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Product Information

Angiotensin II Converting Enzyme (ACE2) Inhibitor Screening Kit

Catalog Number **MAK378**
Storage Temperature -20 °C

TECHNICAL BULLETIN

Product Description

Angiotensin II converting enzyme (ACE2, EC 3.4.17.23), a carboxypeptidase, is part of the renin-angiotensin system (RAS) that controls regulation of blood pressure by cleaving the C-terminal dipeptide of Angiotensin II to convert it into Angiotensin 1-7. It also cleaves Angiotensin I to produce Ang 1-9, of unknown function. ACE2 is a receptor of human coronaviruses, such as SARS and HCoV-NL63. It is expressed on the vascular endothelial cells of lung, kidney and heart. The inhibitors of ACE2 could be able to regulate hypertension by changing vascular permeability. Screening for small molecule and peptide inhibitors might also help in finding treatment for coronavirus mediated infection.

The Angiotensin II Converting Enzyme (ACE2) Inhibitor Screening Kit can be used to screen for potent inhibitors of ACE2 activity. The kit utilizes the ability of an active ACE2 to cleave a synthetic 4-methoxycoumarin-7-acetic acid (MCA) based peptide substrate to release a free fluorophore. The released MCA can be easily quantified using a fluorescence microplate reader. In the presence of an ACE2 specific inhibitor, the enzyme loses its peptidase activity which results in decrease of fluorescence intensity. This assay kit is simple and can be used to identify and characterize ACE2 inhibitors in a high-throughput format.

The kit is suitable for Screening/characterizing inhibitors/ligands of ACE2.

Components

The kit is sufficient for 100 fluorometric assays in 96 well plates.

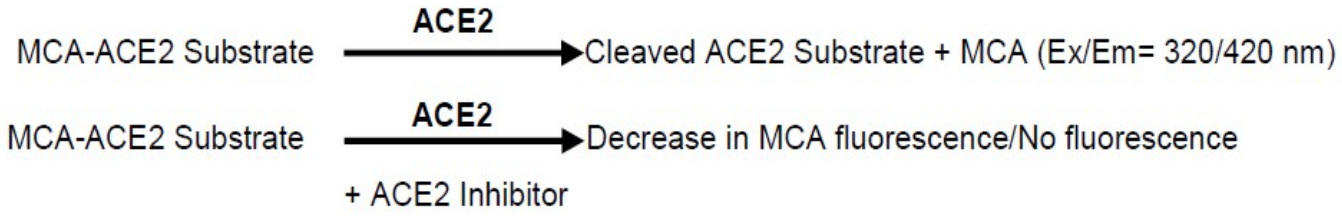
ACE2 Assay Buffer Catalog Number MAK378A	25 mL
ACE2 Dilution Buffer Catalog Number MAK378B	1.5 mL
ACE2 Enzyme Catalog Number MAK378C	20 µL
ACE2 Substrate Catalog Number MAK378D	200 µL
ACE2 Inhibitor (0.5 mM) Catalog Number MAK378E	5 µL

Reagents and Equipment Required but Not Provided.

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

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
The kit is shipped on wet ice. Store components at -20 °C, protected from light. Briefly centrifuge small vials at low speed prior to opening.

Preparation Instructions.

Reagent Preparation

ACE2 Assay Buffer and ACE2 Dilution Buffer: Warm to room temperature prior to use. May be stored at -20 °C or 2-8 °C.

ACE2 Enzyme: Thaw to room temperature prior to use. Avoid multiple freeze/thaw cycles. Use within 3 months.

Diluted ACE2 Enzyme Solution: Add 198 µL of ACE2 Dilution Buffer to the thawed ACE2 enzyme vial. The diluted enzyme can be stored at -20 °C in aliquots.

ACE2 Substrate: Ready to use as supplied. Thaw to room temperature, protected from light, prior to use.

ACE2 Inhibitor: Thaw to room temperature prior to use. Avoid multiple freeze/thaw cycles.

Procedure
Assay Procedure

ACE2 Enzyme Working Solution
Mix enough reagents for the number of assays to be performed. For each well (Enzyme Control (EC), Sample (S), Inhibitor Control (IC) and Solvent Control (SC)), prepare 50 µL of ACE2 Enzyme Working Solution according to Table 1.

