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Product Information

Covidyte™ IF670 Substrate

Catalog Number **MAK376**
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 suffer 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 IF670 is a peptide substrate containing a 12-amino acid sequence (VNSTLQSGLRKM) that can be cleaved by coronavirus proteases. The dark-FRET peptide contains Tide Quencher™ 5 (TQ5) as a quencher and iFluor™ 670 as a fluorescent donor on the N- and C- terminals respectively where the fluorescence of iFluor 670 is effectively quenched by TQ5 when the peptide is intact. When the peptide is hydrolyzed by coronavirus proteases, the iFluor 670 fragment generates significantly enhanced fluorescence since its fluorescence is no longer quenched by TQ5. The activity of coronavirus proteases can be effectively monitored by the fluorescence intensity of iFluor 670.

Covidyte IF670 Substrate is a robust high throughput screening tool for developing methods to screen potential inhibitors of coronavirus proteases. Comparing to the commonly used EDANS substrates (such as Covidyte ED450 Substrate, Catalog Number MAK374), the iFluor 670 substrate has a much stronger and longer fluorescent signal with less interference by colored compounds, reducing false positive results.

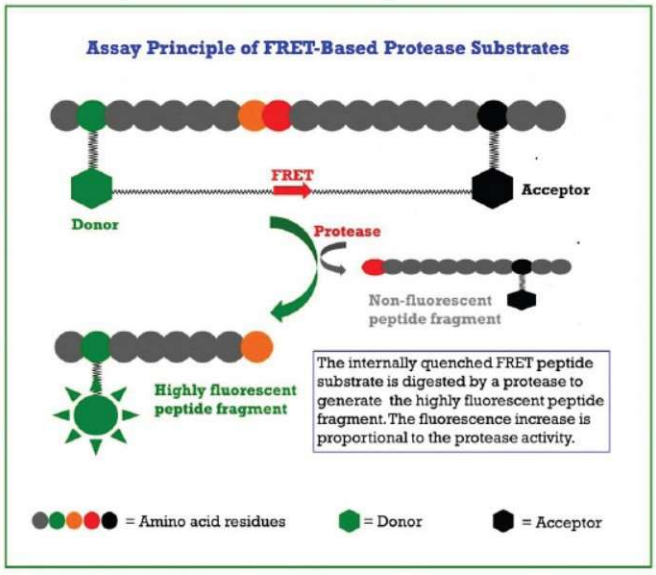

Figure 1. Proteases play essential roles in protein activation, cell regulation and signaling, as well as in the generation of amino acids for protein synthesis or utilization in other metabolic pathways. FRET protease substrates are widely used for detecting protease activities, in particular, for virus 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. Tide Quencher dyes have been proven to be the extremely effective quenchers for developing FRET protease substrates for high throughput screening applications together with the bright Tide Fluor™ and iFluor dyes.

Components

Covidyte IF670 Substrate	1 vial

Sufficient for 100 tests in 96-well plates

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 (λ_{Ex})	350 nm
Emission (λ_{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 IF670 Substrate is shipped at room temperature. Upon receipt, store at -20 °C, protected from light.

Preparation Instructions

Reagent Preparation

Covidyte IF670 Substrate stock solution (200×)- Reconstitute Covidyte IF670 Substrate vial with 25 μL 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 IF670 working substrate solution

Dilute Covidyte IF670 Substrate stock solution (200×) 1:200 in 20 mM Tris buffer, pH 7.5, or buffer of your choice. 50 μL 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 μL of each coronavirus protease dilution to respective wells of a black flat bottom 96-well plate.
2. Add 50 μL of Covidyte IF670 working substrate solution to each well.
3. Mix well.
4. Monitor the fluorescence increase with a fluorescence plate reader at λ_{Ex} = 350 nm/ λ_{Em} = 460 nm; cutoff 660 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. Chen, S., *et al*., Enzymatic activity characterization of SARS coronavirus 3C-like protease by fluorescence resonance energy transfer technique. *Acta Pharmacol. Sin*., **26,** 99–106 (2005).
3. Lamarre, D., *et al.*, An NS3 protease inhibitor with antiviral effects in humans infected with hepatitis C virus. *Nature,* **426**, 186–189 (2003).
4. Patrick, A.K. and Potts, K.E., Protease inhibitors as antiviral agents. *Clin. Microbiol. Rev*., **11**, 614-627 (1998).
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Related Products

Covidyte TF670 Substrate, Catalog Number MAK375
Covidyte ED450 Substrate, Catalog Number MAK374
Covidyte EN450 Substrate, Catalog Number MAK373

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