Manual

Version 2.1

Product name: TEV Protease

Cat #: TEP-100, TEP-200, TEP-OEM, B-TB10

Description:

TEV (Tobacco Etch Virus) Protease is a highly site-specific cysteine protease that recognizes the cleavage site of Glu-Asn-Leu-Tyr-Phe-Gln-Gly and cleaves between Gln and Gly. TEV protease is a very useful enzyme for cleaving fusion proteins due to its high specificity and its high activity rate. TEV Protease contains an N-terminal His-tag, and it can be removed by Ni-NTA column after cleavage reaction.

Protocol:

Recommended Conditions for Cleavage of a Fusion Protein

A Protease-to-target protein ratio (w/w) of 1:50 to 1:200 should provide an affective range for most target proteins. However, the optimal ratio should be determined empirically.
Example of a time course experiment with 10 units TEV Protease at a protease to target protein ratio of 1:100 (w/w) or 10,000 unit (1 mg) TEV protease to 100 mg of target protein is shown below.

1. Add the following to a microcentrifuge tube:

   Fusion Protein 100 µg
   10X TEV Buffer 15 µl
   TEV Protease, (10 units) 1.0 µl
   Water to 150 µl

2. Incubate at 30°C. Remove 30 µl aliquots at 1, 2, 4, and 6 hours.

3. Add 30 µl 2X SDS sample buffer (125 mM Tris-HCl, pH 6.8; 4% SDS; 1.4 M β-mercaptoethanol; 20% (v/v) glycerol; 0.01% bromophenol blue). Keep the samples at -20°C until the experiment is complete.

4. Analyze 40 µl of sample by a SDS-PAGE gel. The percentage of the cleaved protein is determined by analyzing the amount of cleaved products formed and amount of uncleaved protein remaining after digestion. After evaluating the initial results, you may optimize the cleavage reaction for your specific protein by optimizing the amount of TEV Protease, incubation temperature, or reaction time.