PRODUCT INFORMATION

Covidyte™ EN450

Item No. 33765

Purity: ≥95%
Ex./Em. Max: 350/460 nm
Supplied as: A solid
Storage: -20°C
Stability: ≥2 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. Covidyte™ 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 Covidyte™ 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. Covidyte™ EN450 substrate working solution
        i. Dilute the Covidyte™ EN450 400X stock solution 1:200 in 20 mM Tris buffer (pH 7.5) or buffer of choice to make a 2X working solution. Covidyte™ 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 Covidyte™ 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).

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