Sigma-Aldrich.

Product Information

TEV Protease, Biotin tagged

Recombinant protein, aqueous solution

SAE0118

Product Description

TEV protease, originally isolated from Tobacco Etch Virus, is a member of the family of C4 peptidases. TEV protease has high sequence specificity, and has thus become a widely used protease for removing fusion tags from recombinant fusion proteins.^{1,2}

TEV protease specifically cleaves proteins within a seven-residue optimal recognition sequence, as follows:²

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

However, TEV protease is active on a range of substrates with a broader consensus sequence of:

$$E-X-X-Y-X-Q-G/S$$
 (where **X** is any residue)²

One study on various mutants at the P1' residue (G/S) of the TEV protease optimal recognition sequence proposes that other residues such as Ala, Cys and Met can be tolerated at the P1' residue in such TEV protease variants.³

Native TEV protease is optimally active at 30 °C, and is highly effective in the temperature range of 29-34 °C.⁴ However, native TEV protease suffers severe loss of activity at temperatures \geq 37 °C.⁵

TEV protease has activity in the pH range of 6-9. At pH \leq 5, TEV protease is inactive.⁵

This biotinylated TEV protease is expressed in *E. coli* and does not carry any purification tag other than biotin. Biotinylation of this product is done enzymatically with no effect on its proteolytic activity. It is designed to be used for on-column cleavage of fusion proteins that contain a TEV protease recognition sequence. This method specifically cleaves the protein of interest from a column-bound fusion protein, leaving the purification domain or tag bound to the affinity column (such as an Ni-NTA column) and eluting only the protein of interest. This method is advantageous over post-elution cleavage for several reasons:

- It eliminates most of the impurities normally associated with affinity purification.
- It allows much gentler elution conditions, with an added flexibility in the composition of the elution buffer. This can help to prevent protein aggregation and inactivation.

After cleavage, the biotinylated TEV protease can be removed with avidin-conjugated or streptavidin-conjugated beads.

Reagent

This product is supplied as an aqueous buffered glycerol solution containing 20 mM Trizma $^{\circ}$ -HCl (pH 7.5), 50 mM NaCl, 1 mM TCEP, 1 mM EDTA 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.

Storage/Stability

The product retains activity for at least 2 years when stored at -20 °C.

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.

Procedure

1

Activity toward substrate proteins is dependent on the substrate identity and reaction conditions. The use of low concentrations of a reducing agent, such as 0.1-2 mM DTT, in the reaction buffer is suggested, to keep the enzyme active during prolonged incubations.

Although this biotin-tagged TEV protease product does not contain a histidine tag, it has low intrinsic affinity to Ni-NTA resins. It is recommended to use buffers containing low concentrations of imidazole (20-40 mM), to prevent this non-specific interaction with the resin that reduces efficiency of the enzyme towards column-bound proteins.



A good starting point for optimization is to use 40 units of TEV Protease per 1 mg of target protein at 2-8 °C for 12-24 hours. Faster protein cleavage can be achieved by increasing the temperature up to 30 °C or by increasing the amount of TEV Protease to 200 units per 1 mg of target protein. Under such conditions, cleavage may be completed within 1 hour.

On-Column Cleavage Protocol

- 1. Dilute the desired amount of TEV protease in a volume equal to one column volume. A concentration of 100-200 U/mL is recommended as a starting point.
- 2. Inject the protease solution directly onto the column.
- 3. Incubate the column at 4-8 °C for 8-24 hours.
- 4. Elute the cleaved target protein with 1-3 column volumes of elution buffer.
- 5. If the target protein is prone to precipitation at higher concentrations, on-column cleavage can be performed by continually circulating the protease solution in a larger volume through the column in a closed circle, until all target protein is removed from the column.

Depending on the results, the concentration of TEV Protease can be increased or decreased in subsequent experiments.

References

- 1. Dougherty, W.G. et.al., Virology, 171(2), 356-364 (1989).
- 2. Cesaratto, F. et al., J. Biotech., 231(10), 239-249 (2016).
- 3. Kapust, R.B. et.al., Biochem. Biophys. Res. Comm., 294(5), 949-955 (2002).
- 4. Nallamsetty, S. et al., Protein Expr. Purif., 38(1), 108-115 (2004).
- 5. Waugh, D.S., Protein Expr. Purif., 80(2), 283-293 (2011).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

