

Rev. 2025-03-25

## FasTEV<sup>TM</sup> Protease contents

Catalog #	Description	Size	M. W.	Purity	рН	Storage
GE0501	FasTEV <sup>TM</sup>	1,000 units, lyophilized	29,332	> 95%	6.5-8.0 optimal	-20°C, up to 6 months
BA0901	10X Reaction Buffer 5	1 mL			7.5	4 to 25°C

This product is for research use only and not for resale or for any use in the manufacture of a therapeutic or for any diagnostic purpose.

**Product description:** Tobacco Etch Virus (TEV) Protease (EC #3.4.22.44) specifically cleaves the peptide bond between a glutamine and a glycine or serine of the consensus sequence, ENLYFQ $\downarrow$ (G/S).



FasTEV<sup>TM</sup> is an engineered version of the enzyme with multiple mutations that enhance its stability and catalytic activity. It is recombinantly expressed in *Escherichia coli* with an *N*-terminal 8xHis tag.

This product does not contain any detectable activities of nonspecific proteases.

**Unit definition:** One unit is defined as the amount of FasTEV<sup>TM</sup> required to cleave 90% of 0.1 nanomole (5.4  $\mu$ g) of a fusion protein substrate in 1 h at 30°C in 20  $\mu$ L 1X Reaction Buffer 5 (50 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, pH 7.5).

**Product reconstitution:** Dissolve the lyophilized product in 100 μL molecular grade water to make a 10,000 units/mL (GE0501) solution in 1X Reaction Buffer 5. Once reconstituted, FastTEV<sup>TM</sup> can be stored at 4°C for up to 2 weeks or -20°C for up to 3 months. Aliquoting is recommended to avoid repeated freeze-thaw cycles.

## Suggested protocol for fusion tag removal:

1. Mix the following components in a microfuge tube:

Fusion protein substrate 1-10 µg (0.1 nanomole)

10X Reaction Buffer 5 (Cat #BA0901) 2.0 μL

FasTEV<sup>TM</sup> Protease (Cat #GE0501) 0.5  $\mu$ L (5 units) Molecular grade water to 20  $\mu$ L final volume

- 2. Incubate at 30°C for 1 h.
- 3. Analyze by SDS-PAGE mobility shift or other method to determine the extent of fusion protein cleavage.
- 4. (Optional) Run immobilized metal affinity chromatography (IMAC) to remove FasTEV<sup>TM</sup>.

**Reference:** Nam H, et al. FEBS Open Bio. 2020 Apr;10(4):619-626. PMID: 32129006.

**Note:** Reactions may be scaled up to accommodate larger amount and volume of substrate. Titration of the amount of enzyme in a reaction is recommended for each new substrate. FasTEV $^{\text{TM}}$  has been shown experimentally to remain stable and active after 48 h incubation at 37°C.