Product Information

Mpro, 3CL Protease from coronavirus SARS-COV2

Recombinant protein, lyophilized powder

SAE0172

Product Description

Maturation of the SARS-CoV2 virus depends on cleavage of the overlapping large polyproteins 1a and 1ab by two viral proteases:

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

Proteolytic cleavage by Mpro of the 1ab polyprotein 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 (NSP).[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.[2,3] It is noteworthy that Mpro protease from SARS-CoV1 and SARS-CoV2 are functionally identical.[4,5]

This fully active Mpro protease product contains the complete sequence of Mpro protease (Accession: YP_009725301.1) without any additional tags. It is provided as a lyophilized powder for increased stability.

This product has been used in a Surface Plasmon Resonance (SPR) binding assay.[6]

Reagent

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

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.

Storage/Stability

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

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 (Cat. No. 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 25 mM HEPES, 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 kinetics for 5-60 minutes.

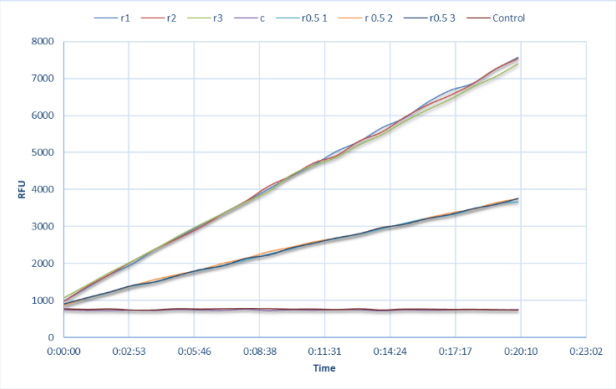

Figure 1. Mpro enzyme kinetics performed with the fluorogenic substrate SAE0180.

- Substrate concentration: 10 µg/mL
- Mpro enzyme concentrations: 0, 2.5 and 5 µg/mL

Experiments with Mpro at 2.5 and 5 µg/mL are performed in triplicate.

References

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2. Xia, B., and Kang, X., *Protein Cell*., **2(4)**, 282-290 (2011).
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4. Goyal, B., and Goyal, D., *ACS Comb Sci*., **22(6)**, 297-305 (2020).
5. Zhang, L. *et al*., *Science*, **368(6489)**, 409-412 (2020).
6. Li, H. *et al*., *J. Agric. Food Chem.*, **69(41)**, 12197-12208 (2021).

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