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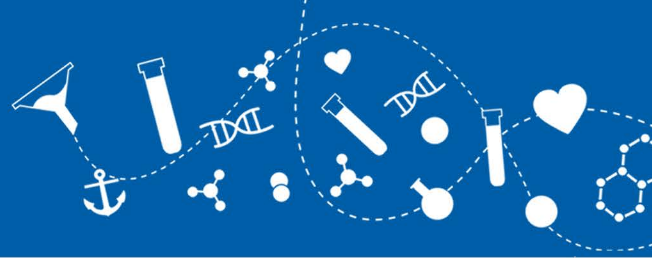

Razor™ TEV Protease

Catalog No:	BM-13002
Lot No:	XXXXX
Source:	*E. coli*
MW:	28.6 kDa
Supplied as:	liquid
Size:	1000 u (100 µl), 10000 u (150 or 300 µl) lotspecific
Stability:	store at -20°C

Background

TEV protease is a highly site-specific cysteine protease that is found in the Tobacco Etch Virus (TEV) often used for easy tag removal. TEV protease cleaves the sequence ENLYFQG/S between the Q and G/S residues with high specificity. Razor™ TEV protease contains an N-terminal His-tag for simple removal from the cleavage reaction by immobilization on metal-affinity resins. This TEV protease contains a S219V mutation against autoproteolysis (self cleavage) and for increased catalytic activity and efficacy compared to the wild type enzyme. Avoid multiple freeze-thaw cycles!

Formulation

7.3 mM Tris-HCl, 36.6 mM NaCl, 50% Glycerol, 0.366 mM TCEP, pH 8.5.

Activity

One unit of TEV Protease cleaves >85% of 3 µg of control substrate in 1 hour at 30°C.

Activity Assay

A 6His tagged fusion protein (43.7 kDa) is incubated at 1 mg/ml in a buffer of 50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 1 mM DTT, at 30°C for 1 hour: (2) without TEV and with a TEV protease – substrate ratio of (3) 1:400, (4) 1:200, (5) 1:150, (6) 1:100, (7) 1:75, (8) 1:50, and (9) 1:37.5 (w/w). The cleaved product is 41.5 kDa. No non-specific cleavage has been observed.

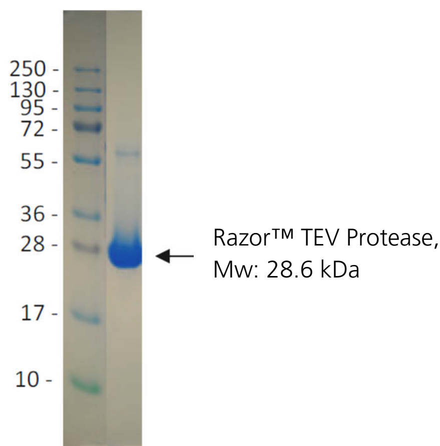

Fig. 1: Coomassie stain of Razor™ TEV protease. Analysis by SDS-PAGE under reducing conditions.

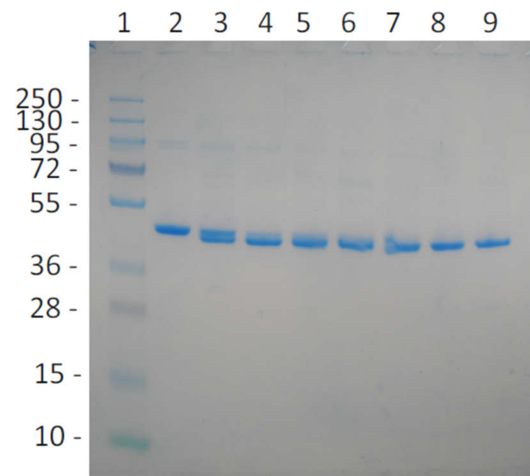

Fig. 2: Coomassie stain of activity assay of Razor™ TEV protease with His tagged substrate protein. Analysis by SDS-PAGE under reducing conditions.

Cleavage Assay

1. Prepare the following assay buffer freshly prior usage: 50 mM Tris, 0.5 mM EDTA, 1 mM DTT, pH 8.0

2. Prepare a dilution of substrate protein (any fusion protein containing the recognition sequence ENLYFQ) in assay buffer to obtain a concentration of substrate of 1 mg/ml and a dilution of TEV protease to obtain a concentration of 1.26 mg/ml.

3. Pipette each reaction mixture in a microcentrifuge tube as indicated below (for control reactions prepare two additional mixtures containing 5 µl of assay buffer replacing the TEV protease):

Reaction mixture:

Component	Volume	Final protein amount
Substrate (dil.)	20 µl	20 µg
Razor™ TEV Protease (dil.)	5 µl	6.3 µg
Assay buffer	175 µl	-

4. Incubate the samples at 30°C or any other temperature below down to 4°C. Leave one of the two control reactions on ice or at 4°C.

5. Remove 20 µl aliquots from every reaction after 1, 2, 4, and 6 hours (and if necessary after over night incubation). Stop the reactions by mixing equal volumes of reaction mixture (including controls) and 2X reducing SDS-PAGE sample buffer and store at –20°C until ready to analyze.

6. Heat the samples for 5 minutes at 95 °C. Analyze 10 µl by SDS-PAGE followed by protein staining.

Note

Cleavage reactions with TEV protease are performed generally in 50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 1 mM DTT for 1 hour at 30°C. Other conditions like lower temperatures and the addition of 1 mM TCEP in place of DTT can be applied. If different conditions have to be used, the amount of TEV protease added, the incubation time and/or the incubation temperature have to be optimized. The optimum salt concentration is 0 mM but an increasing salt concentration up to 200 mM NaCl will only result in a three fold decrease in activity.

References

1) Kapust, R.B., et al. (2002a), Biochem.Biophys. Res. Commun.294: 949-955.
2) Nallamsetty, S. et al. (2004), Protein Expr Purif. 38(1): 108-15.

Usage

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