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# Product Information

**HRV-3C Protease, Biotin-tagged**
recombinant, expressed in *E. coli*

Catalog Number **SAE0110**
Storage Temperature –20 °C

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

## Product Description

HRV-3C protease from human rhinovirus type 14 is a protease that specifically cleaves within an eight-residue recognition sequence. This sequence is:

Leu-Glu-Val-Leu-Phe-Gln↓-Gly-Pro

Proteolytic cleavage occurs between the Gln and Gly residues.[1] The HRV-3C protease is useful for cleaving recombinant proteins that are expressed as fusion proteins with this sequence between the carrier domain and the protein of interest.[2]

This biotinylated HRV-3C protease is intended for on-column cleavage of fusion proteins with an HRV-3C cleavage site. It specifically cleaves the protein of interest from a column-bound fusion protein, leaving the fusion domain or tag bound to the affinity column (e.g. Ni-NTA column) and eluting only the protein of interest. This method is advantageous over post-elution cleavage for several reasons:

- It eliminates most impurities normally associated with purification on Ni-chelating columns.
- It allows gentler elution conditions, with added flexibility in the elution buffer composition. This can mitigate protein aggregation and inactivation.

After cleavage, the protease can be removed with any avidin-conjugated or streptavidin-conjugated beads.

This product has been enzymatically biotinylated with no effect on its proteolytic activity. It has no additional protein purification tags. The product is supplied in aqueous buffer (0.8–1.2 mg/mL) with 20 mM Trizma®-HCl, pH 8.0, 200 mM NaCl, 1 mM TCEP, and 50% (v/v) glycerol.

## Storage/Stability

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

## Precautions and Disclaimer

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.

## Procedure

This product is active under a wide range of pH values, ionic strengths, and temperatures. It 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 use of low concentrations of a reducing agent, e.g. 0.2-1 mM DTT, in the reaction buffer is suggested, to keep the enzyme active in prolonged incubations.

A starting point for optimization is to use 1 µg of this product per 100 µg of target protein, for 1 hour at 0–8 °C, or 1 µg of this product per 500 µg at 0–8 °C for 12–24 hours. Temperatures up to 30 °C can be used for faster digestion. Protease activity is ~5× higher at 30 °C versus 0–8 °C. However, protease and substrate stability might be compromised.

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.

## References

1. Cordingley, M.G. *et al.*, *J. Biol. Chem.*, **265(16)**, 9062-9065 (1990).
2. Waugh, D.S., *Protein Expr. Purif.*, **80(2)**, 283-293 (2011).

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