PRODUCT INFORMATION SHEET

Mca-APK(Dnp) ACE2 substrate

Catalog number: 13555, 13556
Unit size: 1 mg, 10 mg

Component	Storage	Amount (Cat No. 13555)	Amount (Cat No. 13556)
Mca-APK(Dnp) ACE2 substrate	Freeze (< -15 °C), Minimize light exposure	1 mg	10 mg

OVERVIEW

ACE2 (angiotensin-converting enzyme 2) is a metalloproteinase that requires a divalent cation positioned at the active site in order to perform catalysis. It has multiple physiological roles that revolve around its trivalent function: a negative regulator of the renin-angiotensin system, facilitator of amino acid transport, and the severe acute respiratory syndrome-coronavirus (SARS-CoV) and SARS-CoV-2 receptor. ACE2 has recently been identified as the SARS-CoV-2 receptor, the infective agent responsible for the coronavirus disease, providing a critical link between immunity, inflammation, ACE2, and cardiovascular disease. Although sharing a close evolutionary relationship with SARS-CoV, the receptor-binding domain of SARS-CoV-2 differs in several key amino acid residues, allowing for stronger binding affinity with the human ACE2 receptor, which may account for the greater pathogenicity of SARS-CoV-2. The loss of ACE2 function following binding by SARS-CoV-2 is driven by endocytosis and activation of proteolytic cleavage and processing. The ACE2 system is a critical protective pathway against heart failure with reduced and preserved ejection fraction including, myocardial infarction and hypertension, and against lung disease and diabetes mellitus. The control of gut dysbiosis and vascular permeability by ACE2 has emerged as an essential mechanism of pulmonary hypertension and diabetic cardiovascular complications. Mca-APK(Dnp) ACE2 substrate is a FRET peptide for measuring enzymatic activity in cells and tissues. It uses DNP as a quencher molecule to quench the fluorescence of methoxycoumarin (Mca). This interaction is abolished when the enzyme cleaves the proline-lysine residue and restore the fluorescence of Mca. This fluorogenic substrate offers more flexibility and higher throughput than the commonly used HPLC-separation-based methods.

AT A GLANCE

1. Prepare test and blank samples (50 µL)
2. Treat samples with inhibitors as desired
3. Add Mca-APK(Dnp) ACE2 substrate working solution (50 µL)
4. Incubate samples at 37 °C
5. Measure fluorescence at Ex/Em = 320/430 nm (Cutoff = 420 nm)

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.

Mca-APK(Dnp) ACE2 substrate stock solution
Prepare 5-10 mM stock solution with appropriate amount of DMSO.

Note Store solution at -20 °C in single use aliquots.

Note Protect from light.

PREPARATION OF WORKING SOLUTION

Mca-APK(Dnp) ACE2 substrate working solution
Add 5 µL of Mca-APK(Dnp) ACE2 substrate stock solution (10 mM) in 1 mL of assay buffer to make Mca-APK(Dnp) ACE2 substrate working solution.

Note The appropriate concentration for the substrate should be measured empirically.

Note With the given formula, the final concentration is 50 µM.

Note 100 mM Tris-HCl containing 1 M NaCl, pH ~ 7.5 can be used as an assay buffer. 600 µM of $ZnCl_2$ can also be added to the buffer.

SAMPLE EXPERIMENTAL PROTOCOL

The following protocol can be used as guidelines.

1. Prepare and add 50 µL of test samples and blank controls.
2. Add 50 µL of Mca-APK(Dnp) ACE2 substrate working solution into the samples and blank samples.
3. Incubate samples at 37 °C.
4. Measure the fluorescence increase with a fluorescence plate reader at Ex/Em = 320/430 nm (Cutoff = 420 nm).

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

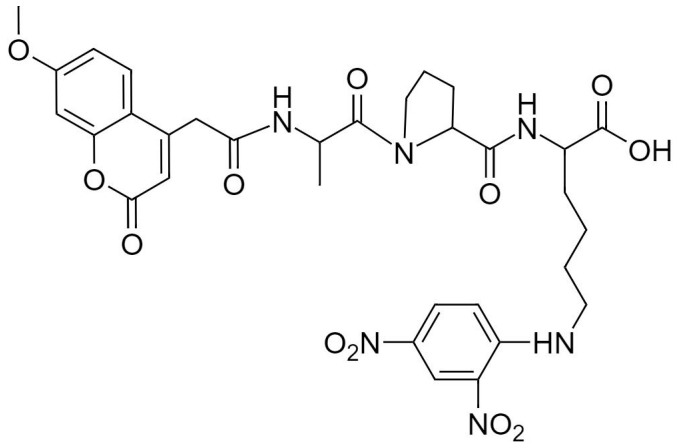

Figure 1. Chemical structure for Mca-APK(Dnp) ACE2 substrate.

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