV Protease specifically recognizes a seven amino acid sequence of the general form Glu-X-X-Tyr-X-Gln↓(Gly/Ser), most commonly Glu-Asn-Leu-Tyr-Phe-Gln↓Gly, and cleaves between glutamine and glycine or serine.

TEV Protease can also be used to cleave the affinity tag from a fusion protein immobilized on the affinity resin. Following digestion, the **TEV Protease** can be easily removed from the cleavage reaction by affinity chromatography using the polyhistidine tag at the N-terminus of the protease.

Features and advantages

- Specific activity: 1 U/µl
- Origin: recombinant TEV Protease expressed in *Escherichia coli*
- Enhanced enzyme stability for prolonged protease activity
- Activity over a broad range of temperatures (4–37°C) and pH (6.5–8.5)
- Contains His-tag to facilitate its removal from digested proteins

Applications

- Removal of affinity tags from fusion proteins

TEV Protease

Usage

1. Mix the following components in a microcentrifuge tube:

Component	Quantity
20x TEV Reaction Buffer	5 µl
100 mM DTT [1]	1 µl
Fusion Protein [2]	10–30 µg [3]
TEV Protease 1 U/µl	10 µl (10 U)
Water	fill up to 100 µl

1) If the target protein is expected to contain disulfide bonds, DTT should not be used in the reaction. In such a case, use 3 mM glutathione or 0.3 mM oxidized glutathione instead. If your target protein contains zinc fingers, it might be a good idea to replace DTT with β-mercaptoethanol (to final concentration of 14 mM) and to replace EDTA with a weaker metal chelator such as citrate (to final concentration of 5 mM).
2) TEV Protease tolerates a range of buffer components: phosphate, MES or acetate.
3) Use more TEV Protease, if the cleavage site of the fusion protein is sterically occluded (when the protease cleavage site is too close to an ordered structure in the target protein) or when the fusion protein exists in the form of soluble aggregates.

2. Incubate the reaction mixture at 16 –30°C for 2 –6 hours. On rare occasions, an overnight incubation may be necessary. If the protein of interest is heat – labile, incubations at 4°C are recommended. Reactions at 4°C will require longer incubation times (overnight) and / or more **TEV Protease**.
3. Remove 20 µl aliquots from the reaction after 2, 4 and 6 hours of incubation. Add an appropriate SDS-PAGE sample buffer to the aliquots and store at -20°C until further analysis. Determine and compare the digestion efficiency by SDS-PAGE analysis.
4. When the cleavage conditions have been optimized at a small scale, scale up proportionally, according to a specific application requirement.

Additional considerations

- A number of variables can be examined to optimize the cleavage of any specific fusion protein:
  - the amount of the enzyme (protease-to-target protein ratio)
  - the temperature of the incubation
  - the time needed for cleavage
- If possible, perform a small-scale reaction first, to check the efficiency of the process.
- The efficiency of cleavage may vary due to the sequences around the cleavage site, the conformation and the solubility of the target protein. Due to its high specificity, more enzyme or longer cleavage time at higher temperature (37°C) may be used to achieve high efficiency without compromising the specificity.
- **TEV Protease** can be removed from the reaction by incubation with an appropriate nickel chelating resin to bind the His-tag (DTT and EDTA should be removed first). Follow the binding instructions for the affinity resin of choice. The cleaved protein of interest will be found in the flow-through or supernatant fraction. If the enzyme is used to remove a polyhistidine tag, this polypeptide will also be removed from the reaction.

Storage buffer

100 mM Tris-HCl (pH 7.5), 500 mM NaCl, 0.5 mM EDTA, 5 mM DTT, 50% (v/v) glycerol

Recommended Reaction Buffer

50 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 1 mM DTT

20x TEV Reaction Buffer

1 M Tris-HCl (pH 8.0), 10 mM EDTA

Quality control

The enzyme is functionally tested for the absence of any non-specific protease activity. Electrophoretic purity over 90% (SDS-PAGE).

Unit definition

One unit of **TEV Protease** cleaves >95% of 3 µg control substrate in one hour at 30°C.

TEV Protease

Components	RP171 1000 U	RP172 10 000 U
TEV Protease 1 U/µl	1 ml	10 x 1 ml
20x TEV Reaction Buffer	1 ml	8 x 1 ml
100 mM DTT	0.5 ml	4 x 0.5 ml

Storage & shipping

Storage conditions
All components should be stored at -20°C.
The buffer can be kept at +4° for short-term storage.

Shipping conditions
Shipping on dry or blue ice.

For research use only

BLIRT S.A.
orders@blirt.eu | www.blirt.eu