Turbo3C Protease

(Catalog number: P5380)

A. 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.

B. Source

E. coli

C. Activity

> 1 Units/µg. 1 Units of Turbo3C (HRV3C) Protease cleaves >95% of 100 µg of target protein at 4°C for 16 hours.

D. Storage Buffer

In 50 mM Tris-HCl, pH8.0, 150 mM NaCl, 1 mM EDTA, 1 mM TCEP, 50% glycerol

E. Storage/Handling

Store at -20°C. For long term storage store at -80°C. Aliquot to avoid repeated freezing and thawing.

F. Protocol

Cleavage in Solution

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 b-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

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protein aggregates in Dialysis Buffer. Save a small aliquot as Un-cut 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 by each user for best performance. 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 Turbo3C cleavage.

Removal of Turbo3C Protease

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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