Turbo3C (HRV3C) Protease, Recombinant

CATALOG #: 9206-1

AMOUNT: 1 mg (1,000 units)

SOURCE: *E. coli*

DESCRIPTION: BioVision's 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 (independent of Pro). The recombinant form of the HRV3C protease is a restriction grade protease that has robust activity at 4˚C with high specific activity 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. This HRV3C Protease is a 47 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.

FORMULATION: 2 mg/ml in 25 mM Tris-HCl, pH8.0, 50 mM NaCl, 1 mM TCEP, and 50% glycerol

SPECIFIC ACTIVITY: > 1 unit/µg. One unit of Turbo3C (HRV3C) Protease cleaves >95% of 100 µg of target protein at 4˚C for 16 hours

STORAGE CONDITIONS: -20˚C. HRV3C Protease is stable at room temperature for at least two weeks without loss of any activity. It retains full activity after incubation at 37˚C for one week.

CLEAVAGE CONDITION: It is recommended to use HRV3C Protease at a protease-to-target protein ratio of 1:100 (w/w) or 1 unit of HRV3C Protease to 100 µg 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 HRV3C Protease to 50-400 µg 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 HRV3C 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.

Removal of HRV3C Protease after Cleavage: The HRV3C Protease contains both GST and His tags. After cleavage of the target protein, HRV3C 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.

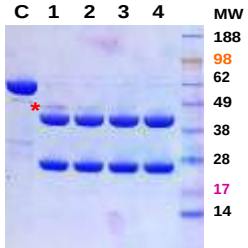

Figure 1. A 68 kDa GST-fusion protein (C) at 1 mg/ml is incubated with HRV3C Protease (*) at a ratio of (1) 1:50, (2) 1:100, (3) 1:200, (4) 1:400 (w/w) in a buffer of 25 mM Tris-HCl, pH8.0, 150 mM NaCl, 14 mM β -mercaptoethanol at 4˚C for 16 hours. The cleaved products are 42 kDa and 26 kDa.

SUGGESTED PROTOCOL:

A) Cleavage in Solution

1. Make fresh cold dialysis buffer in which the target protein is soluble. There should be no protease inhibitor in the dialysis buffer and it 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.

   (An example of dialysis buffer would be 25 mM Tris-HCl, pH 8.0, 150 - 500 mM NaCl, 14 mM β -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 an uncut sample for analysis. EDTA may be added to 0.5 mM final concentration if the target protein pool is eluted from a Ni column and EDTA is compatible with the target protein.

3. Add Turbo3C Protease at a protease-to-target 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 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 a Ni-NTA or GSH 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.

B) 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 a GSH 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.

FOR RESEARCH USE ONLY. Not to be used in humans

RELATED PRODUCTS:

TEV related Protease

- Turbo TEV Protease

Caspase Related Products

- CaspGLOW Active Caspase Staining Kits
- Caspase (1-12) Fluorometric Assay Kits
- Caspase (1-10) Colorimetric Assay Kits
- Caspase Inhibitor Drug Screening Kits
- CaspSELECT Caspase-3 & -7 Immunoassay Kits
- CaspSCREEN Flow Cytometric Caspase Screening Kit
- Human, Mouse, & Rat Active Caspases & Procaspases
- Caspase Inhibitors & Sets (Ready-to-use)
- Caspase Substrates & Sets (Ready-to-use)
- Caspase Assay Buffers & Control Cell Lysates
- Antibodies to Pro- & Active Caspases
- siRNA Apoptosis Vectors
- Bulk Caspase Inhibitors (Powder form)

Calpain Related Products

- Calpain Activity Assay Kit
- Active Human Calpian I 1134-100
- Calpain Inhibitor, Z-Leu-Leu-Tyr-FMK
- Calpain 1 Polyclonal Antibody
- Calpain 2 Polyclonal Antibody
- Calpain 3 Polyclonal Antibody
- Calpain 5 Polyclonal Antibody
- Calpain 6 Polyclonal Antibody
- Calpain 7 Polyclonal Antibody
- Calpain Inhibitor, Z-Leu-Leu-Tyr-FMK
- Cathepsin B & L Inhibitor, Z-Phe-Phe-FMK
- Granzyme B Inhibitor, Z-Ala-Ala-Asp-CMK
- EMAP II Inhibitor, Z-ASTD-FMK
- Biotinylated Caspase-Family Inhibitor, Biotin-VAD-FMK
- Biotinylated Caspase-3 Inhibitor, Biotin-DEVD-FMK
- Biotinylated Caspase-8 Inhibitor, Biotin-DEVD-FMK

Cathepsin Related Products

- Cathepsin B Activity Assaay Kit
- Cathepsin D Activity Assay Kit
- Cathepsin K Activity Assaay Kit
- Cathepsin H Activity Assay Kit
- Cathepsin L Activity Assaay Kit
- Cathepsin S Activity Assaay Kit
- Cathepsin B (Active)
- Cathepsin D (Active)
- Cathepsin H (Active)
- Cathepsin L (Active)
- Cathepsin L (Active)
- Cathepsin L (Active)
- Cathepsin S (Active)
- Cathepsin B&L Inhibitor, Z-Phe-Phe-FMK
- Cathepsin B Polyclonal Antibody
- Cathepsin D Polyclonal Antibody
- Cathepsin F Polyclonal Antibody
- Cathepsin G Polyclonal Antibody
- Cathepsin H Polyclonal Antibody
- Cathepsin K Polyclonal Antibody
- Cathepsin L Monoclonal Antibody
- Cathepsin L Polyclonal Antibody
- Cathepsin L (Cleaved) Polyclonal Antibody
- Cathepsin P Polyclonal Antibody
- Cathepsin S Polyclonal Antibody
- Cathepsin S Polyclonal Antibody
- Cathepsin V Monoclonal Antibody
- Cathepsin V Polyclonal Antibody
- Cathepsin W Monoclonal Antibody

Molecular Biology Products

- EZLys™ Bacterial Protein Extraction Reagent