

blueTEV

Cysteine protease from Tobacco Etch Virus

Cat. no. P2020-138

Product Information

Protein:	<i>blueTEV</i> (~ 53.7 kDa)
Uniprot#:	Q0GDU8
Sequence:	KGPRDYNPISSSICHLTNESDGHTTSLYGIGFGPFIITNKHLFRRNNGTLVVQSLHGVEK VKDTTTLQQHLVDGRDMIIRMPKDFPPFPQKLFREPQREERICLVTTNFQTKSMSSMV SDTSCTFPSGDGIFWKHWIQTkdGQCGSPLVSTRDGFIVGIHSASNFNTNNYFTSVPKN FMELLTNQEAQQWVSGWRLNADSVLWGGHKVFMVKPEEPFQPVKEATQLMNE
	Methionine at pos. 1 might be present due to cloning constraints, C-terminal His-tag and BFP-fusion not shown in sequence.
Source:	Recombinantly expressed in <i>E.coli</i> .
Tag(s):	BFP, 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 Liquid, stored and shipped at -20 °C.
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 <i>blueTEV</i> cleaves > 85 % of 3 μg of a fusion protein in 1 hour at room temperature. Cleavage overnight increases cleavage efficiency to > 99 %. It is recommended to optimize cleavage conditions for each protein by varying the amount of <i>blueTEV</i> , reaction time, or temperature.
Concentration:	Will be determined by BCA-Assay.
Long-term storage:	Recommended at -20 °C.

Background Information:

blueTEV 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. "blue" indicates fusion of the protease to blue fluorescent protein (BFP), which leads to increased stability and solubility of TEV protease. Moreover, *blueTEV* has been optimized by site directed mutagenesis to prevent autocatalytic cleavage. *blueTEV* 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. In biotechnology, *blueTEV* is a versatile enzyme to remove affinity tags from recombinant proteins with high specificity and activity over a wide



Structural model of *blueTEV*

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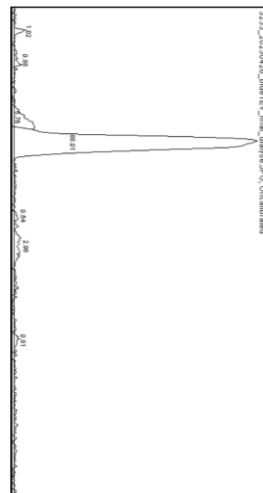
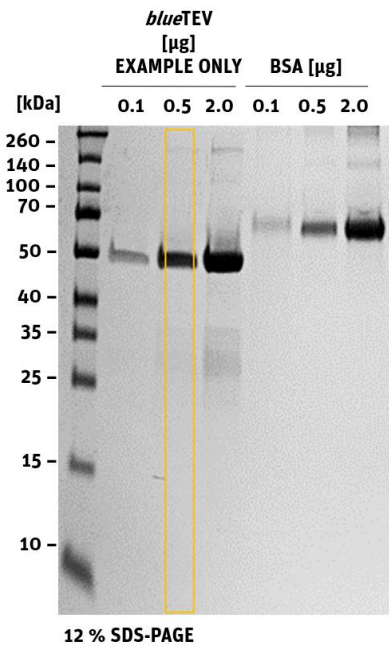
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range of pH, ionic strength and temperatures between 4 °C and 30 °C. The optimal temperature for cleavage is 30 °C. It is recommended to improve cleavage efficiency for each fusion protein by varying the amount of recombinant *blueTEV*, reaction time, or incubation temperature. The great advantage of *blueTEV* 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 *blueTEV* 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 blue fluorescence of *blueTEV* is not suitable and you prefer green fluorescence as readout, check our *greenTEV* protease.

Quality Information (provided for each lot):

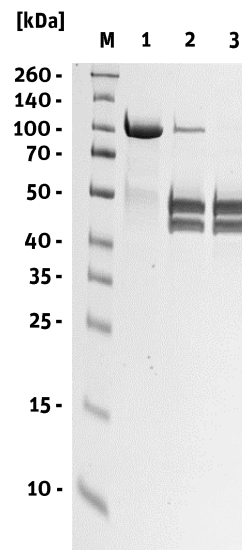


	Area	Percent
1	90.485	1.017
2	78.607	0.884
3	512.335	5.761
4	7827.234	88.007
5	48.071	0.540
6	264.991	2.979
7	72.192	0.812

SDS-PAGE/Coll.Coomassie

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.