Nacalai USA
Innovations for Life Sciences.

**Turbo3C™ (HRV3C Protease)**

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|---|---|---|
| **Cat. No. NU0101P** | 100 units | (0.1mg) |
| **Cat. No. NU0101S** | 1,000 units | (1 mg) |
| **Cat. No. NU0101M** | 10 X 1000 units | (10 mg) |

*For Research Use Only*

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**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. It cleaves between Gln and Gly. Turbo3C™ Protease is a restriction grade recombinant form of the HRV3C 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 is compatible with buffers most suitable for target proteins. Turbo3C™ Protease is a 47 kDa protein with dual GST- and His tags that enable easy removal of Turbo3C™ Protease together with the cleaved GST- or His-tag in a single step by either Ni chelating or Glutathione (GSH) resin.

**Activity and Specificity**

One (1) µg of Turbo3C™ Protease has at least 1 unit activity by the conventional definition: 1 unit of Turbo3C™ protease cleaves >95% of 100 µg of control target protein at 4°C in 16 hours.

Non-specific cleavage of the target protein is not observed under the same condition at 1:10 (w/w) ration of Turbo3C™ Protease to control target protein. Extended incubation of target proteins with Turbo3C™ Protease for several days does not show any non-specific cleavage.

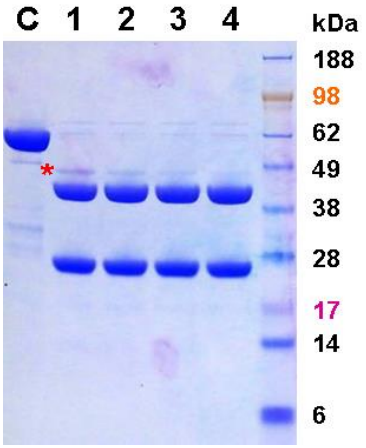


**Figure 1**. A 68 kDa control protein (C) at 1 mg/ml is incubated with Turbo3C (HRV3C) Protease (*) at a ratio of (1) 1:50, (2) 1:100, (3) 1:200, (4) 1:400 (w/w) in a buffer of 25 mM Tris-HCl, pH8.0, 150 mM NaCl, 14 mM β-mercaptoethanol at 4°C for 16 hours. The cleaved products are 42 kDa and 26 kDa.

**Component and Formulation**

Turbo3C™ Protease: 2 mg (2,000 units) /ml in Storage Buffer of 25 mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM TCEP and 50% glycerol. The Storage Buffer is compatible with Ni-chelating resin and GSH resin for on-column digestion.

**Storage and Stability**

Store Turbo3C™ at -20°C. Turbo3C™ Protease is stable at room temperature for at least two weeks without loss of any activity. It retains full activity in Storage Buffer after incubation at 37°C for one week.

**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 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 Turbo3C™ 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 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 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 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 Turbo3C™ cleavage.

**Removal of Turbo3C™ Protease after Cleavage**

The Turbo3C™ Protease has dual GST- and His tags. After cleavage of the target protein, Turbo3C™ Protease can be easily removed in a single step together 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.

**Technical Support**

E-mail: info@nacalaiusa.com
Website: www.nacalaiusa.com

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