Table 1.
Preparation of ACE2 Enzyme Working Solution

Reagent	EC, S, SC and IC
ACE2 Assay Buffer	48 µL
Diluted ACE2 Enzyme Solution	2 µL

Mix well and add 50 µL /well into desired wells in a 96-well microtiter plate. For the background control (BC), add 50 µL of ACE2 Assay Buffer to the appropriate well.

Screening Compounds, Inhibitor Control & Enzyme Control Preparation:

1. Dissolve the candidate inhibitors at ~100× the highest final test concentration using preferred solvent. It is recommended that a pilot experiment with different inhibitor concentrations is performed to determine the optimal concentration.

   Note: High solvent concentrations might affect the enzymatic activity. Prepare parallel well(s) as Solvent Control (SC) to test the effect of the solvent on enzyme activity (same as EC in presence of final solvent concentration). In case SC is significantly different from EC, use its value in the results calculations.
2. Dilute to 10× the desired test concentration with ACE2 Assay Buffer.
3. Add 10 µL of test inhibitors (Sample, S) to appropriate wells.
4. Add 10 µL of ACE2 Assay Buffer to EC and BC wells.
5. Prepare Inhibitor Control by adding 50 µL of ACE2 Assay Buffer to the vial containing ACE2 Inhibitor, mix.
6. For Inhibitor Control (IC), add 10 µL of ACE2 Inhibitor into ACE2 enzyme containing well(s).
7. Incubate at room temperature for 15 minutes.

ACE2 Substrate Mix: Mix enough reagents for the number of assays to be performed. For each well, prepare 40 µL of ACE2 Substrate Mix according to Table 2.

Table 2.
Preparation of ACE2 Substrate Mix

Reagent	Volume
ACE2 Assay Buffer	38 µL
ACE2 Substrate	2 µL

Mix thoroughly. Add 40 µL of ACE2 Substrate Mix into Background Control, Enzyme Control, Solvent Control, Inhibitor Control & Sample (S) wells. Mix well.

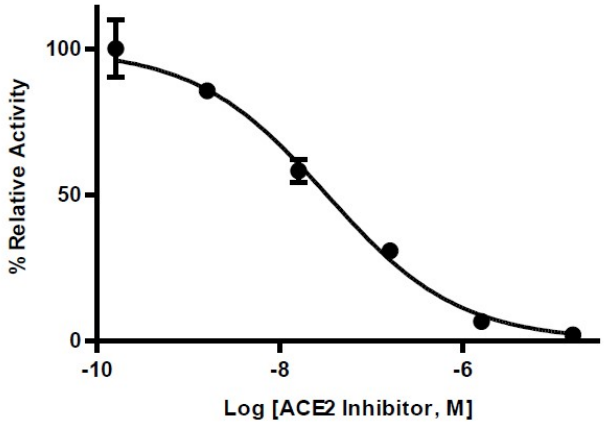
Measurement
Measure fluorescence at λ_{Ex} = 320 nm/λ_{Em} = 420 nm in kinetic mode for one hour at room temperature.

Results

Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 and RFU_2). Calculate the slope for all samples, ΔRFU/ΔT.

$$\%\ \textbf{Relative activity} = \frac{\textbf{Δ RFU of S}}{\textbf{ΔRFU of EC}} \times 100$$

Figure 1.
Inhibition of ACE2 activity by ACE2 Inhibitor, IC50 = 33.0 ± 0.6 nM (n = 3).



Related Products

- Angiotensin II Converting Enzyme (ACE2) Activity Assay Kit (Fluorometric), Catalog Number MAK377

Frequently Asked Questions:

1. **Will the kit work on bacteria or yeast cells?**
   The kit has been standardized for mammalian cells only.

2. **What is the exact volume of sample required for this assay?**
   There is no specific volume or concentration recommended for the candidate inhibitors. It is recommended that a pilot experiment with different inhibitor concentrations is performed to determine the optimal concentration. Additionally, a solvent control for each concentration of the candidate inhibitor tested should be included in the assay.

3. **Can we use an alternate buffer for sample preparation (cell lysis, sample dilutions, etc.)?**
   The kit assay buffers are optimized for the reactions they are designed for. The buffers not only contain detergents for efficient lysis of cells/tissue, but also contain proprietary components required for the further reactions. Therefore, it is highly recommended to use the buffers provided in the kit for the best results.

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