Product Information

TEV Protease

T4455

Product Description

Synonyms: Tobacco Etch Virus protease, TEVp

TEV protease, originally isolated from Tobacco Etch Virus, is a member of the family of C4 peptidases. Three proteolytic enzymes were found as proteins released from the N-terminus of the TEV polyprotein:

- P1
- HC-Pro
- Nuclear Inclusion α (NIα)

In its native form, TEV protease is a 27 kDa catalytic domain that was originally noted as the C-terminal portion of the NIα part of the TEV polyprotein.[1,2]

TEV protease specifically cleaves proteins within a seven-residue optimal recognition sequence. This sequence is:

Glu-Asn-Leu-Tyr-Phe-Gln↓-Gly/Ser (E-N-L-Y-F-Q↓-G/S)

The 7th residue can be either Gly (G) or Ser (S), and proteolytic cleavage occurs between the Glu and Gly/Ser residues.[1] Because of this strict sequence recognition specificity, TEV protease has thus become a useful tool to cleave recombinant proteins that are expressed as fusion proteins with this sequence between the carrier domain and the protein of interest.[2]

Separate crystal structures of an autolysis-resistant mutant of TEV protease, complexed with substrates or product peptides, and of an inactive form of TEV protease, have been published.[3-4]

Native TEV protease has a Cys residue at amino acid position 151. This renders ineffective against TEV protease many common serine protease inhibitors, such as PMSF, AEBSF, TLCK, bestatin, pepstatin A, EDTA and E-64. However, protease inhibitors that target cysteine residues, such as NEM and IAA, are more effective at inhibiting native TEV protease.[2]

Native TEV protease is optimally active at 30 °C, and is highly effective in the temperature range of 29-34 °C.[5] However, native TEV protease suffers severe loss of activity at temperatures ≥ 37 °C.[1]

TEV protease has activity in the pH range of 6-9. At pH ≤ 5, TEV protease is inactive.[1]

Under *in vitro* conditions, native TEV protease has optimal activity in the absence of monovalent salts (such as NaCl).[5] However, TEV protease may be used in the presence of NaCl at concentrations up to 200 mM.[1,5]

TEV protease, in its native form, has limited solubility in aqueous media. Many studies have indicated that the use of different protein tags, to express TEV protease in tagged recombinant form, greatly facilitates the solubility of TEV protease in aqueous media.[2,6]

This product is a recombinant version of TEV protease which contains two distinct protein tags, a GST tag and a six-histidine tag (His-tag). This allows for facile chromatographic purification of the product.

Reagent

This product is supplied at a concentration of ≥ 2 mg/mL, in an aqueous buffer containing 25 mM Trizma®-HCl (pH 8.0), 50 mM NaCl, 1 mM TCEP and 50% (v/v) glycerol.

Unit Definition

One unit of TEV protease cleaves > 85% of 3 µg of control substrate in one hour, at pH 8.0 at 30 °C.

Procedure

Cleavage Protocol

1. Prepare fresh dialysis buffer. Dialysis buffer should be optimized for target protein solubility and contain no protease inhibitors. The dialysis buffer should also be compatible with downstream purification processes, such as minimal EDTA or DTT for removal of the cleaved His-tag with a HIS-Select® column. An example of a suitable dialysis buffer is 25 mM Tris-HCl (pH 8.0), 150-500 mM NaCl, and 14 mM β-mercaptoethanol.

2. Dilute the target protein sample to 1-2 mg/mL with dialysis buffer. This is optional in case the target protein aggregates in dialysis buffer. Save a small aliquot as a control for PAGE analysis. EDTA may be added to a final concentration of 0.5 mM, if the target protein will be eluted from a HIS-Select® column and EDTA is compatible with the target protein.
3. Add TEV protease at a protease:target protein ratio of 1:100 (w/w). An effective general range of the protease:target protein ratio (w/w) is 1:50 to 1:200. However, researchers should empirically determine their optimal protease:target protein ratios.
    - Alternatively, 10,000 units of TEV protease can be added to 100 mg of target protein. If desired, TEV protease can be added directly to the target protein, without changing the buffer or dilution of the TEV protease.
    - An alternative reaction buffer for in vitro reactions with TEV protease is 50 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, and 1 mM DTT.[2]
4. Dialyze against dialysis buffer at 4 °C for ~16 hours. This step is for removal of glutathione or imidazole, if glutathione-affinity or HIS-Select® columns are respectively used to remove the cleaved tag or TEV protease after cleavage.

Removal of TEV Protease

1. The dialyzed target protein and TEV Protease mixture can be applied directly to affinity columns if compatible dialysis/equilibration buffer is used.
    - For His-tagged protein, use HIS-Select® affinity media to remove the cleaved histidine-tag and the TEV Protease.
    - For GST-tagged protein, use glutathione-agarose to remove the cleaved GST-tag and the TEV Protease.
2. If desired, analyze samples using SDS-PAGE analysis. The difference between the tagged and cleaved target protein may be too small to detect by SDS-PAGE. The cleaved His-tag sometimes can be seen at the bottom of the gel.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

References

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6. Zdanov, A.S. *et al*., *Russ. J. Bioorg. Chem*., **29(5)**, 415-418 (2003).

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