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Product Information

Angiotensin II Converting Enzyme (ACE2) Activity Assay Kit (Fluorometric)

Catalog Number **MAK377**
Storage Temperature -20 °C

TECHNICAL BULLETIN

Product Description

Angiotensin II converting enzyme (ACE2, EC 3.4.17.23), a zinc-based metalloprotease, is part of the renin-angiotensin system (RAS) that controls the regulation of blood pressure by cleaving the C-terminal dipeptide of Angiotensin II to convert it into Angiotensin 1-7. 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. ACE2 is a potential therapeutic target for cardiovascular and coronavirus-induced diseases.

The Angiotensin II Converting Enzyme (ACE2) Activity Assay Kit utilizes the ability of an active ACE2 to cleave a synthetic 7-Methoxycoumarin-4-acetic acid (MCA) based peptide substrate to release a free fluorophore. The released MCA can be easily quantified using a fluorescence microplate reader. An ACE2 specific inhibitor is also provided that can differentiate the ACE2 activity from other proteolytic activity. The kit is simple to use, can detect ACE2 concentrations as low as 0.4 mU and can be used in a high-throughput format.

The kit is suitable for the detection of ACE2 activity in tissue (for example lung, heart and kidney) and overexpressed ACE2 in cell lysates, as well as the determination of enzymatic activity of purified ACE2.

Components

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

ACE2 Assay Buffer Catalog Number MAK377A	25 mL
ACE2 Dilution Buffer Catalog Number MAK377B	1.5 mL
ACE2 Lysis Buffer Catalog Number MAK377C	50 mL
ACE2 Positive Control Catalog Number MAK377D	5 µL
ACE2 Substrate Catalog Number MAK377E	200 µL
ACE2 Inhibitor (22 mM) Catalog Number MAK377F	50 µL
MCA-Standard (1mM) Catalog Number MAK377G	15 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
- Refrigerated microcentrifuge capable of RCF ≥16,000 × *g*
- Dounce tissue grinder set (Catalog Number D9063 or equivalent)
- Bicinchoninic Acid Kit for Protein Determination (Catalog Number BCA1)

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

ACE2 Positive Control: Prior to use, add 95 µL of ACE2 Dilution Buffer to the ACE2 Positive Control vial. Unused diluted ACE2 Positive Control can be stored at -20 °C in small aliquots, avoid multiple freeze/thaw cycles. Use within 3 months.

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

ACE2 Inhibitor (22 mM): Bring the ACE2 Inhibitor and the ACE2 Assay Buffer to room temperature. Add 170 µL of ACE2 Assay Buffer to the ACE2 Inhibitor vial and mix thoroughly. Avoid multiple freeze/thaw cycles of the inhibitor. Use within 3 months.

MCA-Standard (1mM): Thaw to room temperature prior to use.

Procedure

Standard Curve Preparation

Prepare a 25 µM MCA Standard solution by diluting 5 µL of the 1 mM MCA-Standard with 195 µL of ACE2 Assay Buffer. Prepare MCA Standards in desired wells of a white flat-bottom 96-well plate according to Table 1.

Table 1.
Preparation of MCA Standards

Well	25 µM Standard Solution	ACE2 Assay Buffer	MCA (pmol/well)
1	0 µL	100 µL	0
2	2 µL	98 µL	50
3	4 µL	96 µL	100
4	6 µL	94 µL	150
5	8 µL	92 µL	200
6	10 µL	90 µL	250

Mix well and measure the fluorescence at λ_{Ex} = 320 nm/λ_{Em} = 420 nm in end point mode.

Note: Do not add Substrate Mix to the Standard wells.

Sample Preparation

Note: It is recommended to use the tissue/cell homogenate immediately to measure the ACE2 activity. If desired, snap freeze the sample lysate and store at -80 °C.

1. Homogenize ~ 100 mg of tissue or 1-2 × 10^6 of pelleted cells with 400 µL of ACE2 Lysis Buffer using a Dounce homogenizer. Incubate on ice for 10 minutes.
2. Gently vortex for 10 seconds, and then incubate on ice for an additional 5 minutes.
3. Centrifuge the homogenate at 16,000 × *g* for 10 minutes at 4 °C. Discard the pellet.
4. Transfer the clarified supernatant to a clean pre-chilled tube and keep on ice.

Protein Determination

Measure the amount of protein in the lysate or purified enzyme using the Bicinchoninic Acid Kit for Protein Determination.

Assay Procedure

1. For Sample (S), add 1-5 µL of lysate into desired well(s) in a 96-well plate. If necessary, dilute the lysate with ACE2 Lysis Buffer.
2. For Background Control (BC), add the same volume of Lysis Buffer as used in the Sample wells (Step 1).
3. For the Positive Control (PC), add 2 µL of the diluted ACE2 Positive Control into desired well(s).
4. For the Negative Control (NC), add 2 µL of the diluted ACE2 Inhibitor to the wells containing Sample and/or ACE2 Positive Control.
5. Adjust the volume of S, BC, NC and PC to 50 µL /well with ACE2 Assay Buffer.
6. Mix well and incubate for 15 minutes at room temperature.

ACE2 Substrate Mix

Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of ACE2 Substrate Mix according to Table 2.

