Certificate of Analysis

ProTEV Plus:

Part# 9PIV610

Revised 1/18

Supplied With:

Cat.#	Size	ProTEV Plus	ProTEV Buffer, 20X	100mM DTT
V6101	1,000 units	V610A	V602A (1ml)	P117B (250µl)
V6102	8,000 units	V610B	V602B (8ml)	P117C (1.25ml)

Description: ProTEV Plus is an engineered form of TEV protease, a highly specific proteolytic enzyme that cleaves within a seven-amino-acid sequence. It can be used to cleave protein fusions that have been engineered to contain the seven-amino-acid sequence at the desired cleavage site. ProTEV Plus also contains an HQ tag located at the N-terminus of the protein, which allows it to be immobilized on affinity resins and removed from the cleavage reaction.

Formulation: ProTEV Plus is supplied at a concentration of 5u/µl in 50mM HEPES (pH 7.5), 300mM NaCl, 1mM DTT, 1mM EDTA, 50% glycerol, 0.1% Triton® X-100. ProTEV Buffer, 20X, contains 1M HEPES (pH 7.0), 10mM EDTA.

Unit Definition: Four units of ProTEV Plus cleaves ≥85% of 20µg of a test fusion protein in 30 minutes at 30°C.

Storage Conditions: Store at –20°C; do not store at –70°C or on dry ice. Avoid exposure to frequent temperature changes. See the expiration date on the product label.

ProTEV Plus protease may precipitate, especially if it is not stored properly. The precipitate only accounts for a fraction of the ProTEV Plus and does not significantly affect activity. However, the aggregates can be hard to resolubilize. Always store ProTEV Plus at –20°C and return to –20°C immediately after use. If precipitation does occur, spin at 4°C at full speed for 5 minutes in a benchtop centrifuge to collect the precipitate and use the cleared supernatant for your experiment.

Quality Control Assays

Purity: ≥90% pure by SDS-PAGE.

Specificity: In an overdigestion assay (10u ProTEV Plus, overnight at 30°C), ProTEV Plus does not cleave proteins that do not contain the TEV protease sequence.

Signed by:

R. Wheeler, Quality Assurance

Promega

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Usage Information

1. Description

ProTEV Plus is an improved 48kDa version of the NIa protease from tobacco etch virus (TEV) that has been engineered to be more stable than native TEV protease for prolonged enzymatic activity (1–3). TEV protease is a highly site-specific proteolytic enzyme that recognizes the seven-amino-acid sequence EXXYXQ(G/S), most commonly ENLYFQG, with cleavage occurring between glutamine and glycine or serine (4,5). The protease is used to cleave affinity tags from fusion proteins after protein purification. The protease cleaves sequences with a variety of amino acids at the G/S (or P1) position (6). This allows the choice from many different amino acids on the newly formed N-terminus after cleavage. Optimum activity is obtained at pH 7.0 and 30°C, but ProTEV Plus is active over a wide range of pH values (5.5–8.5) and temperatures (4–30°C), allowing a choice of conditions amenable to the protein of interest. ProTEV Plus is removed easily from the cleavage reaction after cleavage using the HQ tag located at the N-terminus of the protein. ProTEV Plus also can be used to cleave the affinity tag from a fusion protein immobilized on the affinity resin.

2. General Protocol

ProTEV Plus reactions are carried out in 1X ProTEV Buffer plus 1mM DTT. Assemble the following components in a microcentrifuge tube:

Component	Volume
ProTEV Buffer, 20X	5µl
100mM DTT	1µl
fusion protein	20µg
ProTEV Plus (10u)	2µl
Water to	100µl

Incubate samples at 30°C. If preferred, the fusion protein can be cleaved at a lower temperature. Remove 20µl aliquots from the digest at 1, 2, 4 and 6 hours. Add an appropriate SDS-PAGE sample buffer to the aliquots, and store at –20°C until ready to analyze. Analyze 10–20µl by SDS-PAGE, and determine percent cleavage by monitoring the disappearance of the full-length fusion protein and appearance of the cleaved products.

Notes:

1. If the fusion protein is too dilute to include 20µg in a 100µl reaction, either decrease the amount of fusion protein cleaved in the assay or increase the volume of the reaction to accommodate the desired amount of fusion protein.
2. ProTEV Plus has a molecular weight of approximately 48kDa on reducing SDS-PAGE gels.

Cleavage of the fusion protein can be optimized by changing the amount of ProTEV Plus added to the reaction, changing the incubation time, and/or changing the incubation temperature of the reaction.

3. Removal of ProTEV Plus from Cleavage Reactions

ProTEV Plus has an HQ tag at the N-terminus. After cleaving the fusion protein, ProTEV Plus can be removed from the reaction by incubating with metal-affinity resins like MagneHis™ Ni-Particles (Cat.# V8565) or HisLink™ Protein Purification Resin (Cat.# V8823). Follow the binding instructions for the metal-affinity resin of choice. The cleaved protein of interest will be found in the resin flowthrough or supernatant fraction. If ProTEV Plus was used to remove an HQ or polyhistidine tag, this polypeptide also will be removed from the reaction.

4. Cleavage of Fusion Proteins During Affinity Purification

ProTEV Plus can be used to cleave fusion proteins at the end of affinity purification procedures. Rather than eluting the fusion protein, ProTEV Plus will cleave the fusion protein while it is still bound to the resin, leaving the affinity tag bound to the resin and the protein of interest in the column flowthrough. This process will need to be optimized for each fusion protein with respect to the amount of protease used and time required for cleavage. Optimization can begin using the conditions optimized for the digestion of the fusion protein in solution and varying the assay time and temperature as needed.

In general, after binding the fusion protein to the affinity resin and washing according to the manufacturer's instructions, equilibrate the column with 1X ProTEV Buffer plus 1mM DTT. Leave enough of the buffer in the column to allow free movement of the resin while rocking or rotating the column/tube. Add ProTEV Plus to the column. Using the incubation conditions determined during optimization, incubate while rocking or rotating the resin to keep it resuspended in the buffer. The column flowthrough/supernatant will contain the protein of interest without the affinity tag.

Note: More ProTEV Plus may be required for column cleavage than for solution cleavage of the same fusion protein.

5. References

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2. Carrington, J.C. *et al.* (1993) Internal cleavage and trans-proteolytic activities of the VPg-proteinase (NIa) of tobacco etch potyvirus in vivo. *J. Virol.* **67**, 6995–7000.
3. Kapust, R.B. *et al.* (2001) Tobacco etch virus protease: Mechanism of autolysis and rational design of stable mutants with wild-type catalytic proficiency. *Protein Eng.* **14**, 993–1000.
4. Dougherty, W.G. *et al.* (1989) Characterization of the catalytic residues of the tobacco etch virus 49-kDa proteinase. *Virology* **172**, 302–10.
5. Carrington, J.C. and Dougherty, W.G. (1988) A viral cleavage site cassette: Identification of amino acid sequences required for tobacco etch virus polyprotein processing. *Proc. Natl. Acad. Sci. USA* **85**, 3391–5.
6. Kapust, R.B. *et al.* (2002) The P1 specificity of tobacco etch virus protease. *Biochem. Biophys. Res. Commun.* **294**, 949–55.

6. Related Products

Product	Size	Cat.#
MagneHis™ Ni-Particles	10ml	V8565
HisLink™ Protein Purification Resin	5ml	V8823

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