Covidyte™ ED450

Catalog number: 13537, 13538
Unit size: 100 tests, 1000 tests

Component	Storage	Amount (Cat No. 13537)	Amount (Cat No. 13538)
Covidyte™ ED450	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. The virus rapidly spread worldwide. It has infected more than a million people, and Covid-19 has claimed more than seventy thousand fatalities (as of April 6, 2020). Currently, there are not any specific and effective options available for treating Covid-19. At present the clinical treatment of Covid-19 is mainly symptomatic combined with repurposing of already marketed antiviral drugs such as Remdesivir and antibiotics to treat secondary infections. There is an extremely urgent need for the development of specific antiviral therapeutics and vaccines against SARS-CoV-2. The coronavirus main protease, which plays a pivotal role in viral gene expression and replication through the proteolytic processing of replicase polyproteins, is an attractive target for anti-CoV drug design. The inhibition of viral proteases necessary for proteolytic processing of polyproteins has been a successful strategy in the treatment of human immunodeficiency virus (HIV) and hepatitis C respectively, proving the potential of protease inhibitors for the treatment of viral infections. Similarly, the main protease of SARS-CoV-2 is thought to be essential for viral replication and, therefore, is regarded as promising target for antiviral therapy of Covid-19. Covidyte™ ED450 is a peptide substrate containing 12 amino acid sequence (VNSTLQSGLRKM) that can be cleaved by coronavirus proteases. The dark-FRET peptide contains Dabcyl (quencher) and Edans (donor) 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. Covidyte™ ED450 is a convenient tool for screening inhibitors of coronavirus proteases.

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.

Covidyte™ ED450 stock solution (200X)
Add 25 µL (**For cat# 13537**) or 250 µL (**For cat# 13538**) DMSO to Covidyte™ ED450 vial.

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

PREPARATION OF WORKING SOLUTION

1. Covidyte™ ED450 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 Covidyte™ ED450 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

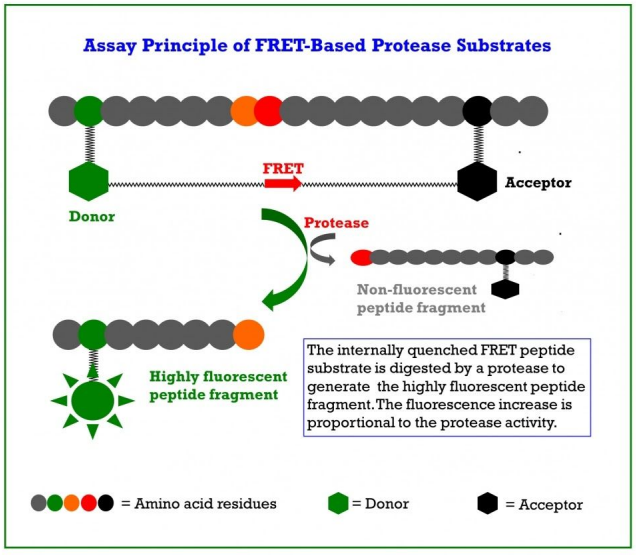

Figure 1. Proteases play essential roles in protein activation, cell regulation and signaling, as well as in the generation of amino acids for protein synthesis or utilization in other metabolic pathways. FRET protease substrates are widely used for detecting protease activities, in particular, for virus protease that often require a long peptide sequence for optimal binding such as coronavirus, HIV and HCV proteases. The internally quenched FRET peptide substrate is digested by a protease to generate the highly fluorescent peptide fragment. The fluorescence increase is proportional to the protease activity. EDANS and DABCYL are a common pair for developing FRET protease substrates.

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