Table 2.
Preparation of ACE2 Substrate Mix

Reagent	ACE2 Substrate Mix
ACE2 Assay Buffer	48 µL
ACE2 Substrate	2 µL

Mix thoroughly. Add 50 µL of ACE2 Substrate Mix into each of the S, BC, PC and NC wells. Do not add Substrate Mix to the Standard wells. Mix well.

Measurement

Measure fluorescence at λ_{Ex} = 320 nm/λ_{Em} = 420 nm in kinetic mode for 30 minutes to two hours at room temperature. Choose any 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 ΔRFU/ΔT.

Results

1. Subtract 0 Standard reading from all readings.
2. Plot the MCA-Standard Curve and obtain the slope of the curve (ΔRFU/pmol).
3. If the Sample Background Control reading is significant, then subtract the Background Control reading from Sample readings.
4. To calculate the specific ACE2 activity of a Sample, subtract ΔRFU of the Negative Control (ΔRFUNC) from the Sample (ΔRFUS).

ACE2 Activity (pmol/minute/mg or mU/mg) =

$$\frac{B \times D}{\Delta T \times P}$$

where:
B = Released MCA in the Sample based on the standard curve slope (pmol)
ΔT = Reaction time (T_2 - T_1 in minutes)
P = Sample used (in mg)
D = Sample dilution factor (D = 1 when samples are undiluted)

Unit Definition: One unit of ACE2 activity is the amount of enzyme that catalyzes the release of 1 nmol of MCA per minute from the substrate under the assay conditions at room temperature.

Figure 1.
Typical MCA Standard Curve (0-300 pmol), error bars indicate SD (n=3).

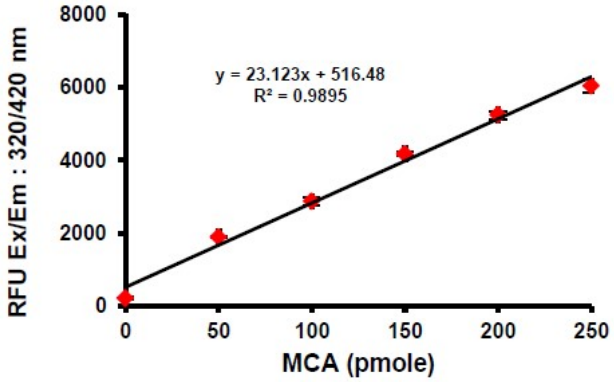


Figure 2.
Kinetic activity curves using different amounts of ACE2 Positive Control in the assay.

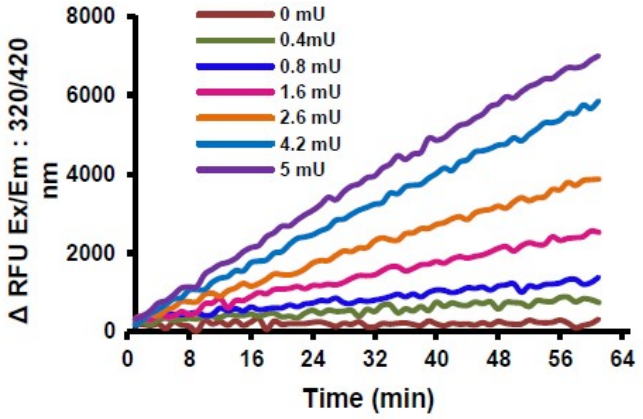


Figure 3.
ACE2 activity was measured for different types of rat tissue samples (total protein in lung and kidney; 17 µg and 23 µg respectively), and human kidney tissue sample (10 µg total protein) in presence (*+Inhibitor*) and absence (*-Inhibitor*) of ACE2 Inhibitor.

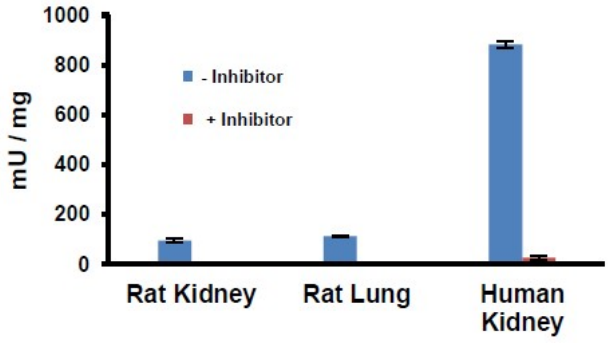
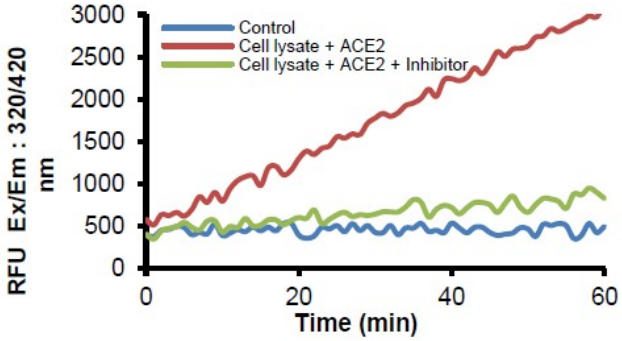


Figure 4.
Spiked ACE2 activity and inhibition measured in HEK293 cell lysate (total protein: 37 µg). