Turbo3C (HRV3C Protease)

Cat. No. NU0101S 1,000 units (1 mg)
Cat. No. NU0101M 10,000 units (10 mg)

For Research Use Only

Introduction

Human rhinovirus 3C protease (HRV3C Protease) is a cysteine protease that recognizes the cleavage site of Leu-Glu-Val-Leu-Phe-Gln/Gly-Pro, commonly referred to as the PreScission Site. It cleaves between Gln and Gly. The recombinant Turbo3C Protease is a restriction grade HRV3C protease that has robust activity at 4°C and high specific activity. Turbo3C Protease is a 47 kDa protein with both GST and His tags so it can be removed by either Ni chelating or Glutathione (GSH) resin.

Activity and Specificity

The activity of Turbo3C Protease is tested using a control target protein. 1 ug of Turbo3C Protease has at least 1 unit activity conventionally used by other suppliers (1 unit of HRV3C protease cleaves >95% of 100 ug of control target protein at 4°C for 16 hours). No non-specific activity has been observed under the same condition with Turbo3C Protease to control target protein ratio of 1:10. Prolonged incubation (several days) under the same condition does not show any non-specific cleavage.

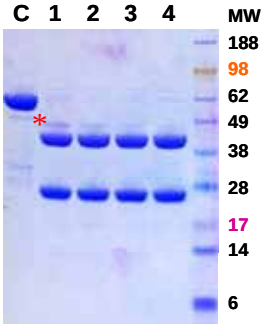

Figure 1
A 68 kDa GST-fusion protein (C) at 1 mg/ml is incubated with Turbo3C Protease (*) at a ratio of (1) 1:50, (2) 1:100, (3) 1:200, (4) 1:400 (w/w) in a buffer of 25 mM Tris-HCl, pH8.0, 150 mM NaCl, 14 mM 2-mercaptoethanol at 4°C for 16 hours. The cleaved products are 42 kDa and 26 kDa

Formulation and Storage

2 mg/ml in 50 mM Tris-HCl, pH8.0, 150 mM NaCl, 1 mM EDTA, 1 mM TCEP, 50% glycerol. Store at -20°C. Shipped in gel packs.

Soruce

Purified from *E. coli* expressing the Turbo3C protease gene.

Procedures - Cleavage Condition

It is recommended to use Turbo3C Protease at a protease-to-target protein ratio of 1:100 (w/w) or 1 unit of Turbo3C Protease to 100 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, target proteins are completely cleaved with a protease-to-target protein ratio of 1:50 to 1:400 or 1 unit Turbo3C Protease to 50-400 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 Turbo3C 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
   Turbo3C has the same activity in 150 mM NaCl or 500 mM NaCl and 400 mM imidazole.
2. Dilute the 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 Turbo3C Protease at a Protease:target protein ratio of 1:100 (w/w) or 1,000 unit Turbo3C Protease to 100 mg of target protein. There is no need to calculate the molar ratio. Turbo3C Protease can be added directly to the target protein. There is no need to change buffer or dilute Turbo3C 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 Turbo3C Protease after cleavage. If desired, the target protein pool can be buffer exchanged first before Rurbo3C cleavage.

Procedures – Removal of Turbo3C Protease after Cleavage

The Turbo3C Protease contains both GST and His tags. After cleavage of the target protein, Turbo3C Protease can be easily removed along with the tags from the cleavage reaction by affinity chromatography on a Ni-chelating resin for His-tagged target protein or GSH resin for GST-tagged target protein.

1. The dialyzed target protein and Turbo3C 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 Turbo3C Protease. For GST-tagged protein, use glutathione column to remove the cleaved GST-tag and Turbo3C 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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