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Product Information

# **HRV-3C Protease**

*N*-Terminal His-tagged recombinant protein

#### **SAE0045**

## **Product Description**

Synonym: Rhinovirus (serotype 14 LP) genomic region encoding protease

HRV-3C protease from human Rhinovirus Type 14 is a protease that specifically cleaves within the following eight-residue recognition sequence:

1

Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro

Proteolytic cleavage occurs between the Gln and Gly residues.<sup>1</sup> HRV-3C protease is a useful tool to cleave recombinant proteins that are expressed as fusion proteins with this sequence between the carrier domain and the protein of interest.<sup>2</sup>

This recombinant HRV-3C protease has a molecular mass of ~21 kDa. It contains a six-histidine tag (His-tag) to allow for removal by IMAC chromatography, such as with HIS-Select® or His◆Bind® products. Several theses³-⁴ and references⁵-⁶ cite use of this SAE0045 HRV-3C protease product in their research protocols.³-⁴

#### Reagent

The product is supplied in aqueous buffer, in a protein concentration range of 0.8-1.2 mg/mL, containing 50 mM Trizma-HCl (pH 7.5), 0.15 M NaCl, 1 mM TCEP, and 50% (v/v) glycerol. For the exact concentration, please refer to the lot-specific Certificate of Analysis for your specific lot number.

One unit of HRV-3C protease is defined as the amount of enzyme needed to digest 1 nmole of the substrate peptide H-Glu-Ala-Leu-Phe-Gln-pNA per hour at 0 °C, in a reaction buffer containing 25 mM HEPES (pH 7.5), 150 mM NaCl, 1 mM EDTA, and 1 mM DTT.

#### Storage/Stability

The product retains activity for at least 2 years when stored at -20 °C.

### Procedure

HRV-3C protease is active under a wide range of pH values, ionic strengths, and temperatures. This protease retains high activity even at 0 °C, making it an optimal choice for temperature-sensitive proteins. However, the activity toward substrate proteins may differ depending on the substrate identity and reaction conditions.

The presence of low concentrations of a reducing agent in the reaction buffer is highly desirable, to keep the enzyme active in prolonged incubations. It is recommended to use 0.2-1 mM DTT in the reaction buffer for optimal results.

A good starting point for optimization is to use 1  $\mu$ g of HRV-3C protease per 100  $\mu$ g of target protein for 1 hour at 0-8 °C, or 1  $\mu$ g of HRV-3C protease per 500  $\mu$ g at 0-8 °C for 12-24 hours.

Temperatures up to 30 °C can be used for faster digestion. Protease activity is  $\sim 5 \times$  higher at 30 °C versus 0-8 °C. However, protease and substrate stability might be compromised.

#### Precautions and Disclaimer

1

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.



#### References

- 1. Cordingley, M.G. et al., J. Biol. Chem., **265(16)**, 9062-9065 (1990).
- Waugh, D.S., Protein Expr. Purif., 80(2), 283-293 (2011).
- Aguilera, Marcos Moreno, "Estudio de Nrn1 en la patología de esquizofrenia" ("Study of Nrn1 in the pathology of schizophrenia"). Universidad de Barcelona, M.Sc. thesis, p. 9 (2017).
- 4. Gao, Mengjin, "The expression and purification of zinc metalloprotease STE24 (ZMPSTE24) and study of the interactions between the protein-inhibitor binding". McGill University, M.Sc. thesis, p. 41 (2021).
- 5. Lindesmith, L.C. *et al.*, *Immunity*, **50(6)**, 1530-1541.e8 (2019).
- 6. Yukawa, M. *et al.*, *J. Exp. Med.*, **217(1)**, e20182009 (2020).

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