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Product Information

TEV Protease, Biotin-tagged
recombinant, expressed in *E. coli*

Catalog Number **SAE0118**
Storage Temperature –20 °C

Synonyms: Tobacco Etch Virus protease, TEVp

Product Description
TEV protease is a highly sequence-specific serine protease from Tobacco Etch Virus. Due to its high specificity, TEV protease is popular for cleavage of recombinant fusion proteins to remove fusion tags. The optimal sequence for TEV protease cleavage is ENLYFQ\S. However, TEV protease is active on a range of substrates with a consensus sequence of EXLYΦQ\ϕ, where:

- X is any residue
- Φ is any large or medium hydrophobic residue
- ϕ is any small hydrophobic or polar residue (i.e. glycine, serine, alanine, valine, cysteine)[1]

Biotinylation of this product is done enzymatically with no effect on its proteolytic activity. This biotin-tagged TEV protease does not carry any purification tag other than biotin. 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 (e.g. Ni-NTA column) and eluting only the protein of interest.

This method is advantageous to 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 any avidin-conjugated or streptavidin-conjugated beads.

This product is supplied at a concentration of ≥10,000 units/mL in an aqueous buffer containing 20 mM Trizma® HCl, pH 7.5, with 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.

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.

Storage/Stability
The product retains activity for at least 2 years when stored at –20 °C.

Procedure
TEV Protease is active over a wide range of pH values, ionic strengths, and temperatures. It retains significant activity at temperatures as low as 4 °C, making it a good choice for temperature-sensitive proteins. However, activity toward substrate proteins is dependent on the substrate identity and reaction conditions. Since TEV protease is a cysteine protease, the use of low concentrations of a reducing agent, e.g. 0.1–2 mM DTT, in the reaction buffer is suggested, to keep the enzyme active during prolonged incubations.

Although biotin-tagged TEV protease 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.

To perform on-column cleavage:

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. Kapust, R.B. *et.al.*, *Biochem. Biophys. Res. Comm.,* **294(5)**, 949-955 (2002).

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