AMID BIOSCIENCES

Product Manual

Product name: Tobacco Etch Virus Protease (TEV Protease), Recombinant, His Tag
Catalog# TVP-301-1, **Size**: 1,000 Units; **Catalog#** TVP-301-5, **Size**: 5,000 Units

TEV protease is supplied at a concentration of 10 Units/µl in 50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM DTT, and 50% Glycerol.

Components	Size
TEV Protease	1000 Units or 5000 Units
20 X TEV Buffer (1 M Tris-HCl, pH 8.0, 10 mM EDTA)	1 ml
100 mM DTT	0.1 ml

Product Description
Amid Biosciences TEV Protease is a 27 kDa engineered form of Tobacco Etch Virus (TEV) protease. The optimum recognition site for TEV protease is the seven-amino-acid sequence Glu-Asn-Leu-Tyr-Phe-Gln-(Gly/Ser) [ENLYFQ(G/S)] with cleavage occurring between the Gln and Gly/Ser residues. The enzyme is highly specific and active for its seven-amino acid sequence with minimal off-target effects which makes it an ideal choice to remove affinity tags from fusion proteins after protein purification. The optimal temperature for cleavage is 30℃; however, the enzyme is active over wide ranges of temperature (4℃ to 30℃) and pH (pH 6.0-8.5). Recombinant TEV protease contains an N-terminal His tag and can be easily removed by immobilized metal affinity chromatography from the cleavage reaction. The enzyme is compatible for both in-solution and on-column cleavage reactions.

Recommended Conditions for Cleavage of a Fusion Protein
One unit of TEV protease cleaves >85% of 3 µg of control substrate in 1 hour at pH 8.0 at 30℃. However, because each target protein has the different position of cleavage site, it is recommended to optimize the concentrations of TEV, incubation time and temperatures in order to find the best cleavage condition.

The following reagents or conditions interfere with cleavage reaction:
1. > 2 M urea, >0.5 M guanidine hydrochloride, or > 50 mM imidazole.
2. pH values for the buffer below 6 and above 9.
3. cysteine protease inhibitors

Example of a time course experiment with 10 units of TEV Protease is shown below.

1. Add the following components to a microcentrifuge tube:

Fusion Protein	40 µg
20X TEV Buffer	5 µl
TEV Protease (10 units)	1.0 µl
0.1 M DTT	1.0 µl
Water to 100 µl	

2. Incubate at 30℃. If preferred, the fusion protein can be cleaved at a lower temperature. Remove 20 µl aliquots at 1, 2, 4, and 6 hours.
3. Add an appropriate SDS-PAGE sample buffer to the aliquots. Keep the samples at -20℃ until the experiment is complete.
4. Analyze 20-30 µl of sample by a SDS-PAGE gel to determine the best cleavage result. The percentage of the cleaved protein is determined by analyzing the amount of cleaved products formed and amount of uncleaved protein remaining after digestion.
Cleavage reaction for specific protein can be optimized by varying the amount of TEV Protease, incubation temperature, or reaction time.

Removal of TEV Protease after Cleavage
Amid Biosciences TEV Protease contains a polyhistidine tag at the N-terminus of TEV. Protease can be removed from the cleavage reaction by affinity chromatography on Ni^{2+} - or Co^{2+}-chelating resins. The cleaved protein will be in the flow-through fractions.