Covipyte™ EN450

Catalog number: 13545, 13546
Unit size: 100 tests, 1000 tests

Component	Storage	Amount (Cat No. 13545)	Amount (Cat No. 13546)
Covipyte™ EN450	Freeze (< -15 °C), Minimize light exposure	100 tests	1000 tests

OVERVIEW

Coronaviruses (CoVs) can infect humans and multiple species of animals, causing a wide spectrum of diseases. In late 2019, a novel coronavirus, termed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was determined as a cause for several cases of respiratory disease (Covid-19). Even though most infected patients only suffer from mild symptoms such as fever and cough associated with a good prognosis, the disease can progress into fatal cases of pneumonia and acute respiratory failure, especially in older males with comorbidities. Covid-19 rapidly spread worldwide. It has infected more than 4.3 million people and claimed more than three hundred thousand fatalities (as of May 14, 2020). Coronavirus is a single-stranded RNA positive-strand envelope type B coronavirus. Like the other two coronaviruses that cause SARS (Severe Acute Respiratory Syndrome) and MERS (Middle East Respiratory Syndrome), SARS-CoV-2 encodes non-structural, structural, and accessory proteins. Non-structural proteins include 3-chymotrypsin-like protease (3CLpro), papain-like protease, helicase, and RNA-dependent RNA polymerase (RNA -dependent RNA polymerase (RdRp). Structural proteins include spike glycoproteins. Papain in coronavirus operates on more than 11 cleavage sites on the large polyprotein 1ab. Processing of polyproteins translated from viral RNA is essential, therefore, the main proteases are identified as an attractive drug targets for preventing virus imitation. Papain-like protease (PLpro) of coronaviruses carries out proteolytic maturation of non-structural proteins that play a role in replication of the virus and performs deubiquitination of host cell factors to scuttle antiviral responses. Covipyte™ EN450 is a peptide substrate containing 9 amino acid sequence (RELNGGAPI) that can be cleaved by coronavirus PLpro. The dark-FRET peptide contains Edans (donor) and Dabcyl (quencher) on the N-and C-terminals respectively where the fluorescence of Edans is effectively quenched by Dabcyl when the peptide is intact. When the peptide is hydrolyzed by coronavirus proteases, the Edans fragment generates significantly enhanced fluorescence since its fluorescence is no longer quenched by Dabcyl. The activity of coronavirus proteases can be effectively monitored by the fluorescence intensity of Edans. Covipyte™ EN450 is a convenient tool for screening and studying kinetics of PLpro inhibitors.

KEY PARAMETERS

Fluorescence microplate reader

Excitation	350 nm
Emission	460 nm
Cutoff	420 nm
Recommended plate	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

Covipyte™ EN450 stock solution (200X)
Add 25 µL (**For cat# 13545**) or 250 µL (**For cat# 13546**) DMSO to Covipyte™ EN450 vial.

Note Make single use aliquots and store at -20 °C.

PREPARATION OF WORKING SOLUTION

1. Covipyte™ EN450 working solution

Dilute substrate stock solution at 1:200 in 20 mM Tris buffer (pH 7.5) or buffer of your choice. Use 50 µL of substrate solution per assay in a 96-well plate.

2. Coronavirus proteases dilution
Dilute the coronavirus proteases as desired.

SAMPLE EXPERIMENTAL PROTOCOL

Sample Protocol for One 96-well plate

1. Add 50 µL of EACH protease dilution to respective wells of the assay plate.
2. Add 50 µL of Covipyte™ EN450 working solution to each protease dilution.
3. Monitor the fluorescence increase with a fluorescence plate reader at Ex/Em = 350/460 nm (cutoff 420nm).

For kinetic reading: Immediately start measuring fluorescence intensity continuously and record data every 5 minutes for 30-120 minutes.
For end-point reading: Incubate the reaction at a desired temperature for 30 to 120 minutes, protected from light. Then measure the fluorescence intensity.

EXAMPLE DATA ANALYSIS AND FIGURES

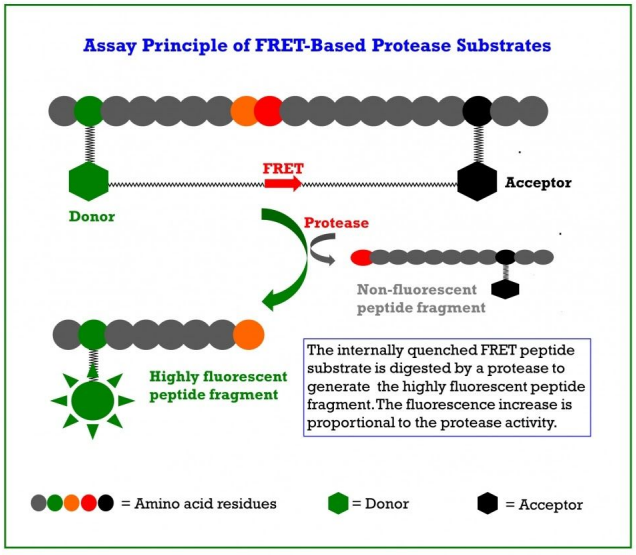

Figure 1. Covipyte™ EN450 is a peptide substrate containing 9 amino acid sequence (RELNGGAPI) that can be cleaved by coronavirus PLpro. The dark-FRET peptide contains Edans (donor) and Dabcyl (quencher) on the N-and C-terminals respectively where the fluorescence of Edans is effectively quenched by Dabcyl when the peptide is intact. When the peptide is hydrolyzed by coronavirus proteases, the Edans fragment generates significantly enhanced fluorescence since its fluorescence is no longer quenched by Dabcyl. The activity of coronavirus proteases can be effectively monitored by the fluorescence intensity of Edans. Covipyte™ EN450 is a convenient tool for screening and studying kinetics of PLpro inhibitors.

DISCLAIMER

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