

# PRODUCT INFORMATION



## Covipyte™ EN450

Item No. 33769

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

Papain-like protease (PL<sup>pro</sup>) is a cysteine viral protease and component of the replicase complex in severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2, the causative agents of SARS and COVID-19, respectively.<sup>1,2</sup> PL<sup>pro</sup> processes SARS-CoV and SARS-CoV-2 non-structural proteins 1-3, which, together with non-structural proteins 4-16, form the viral replicase complex, at three conserved sites, beginning with autolytic cleavage of itself.<sup>1-3</sup> Covipyte™ EN450 is a fluorogenic substrate for PL<sup>pro</sup> that contains 9 amino acids, RELNGGAPI, which are recognized by PL<sup>pro</sup>. Upon enzymatic cleavage by PL<sup>pro</sup>, EDANS is separated from the Dabcyl quencher, displays excitation/emission maxima of 350/460 nm, respectively, and can be used to quantify PL<sup>pro</sup> activity.

### Assay Protocol

- Preparation of stock solution
  - Add 25 µl of DMSO to the Covipyte™ EN450 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
  - Covidyte™ EN450 substrate working solution
    - Dilute the Covipyte™ EN450 400X stock solution 1:200 in 20 mM Tris buffer (pH 7.5) or buffer of choice to make a 2X working solution. Covipyte™ EN450 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 Covipyte™ EN450 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 = 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.

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, 02/08/2024

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

# PRODUCT INFORMATION



## References

---

1. Barretto, N., Jukneliene, D., Ratia, K., *et al.* The papain-like protease of severe acute respiratory syndrome coronavirus has deubiquitinating activity. *J. Virol.* **79**(24), 15189-15198 (2005).
2. Anirudhan, V., Lee, H., Cheng, H., *et al.* Targeting SARS-CoV-2 viral proteases as a therapeutic strategy to treat COVID-19. *J. Med. Virol.* 1-13 (2021).
3. Romano, M., Ruggiero, A., Squeglia, F., *et al.* A structural view of SARS-CoV-2 RNA replication machinery: RNA synthesis, proofreading and final capping. *Cells* **9**(5), 1267 (2020).

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