Nacalai USA
Innovations for Life Sciences.

TurboTEV (TEV Protease)

Cat. No. NU0102S	1,000 units (0.1 mg)
Cat. No. NU0102M	10,000 units (1 mg)
Cat. No. NU0102L	100,000 units (10 mg)

For Research Use Only

Introduction

TurboTEV Protease contains an enhanced form of a catalytic fragment of the N1a protein of Tobacco etch virus (TEV), a cysteine protease that recognizes the cleavage site of Glu-Asn-Leu-Tyr-Phe-Gln-Gly and cleaves between Gln and Gly. TurboTEV Protease is a restriction grade protease that has a robust activity at 4°C with high specificity and great stability. It does not require any special buffer for its activity and can be used in a buffer most suitable for the target protein. TurboTEV Protease is a 52 kDa protein with both GST and His tags so it can be easily removed by either Ni-chelating or Glutathione (GSH) resin along with the cleaved tag.

Activity and Specificity

TurboTEV Protease has a specific activity of 10,000 units/mg, using the conventionally defined activity unit (One unit cleaves ≥85% of 3 µg control substrate in 1 h at 30°C). In practice, 1 mg (10,000 units) of TurboTEV Protease cleaves >90% of 100 mg of a target fusion protein at 4°C in 16 hours. No non-specific cleavage has been observed under the same condition when TurboTEV Protease and target fusion protein was mixed at 1:10 ratio. TurboTEV Protease retains >80% activity after storage at room temperature for over 65 hours.

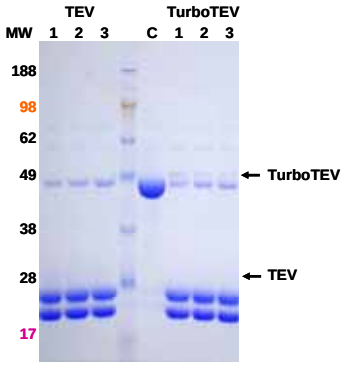

Figure 1

A 49 kDa GST-fusion protein (C) at 1 mg/ml is incubated with TurboTEV or TEV Protease at a ratio of (1) 1:50, (2) 1:100, (3) 1:200 (w/w) in a buffer of 25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 14 mM 2-mercaptoethanol at 4°C for 16 hours. The cleaved products are 27 kDa and 22 kDa. TEV is a competitor TEV product.

Formulation and Storage

2 mg/ml in 25 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM TCEP, and 50% glycerol. Store at -20°C. Shipped in gel packs.

Source

Purified from *E. coli* expressing the TurboTEV protease gene.

Procedures - Cleavage Condition

It is recommended to test TurboTEV Protease cleavage with a protease-to-target protein ratio of 1:100 (w/w) or 1 unit of TurboTEV to 10 ug of target protein in a buffer suitable for the target protein at 4°C overnight, with the target protein concentration at 1-2 mg/ml. In most cases, >90% of target protein is cleaved with a TurboTEV-to-target protein ratio of 1:50 to 1:200 or 1 unit TurboTEV to 5-20 ug of target protein (as shown in Figure 1). The efficiency of cleavage may vary due to the sequences around the cleavage site, the conformation and the solubility of the target protein. Due to its high specificity, more TurboTEV Protease (at 1:10 ratio) or longer cleavage time (over a weekend) at higher temperature (37°C) can be used to achieve high cleavage efficiency without non-specific cleavage of target proteins.

1. Make fresh cold Dialysis Buffer. Dialysis Buffer should be a buffer in which the target protein is soluble. There should be no protease inhibitor in the Dialysis Buffer. The Dialysis Buffer should be compatible with downstream purification processes, e.g. minimal amount of EDTA or DTT if Ni column will be used to remove the cleaved His-tag.
   Here is an example of Dialysis Buffer. 25 mM Tris-HCl, pH 8.0, 150 - 500 mM NaCl, 14 mM 2-mercaptoethanol
   TurboTEV has the same activity in 150 mM NaCl or 500 mM NaCl and 400 mM imidazole.
2. Dilute the target protein pool to 1-2 mg/ml with Dialysis Buffer. This is optional in case the target protein aggregates in Dialysis Buffer. Save a small aliquot as Uncut sample for analysis. EDTA may be added to 0.5 mM final concentration if the target protein pool is eluted from Ni column and EDTA is compatible with the target protein.
3. Add TurboTEV Protease at a Protease:target protein ratio of 1:100 (w/w) or 10,000 unit (1 mg) TurboTEV Protease to 100 mg of target protein. There is no need to calculate the molar ratio. TurboTEV Protease can be added directly to the target protein. There is no need to change buffer or dilute TurboTEV Protease. The optimal ratio should be determined empirically. A Protease-to-target protein ratio (w/w) of 1:50 to 1:200 should work for most target proteins.
4. Dialyze against the Dialysis Buffer at 4°C overnight (about 16 hrs). Dialysis is to remove imidazole or glutathione if Ni or glutathione column is used to remove the cleaved tag or TurboTEV Protease after cleavage. If desired, the target protein pool can be buffer exchanged first before TurboTEV cleavage.

Procedures – Removal of TurboTEV Protease after Cleavage

TurboTEV Protease contains both GST and His tags. After cleavage of the target protein, TurboTEV Protease is easily removed along with the tags from the cleavage reaction by affinity chromatography using Ni-chelating resin for His-tagged target protein or GSH resin for GST-tagged target protein.

1. The dialyzed target protein and TurboTEV Protease mixture can be applied directly to affinity columns if compatible Dialysis Buffer is used. For His-tagged protein, use IMAC to remove the cleaved His-tag and TurboTEV Protease. For GST-tagged protein, use glutathione column to remove the cleaved GST-tag and TurboTEV Protease.
2. If desired, analyze samples using SDS-PAGE analysis. The difference between the tagged and cleaved target protein may be too small to detect by SDS-PAGE. The cleaved His-tag sometimes can be seen at the bottom of the gel.

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Rev. 06/2009