

Product Information

SARS-CoV2 Main Protease fluorogenic substrate peptide

AFC-peptide substrate, lyophilized

SAE0180

Product Description

This hexapeptide, Thr-Ser-Ala-Val-Leu-Gln-AFC, is a substrate peptide for both the Main protease (Mpro) from the SARS coronavirus, and also the Mpro of SARS-CoV2.¹⁻³ This substrate allows measurement of the activity of Mpro, using a fluorescent plate reader or fluorometer. Proteolytic cleavage by Mpro will release the fluorescent amino trifluoromethyl coumarin (AFC) group from the peptide:



Increase in fluorescence should be measured using the following wavelengths:

- excitation (λ_{ex}) = 400 nm
- emission (λ_{em}) = 505 nm

This product is lyophilized from 0.1% TFA in H₂O.

Storage/Stability

The product is stable in lyophilized form for at least 5 years when stored at -20 °C.

The product has a limited lifetime in solution. Long-term storage in solution should be avoided.

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

Solubilization:

The peptide is soluble in DMSO at a concentration of 20-50 mg/mL. Solubilized peptide can be stored at 2-8 °C for at least one month.

Procedure

Working reagent concentrations:

Peptide: suggested working concentration = 10 µg/mL

Mpro: we recommend using Mpro, Cat. No. SAE0172, at a working concentration of 1-5 µg/mL.

Assay buffer: Mpro protease is active under a wide variety of conditions and temperatures. Recommended assay buffers are 25 mM HEPES or Tris-HCl buffer, pH 7.0-7.5.

Reaction temperature: 0-30 °C

Sample protocol:

1. Prepare substrate solution for 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 excitation (λ_{ex}) = 400 nm, and emission (λ_{em}) = 505 nm.
3. Use 200 µL of substrate solution per well for 96-well plate format.
4. Add 2-20 µg/mL Mpro (Cat. No. SAE0172).
5. Read kinetics for 5-60 minutes.

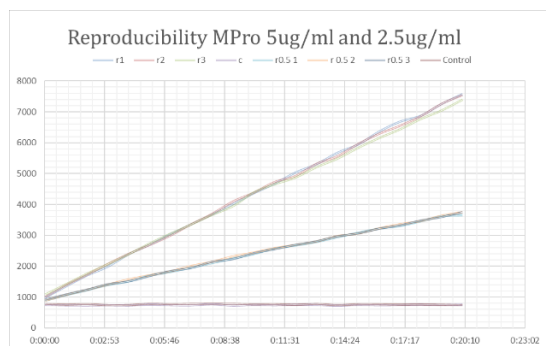


Figure 1. Mpro enzyme (SAE0172) kinetics performed with the fluorogenic substrate SAE0180.

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

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

References

1. Liu, P. *et al.*, Potent inhibitors of SARS-CoV-2 3C-like protease derived from N-substituted isatin compounds. *Eur. J. Med. Chem.*, **206**, 112702 (2020).
2. Liu, Z. *et al.*, Virtual screening of novel noncovalent inhibitors for SARS-CoV 3C-like proteinase. *J. Chem. Inf. Model.*, **45(1)**, 10-17 (2005).
3. Li, C. *et al.*, Maturation mechanism of severe acute respiratory syndrome (SARS) coronavirus 3C-like proteinase. *J. Biol. Chem.*, **285(36)**, 28134-28140 (2010).

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