

## Product Information

### Covidyte™ EN450 Substrate

Catalog Number **MAK373**

Storage Temperature -20 °C

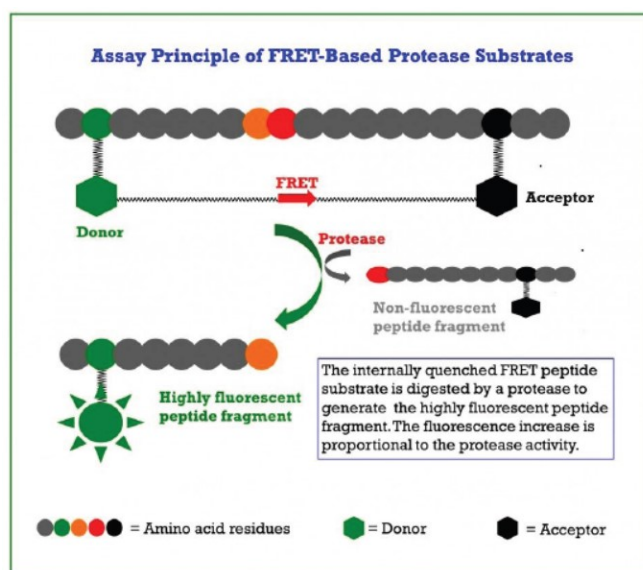
#### Product Description

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. As of 2020, the clinical treatment of Covid-19 is mainly symptomatic combined with repurposing of established antiviral drugs and antibiotics to treat secondary infections. There is an 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 is therefore regarded as promising target for antiviral therapy of Covid-19.

Covidyte EN450 is a peptide substrate containing a 14 amino acid sequence 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 EN450 Substrate is a convenient tool for developing methods to screen potential inhibitors of coronavirus proteases.



**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.

#### Components

Covidyte EN450 Substrate  
Sufficient for 100 tests in 96-well plates

1 vial

### Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Black flat bottom 96-well plates
- Fluorescence multiwell plate reader capable of:
 

Excitation ( $\lambda_{Ex}$ )	350 nm
Emission ( $\lambda_{Em}$ )	460 nm
Cutoff	420 nm
- Dimethyl Sulfoxide (DMSO), anhydrous, Catalog Number 276855 or equivalent
- 20 mM Tris buffer, pH 7.5, or alternative buffer of choice

### Precautions and Disclaimer

For Research Use Only. Not for use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

Covidyte EN450 Substrate is shipped at room temperature. Upon receipt, store at -20 °C, protected from light.

### Preparation Instructions

#### Reagent Preparation

Covidyte EN450 Substrate stock solution (200×): Reconstitute Covidyte EN450 substrate vial with 25  $\mu$ L of DMSO.

**Note:** Unused stock solution should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

#### Covidyte EN450 working substrate solution Preparation

Dilute Covidyte EN450 Substrate stock solution (200×) 1:200 in 20 mM Tris buffer, pH 7.5, or buffer of your choice. 50  $\mu$ L of working substrate solution is required per assay well.

#### Sample Preparation

Dilute the coronavirus proteases as desired in preferred protease diluent.

### Procedure

#### Assay Reaction

1. Add 50  $\mu$ L of each coronavirus protease dilution to respective wells of a black flat bottom 96-well plate.
2. Add 50  $\mu$ L of Covidyte EN450 working substrate solution to each well.
3. Mix well.
4. Monitor the fluorescence increase with a fluorescence plate reader at ( $\lambda_{Ex}$  = 350 nm/ $\lambda_{Em}$  = 460 nm; cutoff 420 nm).

**For kinetic reading:** Immediately start measuring fluorescence intensity continuously and record data every 5 minutes for 30 to 120 minutes.

**For end-point reading:** Incubate the reaction at a desired temperature for 30 to 120 minutes, protected from light. Measure the fluorescence intensity at the end of the incubation period.

### References

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2. Hsu, John T-A, *et al.*, Evaluation of metal-conjugated compounds as inhibitors of 3CL protease of SARS-CoV. *FEBS Lett.*, **574**, 116-20 (2004).
3. Zhang, L., *et al.*, Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved  $\alpha$ -ketoamide inhibitors. *Science*, **368**, 409–412 24 (2020).
4. Kumar, V., *et al.*, Identification and evaluation of potent Middle East respiratory syndrome coronavirus (MERS-CoV) 3CLPro inhibitors / *Antiviral Res.*, **141**, 101-106 (2017).
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7. Patrick, A.K. and Potts, K.E., Protease inhibitors as antiviral agents. *Clin. Microbiol. Rev.*, **11**, 614-627 (1998).
8. Medintz, I., *et al.*, Proteolytic activity monitored by fluorescence resonance energy transfer through quantum-dot-peptide conjugates. *Nature Mater.*, **5**, 581–589 (2006).
9. Park, J.-Y., *et al.*, Evaluation of polyphenols from *Broussonetia papyrifera* as coronavirus protease inhibitors, *J. Enzyme Inhib. Med. Chem.*, **32**, 504-512, (2017).
10. Chou, C.-Y., *et al.*, Thiopurine analogues inhibit papain-like protease of severe acute respiratory syndrome coronavirus. *Biochem. Pharma.*, **75**, 1601-9 (2008).

**Related Products**

Covidyte IF670 Substrate, Catalog Number MAK376  
Covidyte TF670 Substrate, Catalog Number MAK375  
Covidyte ED450 Substrate, Catalog Number MAK374

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