Razor™ TEV Protease

Catalog No: BM-13002
Lot No: XXXXX
Source: E. coli
MW: 28.6 kDa
Supplied as: liquid
Size: 1000 u (30 µl), 10000 u (300 µl)
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 0.12 mg/ml (or 1 unit/µl).

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:**
   
<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
<th>Final protein amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate (dil.)</td>
<td>20 µl</td>
<td>20 µg</td>
</tr>
<tr>
<td>Razor™ TEV Protease (dil.)</td>
<td>5 µl</td>
<td>0.6 µg</td>
</tr>
<tr>
<td>Assay buffer</td>
<td>175 µl</td>
<td>-</td>
</tr>
</tbody>
</table>

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**


**Usage**

This product is offered by Biomol for research purposes only. Not for diagnostic purposes or human use. It may not be resold or used to manufacture commercial products without written approval of Biomol GmbH.