FasTEV™ Protease contents

Catalog #	Description	Size	M. W.	Purity	pH	Storage
GE0501-1KU	FasTEV™	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↓(G/S).

FasTEV™ 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™ required to cleave 90% of 0.1 nanomole (5.4 µg) of a fusion protein substrate in 1 h at 30°C in 20 µL 1X Reaction Buffer 5 (50 mM Tris, 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 (GE0501-1KU) solution in 1X Reaction Buffer 5 (50 mM Tris, 50 mM NaCl, 1 mM EDTA, pH 7.5). Once reconstituted, FastTEV™ 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™ Protease (Cat #GE0501-1KU)	0.5 µL (5 units)
Molecular grade water	to 20 µL 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™.

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™ has been shown experimentally to remain stable and active after 48 h incubation at 37°C.*

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