

Product Information

Mpro, 3CL Protease from MERS Coronavirus

Recombinant protein, Lyophilized powder

SAE0223

Product Description

Maturation of the MERS coronavirus depends on cleavage of the two large polyproteins (pp1a and pp1ab) by two viral proteases:

- Mpro (main protease)
- PLpro (Papain-like protease)

Proteolytic cleavage by Mpro of the polyproteins occurs at 11 sites:

- 7 sites within the 1a polyprotein
- 4 sites within the 1ab polyprotein

This results in maturation of a total of 16 viral non-structural proteins.^{1,2}

Mpro protease forms a functional homodimer. Both the *N*-terminus and the *C*-terminus of Mpro have been shown to be critical for dimer formation and for enzyme function.²

The Mpro protease is an ideal target for antiviral drug design due to its high conservation between different coronavirus strains and absence of functional analogs in the human proteome.^{3, 4}

This fully active Mpro protease product contains the complete sequence of Mpro protease from MERS coronavirus (Accession: YP_009944296.1) without any additional tags. It is provided as a lyophilized powder for increased stability.

Reagent

The product is supplied lyophilized from 20 mM HEPES (pH 7.3), 25 mM NaCl, 1 mM DTT, 2.5% Trehalose, and 0.05% TWEEN® 20.

Storage/Stability

The product retains activity for at least 2 years when stored lyophilized 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.

Preparation Instructions

It is suggested to reconstitute the product in water or in 10% aqueous glycerol solution at a protein concentration of 1-2 mg/mL. Aliquot the protein solution and store the solution aliquots at -20 °C. Avoid freeze-thaw cycles.

Procedure

Activity measurement using fluorogenic substrate peptide (SAE0180)

Prepare the stock solution of the fluorogenic peptide in DMSO at a concentration of 20 mg/mL.

Depending on the hardware set-up, the fluorogenic peptide can be used at working concentrations of 10-100 µg/mL.

Sample Protocol

1. Prepare the substrate solution at a final concentration of 10 µg/mL in 50 mM Tris, 1 mM EDTA, pH 7.0.
2. Set up the fluorimeter (fluorescent plate reader) for reading at an excitation (λ_{ex}) of 400 nm, and emission (λ_{em}) at 505 nm.
3. Use 200 µL of substrate solution per well for the 96-well plate format.
4. Add 2-20 µg/mL Mpro.
5. Read fluorescence kinetics for 5-60 minutes.

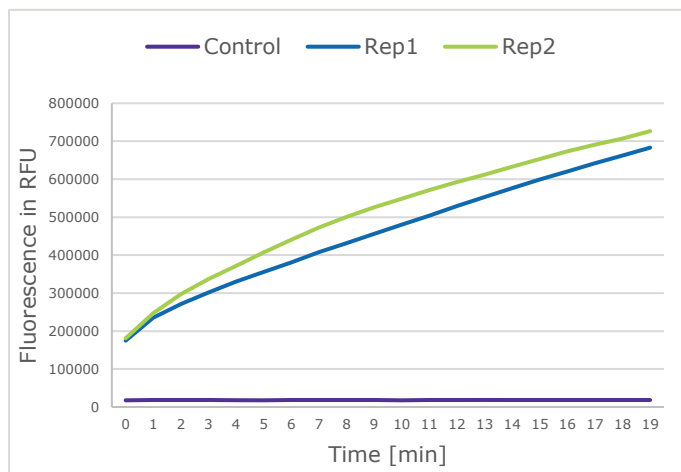


Figure 1. Mpro from MERS enzyme kinetics performed with the fluorogenic substrate SAE0180.

Substrate concentration: 10 µg/mL, Mpro enzyme concentrations: 0 (control), and 5 µg/mL (2 repeats).

References

1. Kilianski, A., Mielech, A. M., Deng, X., & Baker, S. C. (2013). Assessing activity and inhibition of Middle East respiratory syndrome coronavirus papain-like and 3C-like proteases using luciferase-based biosensors. *Journal of virology*, 87(21), 11955-11962.
2. Ho, B. L., Cheng, S. C., Shi, L., Wang, T. Y., Ho, K. I., & Chou, C. Y. (2015). Critical assessment of the important residues involved in the dimerization and catalysis of MERS coronavirus main protease. *PLoS One*, 10(12), e0144865.
3. Xia, B., & Kang, X. (2011). Activation and maturation of SARS-CoV main protease. *Protein & cell*, 2, 282-290.
4. Yang, H., Bartlam, M., & Rao, Z. (2006). Drug design targeting the main protease, the Achilles' heel of coronaviruses. *Current pharmaceutical design*, 12(35), 4573-4590.

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