Razor™ TVMV Protease

Catalog No: BM-13001
Lot No: XXXX
Source: *E. coli*
MW: 27.4 kDa
Supplied as: liquid
Size: 1000 u (9.5 µl), 10000 u (95 µl)
Stability: store at -20°C

Background

TVMV Protease (Tobacco Vein Mottling Virus Protease C4) cleaves the sequence ETVRFQS, with cleavage occurring between Q and S residues with high specificity. It is mainly used for removing affinity tags from proteins. Razor™ TVMV Protease is fused to an N-terminal His-tag for simple removal from the cleavage reaction by immobilization on metal-affinity resins. Avoid multiple freeze-thaw cycles!

Formulation

20 mM Tris, 100 mM NaCl, 50% Glycerol, 1 mM TCEP, pH 8.5.

Activity

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

Activity Assay

A MBP-tagged fusion protein (77.9 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 TVMV and with TVMV at a ratio of (3) 1:150, (4) 1:75, (5) 1:50, (6) 1:37.5, (7) 1:30, (8) 1:25, and (9) 1:18.5 (w/w). The cleaved products are 35.5 and 42.4 kDa. No non-specific cleavage has been observed.

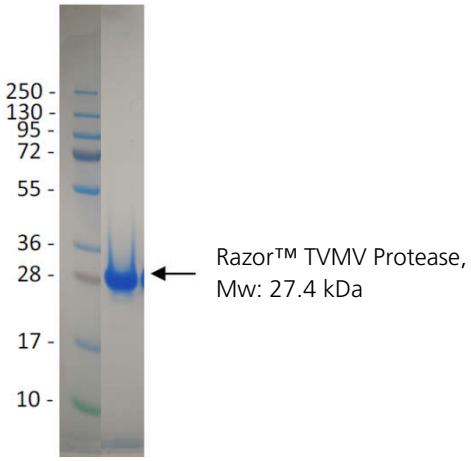

Fig. 1: Coomassie stain of Razor™ TVMV protease. Analysis by SDS-PAGE under reducing conditions.

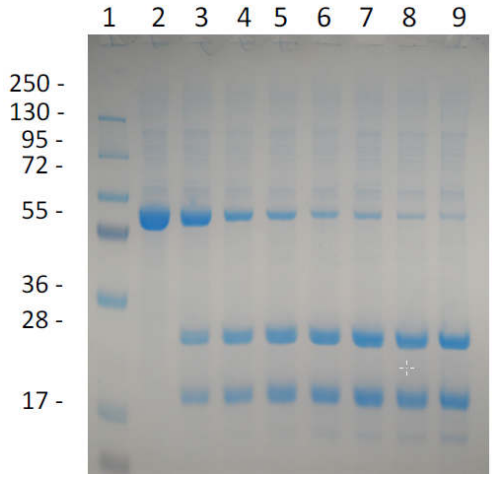

Fig. 2: Coomassie stain of activity assay of Razor™ TVMV protease with His tagged substrate protein. Analysis by SDS-PAGE under reducing conditions.

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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 ETVRFQS) in assay buffer to obtain a concentration of substrate of 1 mg/ml and a dilution of TVMV protease to obtain a concentration of 0.04 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 TVMV protease):

Reaction mixture:

Component	Volume	Final protein amount
Substrate (dil.)	20 µl	20 µg
Razor™ TVMV Protease (dil.)	5 µl	0.2 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 TVMV 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 TVMV 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