

PRODUCT INFORMATION



CovidyTM EN450

Item No. 33765

Purity:	≥95%
Ex./Em. Max:	350/460 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^{pro}), also known as 3C-like protease (3CL^{pro}), 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.^{1,2} M^{pro} 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. CovidyTM EN450 is a fluorogenic substrate for M^{pro} that contains 14 amino acids, KTSAVLQSGFRKME, which are recognized by M^{pro}. Upon enzymatic cleavage by M^{pro}, EDANS is separated from the Dabcyl quencher, displays excitation/emission maxima of 350/460 nm, respectively, and can be used to quantify M^{pro} activity.

Assay Protocol

1. Preparation of stock solution
 - a. Add 25 µl of DMSO to the CovidyTM EN450 vial to make a 400X stock solution. This is sufficient for 100 tests in a 96-well plate format.
 - b. Aliquot in volumes sufficient for single use and store at -20°C. Avoid repeated freeze-thaw cycles.
2. Preparation of working solutions
 - a. CovidyTM EN450 substrate working solution
 - i. Dilute the CovidyTM EN450 400X stock solution 1:200 in 20 mM Tris buffer (pH 7.5) or buffer of choice to make a 2X working solution. CovidyTM EN450 2X working solution will be used at a volume of 50 µl per well in a 96-well plate.*
 - b. M^{pro} enzyme dilution series
 - i. Dilute M^{pro} enzyme to make a 2X working solution for each desired final enzyme concentration. The M^{pro} 2X working solution will be used at a volume of 50 µl per well in a 96-well plate.*
3. Experimental protocol
 - a. Add 50 µl of each M^{pro} 2X working solution to a 96-well plate.*
 - b. Add 50 µl of CovidyTM EN450 2X working solution to each M^{pro} well in the 96-well plate.*
 - c. Monitor the fluorescence increase with a fluorescence plate reader at ex/em = 350/460 nm, respectively.

*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.

1. Kuo, C.-J., Chi, Y.-H., Hsu, J.T.-A., et al. Characterization of SARS main protease and inhibitor assay using a fluorogenic substrate. *Biochem. Biophys. Res. Commun.* **318**(4), 862-867 (2004).
2. Hung, H.-C., Ke, Y.-Y., Huang, S.Y., et al. Discovery of M protease inhibitors encoded by SARS-CoV-2. *Antimicrob. Agents Chemother.* **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.

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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