

## Product Information

# Mpro, 3CL Protease from Coronavirus SARS

Recombinant protein, Lyophilized powder

**SAE0224**

## Product Description

Maturation of the SARS coronavirus 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 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.<sup>1</sup>

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.<sup>2</sup>

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.<sup>1,3</sup> It is noteworthy that Mpro protease from SARS-CoV1 and SARS-CoV2 are functionally identical.<sup>4,5</sup>

This fully active Mpro protease product contains the complete sequence of Mpro protease from SARS-COV (Accession: WEG19431.1, residues 3230-3535) 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.

## 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.

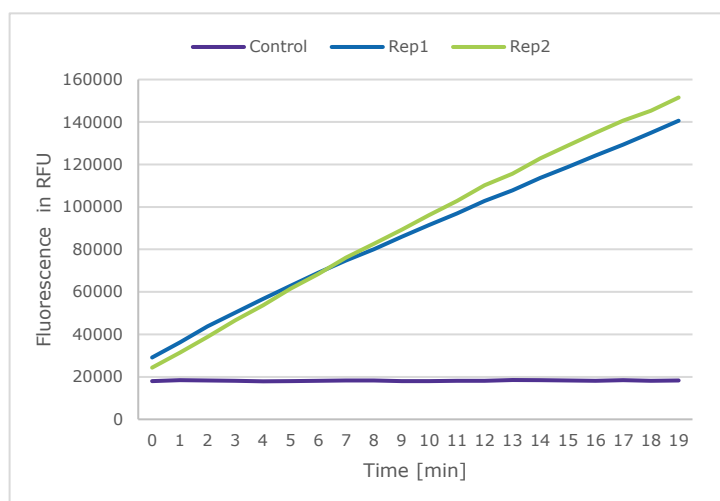
## Procedure

### Activity measurement using fluorogenic substrate peptide (SAE0180)

1. Prepare the stock solution of the fluorogenic peptide in DMSO at a concentration of 20 mg/mL.
2. 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 ( $\lambda_{ex}$ ) of 400 nm, and emission ( $\lambda_{em}$ ) 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 SARS 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. Xia, B., Kang, X. Activation and maturation of SARS-CoV main protease. Protein Cell 2, 282–290 (2011). <https://doi.org/10.1007/s13238-011-1034-1>.
2. Wu, C.-G., Cheng, S.-C., Chen, S.-C., Li, J.-Y., Fang, Y.-H., Chen, Y.-H. & Chou, C.-Y. (2013). Acta Cryst. D69, 747-755.
3. Yang, H. et al., Curr. Pharm. Des., 12(35), 4573-4590 (2006).
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).

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