# Manual

Version 3.1

## Product name: TurboTEV Protease

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

## Description:

TurboTEV (Tobacco Etch Virus) Protease is a highly enhanced site-specific cysteine protease that recognizes the cleavage site of Glu-Asn-Leu-Tyr-Phe-Gln-Gly and cleaves between Gln and Gly. TurboTEV protease is resistant to auto-inactivation under normal reaction conditions and works as a better catalyst than the wild-type enzyme. It is a very useful enzyme for cleaving fusion proteins due to its high specificity and high activity rate without the requirement for specialized buffer. It has both His-tag and GST tag, which allow to be removed by either Ni-chelating or GSH resins.

## 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 Tubo TEV Buffer 15 µl
   Turbo TEV Protease, (10 units) 1.0 µl
   Water to 150 µl

2. Incubate at 30°C. Remove 30 µl of 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 protein cleavage is determined by analyzing the amount of cleaved products formed and amount of uncleaved protein remaining after the 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.