

Product Information

HRV-3C Protease, Biotin-tagged

Recombinant protein, aqueous solution

SAE0110

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:

↓
Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro

Proteolytic cleavage occurs between the Gln and Gly residues.¹ 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.²

This biotinylated HRV-3C protease is intended for on-column cleavage of fusion proteins with an HRV-3C cleavage site. This enzyme specifically cleaves the protein of interest from a column-bound fusion protein, leaving the fusion domain or tag bound to the affinity column (such as an 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.

Several references cite use of this SAE0110 product in their research protocols.³⁻⁶

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.

Reagent

This recombinant HRV-3C protease has been enzymatically biotinylated with no effect on its proteolytic activity. It has no additional protein purification tags. This product has a molecular weight of 22 kDa. 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.

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

This product is active under a wide range of pH values, ionic strengths, and temperatures. It retains activity even at 0 °C, making it a useful 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, such as 0.2-1 mM DTT, in the reaction buffer is suggested, to keep the enzyme active in prolonged incubations.

Starting points for optimization would be to use:

- 1 µg of this product per 100 µg of target protein, at 0-8 °C for 1 hour, or:
- 1 µg of this product per 500 µg of target protein, 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.

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).
3. Liu, C. *et al.*, *Antiviral Res.*, **187**, 105020 (2021).
4. Cousin, M.A. *et al.*, *Nat. Genet.*, **53**(7), 1006-1021 (2021).
5. Scholle, M.D. *et al.*, *SLAS Discov.*, **26**(8), 974-983 (2021).
6. Wellner, A. *et al.*, *Nat. Chem. Biol.*, **17**(10), 1057-1064 (2021).

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

The life science business of Merck operates as MilliporeSigma in the U.S. and Canada.

Merck and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

© 2021 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

SAE0110pis Rev 01/22 DT,GCY,SM,MAM

The Merck logo, consisting of the word "MERCK" in a bold, red, sans-serif font.