

greenTEV, lyophilized formulation

Cysteine protease from Tobacco Etch Virus

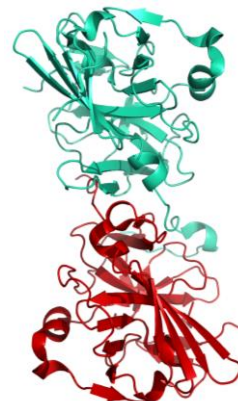
Cat. no. P2020-142

Product Information

| | |
|--------------------|---|
| Protein: | greenTEV, lyophilized formulation (~ 53.7 kDa) |
| Uniprot#: | Q0GDU8 |
| Sequence: | KGPRDYNPISSSICHLTNESDGHTTSLYGIGFGPFIITNKHLFRRNNGTLVVQSLHGVEK VKDTTTTLQQHLVDGRDMIIRMPKDFPPFPQKLKFRPQREERICLVTTNFQTKSMSSMV SDTSCTFSGDGIWFKHWIQTKDGQCGSPLVSTRDGFIVGIHSASNFTNTNNYFTSVPKN FMELLTNQEAQQWVSGWRLNADSVLWGGHKVFMVKPEEPFPVKEATQLMNE |
| | Methionine at pos. 1 present due to cloning constraints, C-terminal His-tag and GFP-fusion not shown in sequence. |
| Source: | Recombinantly expressed in <i>E.coli</i> . |
| Tag(s): | GFP, N-terminal and His-Tag, C-terminal |
| Purification: | Purified by affinity chromatography and subsequent buffer exchange. |
| Formulation: | 50 mM Tris, 150 mM NaCl, 0.5 mM EDTA, 40% Glycerol; pH 8.0. Lyophilized, stored at -80 °C and shipped at ambient temperature. We recommend reconstituting the enzyme in 40 % Glycerol (w/v). In case of 0.2 kU, add 10 µl of 40 % Glycerol (w/v) In case of 1 kU, add 50 µl of 40 % Glycerol (w/v). In case of 10 kU, add 500 µl of 40 % Glycerol (w/v). |
| Purity: | >85 % (will be determined by densitometry of Coomassie stained gel, example next page) |
| Specific Activity: | ≥20 Units/µl (determined by cleavage of control protein) ≥0.25 µmol/min/mg (determined by cleavage of labeled peptide (Fluorometric assay), TEV Protease Activity Kit (Abcam)) |
| Unit definition: | One unit of greenTEV will cleave 3 µg of a fusion protein to 98 % in 1 hour at room temperature. It is recommended to optimize cleavage conditions for each fusion protein by varying the amount of greenTEV, reaction time, or temperature. |
| Concentration: | Will be determined by BCA-Assay. |
| Long-term storage: | Recommended at -20 °C. |

Background Information:

greenTEV represents the catalytic domain of the nuclear inclusion a (NIa) protein with a molecular weight of 27 kDa encoded by the plant virus Tobacco Etch Virus. "green" indicates fusion of the protease to green fluorescent protein (GFP), which leads to increased stability and solubility of TEV protease. Moreover, greenTEV has been optimized by site directed mutagenesis to prevent autocatalytic cleavage. greenTEV is a highly site-specific cysteine protease that recognizes the amino acid sequence Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser) [ENLYFQ(G/S)] and cleaves between the residues Gln and Gly/Ser. The most commonly used recognition sequence is ENLYFQG.



Structural model of greenTEV, lyophilized formulation

greenTEV, lyophilized formulation

Cysteine protease from Tobacco Etch Virus

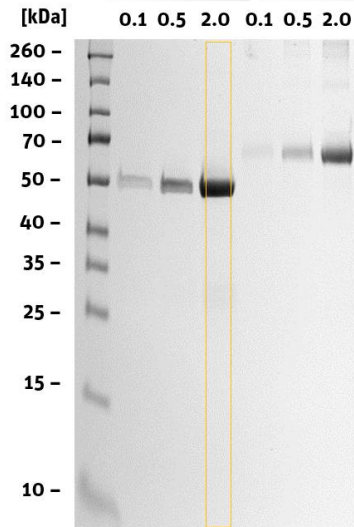
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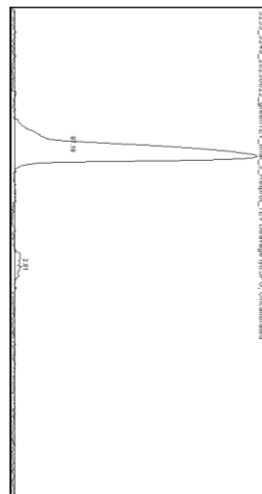
In biotechnology, *greenTEV* is a versatile enzyme to remove affinity tags from recombinant proteins with high specificity and activity over a wide range of pH, ionic strength and temperatures between 4 °C and 30 °C. The optimal temperature for cleavage is 30 °C. It is recommended to improve cleavage efficiency for each fusion protein by varying the amount of recombinant *greenTEV*, reaction time, or incubation temperature. The great advantage of *greenTEV* is its facile removal after cleavage reaction by immobilized metal affinity chromatography (IMAC) since it is equipped with a His-tag. Furthermore, the removal of *greenTEV* can be monitored instantly by detection of fluorescence in solution - this easy, fast and sensitive method omits time-consuming SDS-PAGE or Western blot analysis. If the green fluorescence of *greenTEV* is not suitable and you prefer blue fluorescence as readout, check our *blueTEV* protease.

Quality Information (provided for each lot):

greenTEV
[µg]
EXAMPLE ONLY BSA [µg]
0.1 0.5 2.0 0.1 0.5 2.0



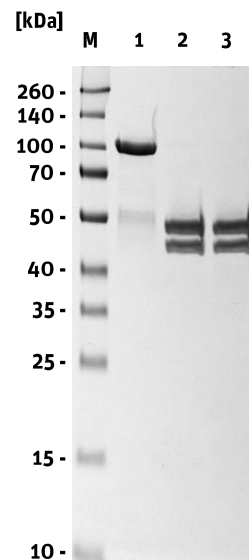
12 % SDS-PAGE



| | Area | Percent |
|---|----------|---------|
| 1 | 8906.619 | 97.194 |
| 2 | 257.092 | 2.806 |

Histogram (of marked lane in gel picture)

Test Cleavage:



P2020-131 Cleavage and tag control protein (90.5 kDa):

- 1 : Start of cleavage reaction
- 2 : Cleavage reaction (1 h)
- 3 : End of cleavage reaction (24 h)

12 % SDS-PAGE & coll. Coomassie staining.