

# PRODUCT INFORMATION



## Covidy<sup>TM</sup> IF670

Item No. 33768

Purity:	≥95%
Ex./Em. Max:	656/670 nm
Supplied as:	A solid
Storage:	-20°C
Stability:	≥4 years

Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

### Description

Viral main protease (M<sup>pro</sup>), also known as 3C-like protease (3CL<sup>pro</sup>), is a viral protease that mediates the replication and transcription of severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2, the causative agents of SARS and COVID-19, respectively.<sup>1,2</sup> M<sup>pro</sup> digests SARS-CoV and SARS-CoV-2 viral polyproteins at 11 conserved sites, beginning with autolytic cleavage of itself, to release the functional peptides required for viral replication and transcription. Covidy<sup>TM</sup> IF670 is a fluorogenic substrate for M<sup>pro</sup> that contains 12 amino acids, VNSTLQSGLRKM, which are recognized by M<sup>pro</sup>. Upon enzymatic cleavage by M<sup>pro</sup>, the fluorophore, iFluor<sup>TM</sup> 670, is separated from the quencher, TideQuencher<sup>TM</sup> 5 (TQ5), and displays excitation/emission maxima of 656/670 nm, respectively, and can be used to quantify M<sup>pro</sup> activity.

### Assay Protocol

- Preparation of stock solution
  - Add 25 µl of DMSO to the Covidy<sup>TM</sup> IF670 vial to make a 400X stock solution. This is sufficient for 100 tests in a 96-well plate format.
  - Aliquot in volumes sufficient for single use and store at -20°C. Avoid repeated freeze-thaw cycles.
- Preparation of working solutions
  - Covidy<sup>TM</sup> IF670 substrate working solution
    - Dilute the Covidy<sup>TM</sup> IF670 400X stock solution 1:200 in 20 mM Tris buffer (pH 7.5) or buffer of choice to make a 2X working solution. Covidy<sup>TM</sup> IF670 2X working solution will be used at a volume of 50 µl per well in a 96-well plate.\*
  - M<sup>pro</sup> enzyme dilution series
    - Dilute M<sup>pro</sup> enzyme to make a 2X working solution for each desired final enzyme concentration. The M<sup>pro</sup> 2X working solution will be used at a volume of 50 µl per well in a 96-well plate.\*
- Experimental protocol
  - Add 50 µl of each M<sup>pro</sup> 2X working solution to a 96-well plate.\*
  - Add 50 µl of Covidy<sup>TM</sup> IF670 2X working solution to each M<sup>pro</sup> well in the 96-well plate.\*
  - Monitor the fluorescence increase with a fluorescence plate reader at ex/em = 656/670 nm.

\*Adjust to lower volumes of substrate and enzyme 2X working solutions for low-volume 96-well or higher density-format plates.

**NOTE for kinetic reading:** Immediately start a continuous measurement of fluorescence intensity, recording data every five minutes for 30-120 minutes.

**NOTE for end-point reading:** Incubate the reaction at the desired temperature for 30-120 minutes, protected from light. After incubation, measure the fluorescence intensity.

### References

- Kuo, C.-J., Chi, Y.-H., Hsu, J.T.-A., et al. *Biochem. Biophys. Res. Commun.* **318(4)**, 862-867 (2004).
- Hung, H.-C., Ke, Y.-Y., Huang, S.Y., et al. **64(9)**, e00872-20 (2020).

WARNING  
THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

SAFETY DATA  
This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent via email to your institution.

WARRANTY AND LIMITATION OF REMEDY  
Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

Copyright Cayman Chemical Company, 11/15/2022

CAYMAN CHEMICAL  
1180 EAST ELLSWORTH RD  
ANN ARBOR, MI 48108 · USA  
PHONE: [800] 364-9897  
[734] 971-3335  
FAX: [734] 971-3640  
CUSTSERV@CAYMANCHEM.COM  
WWW.CAYMANCHEM.COM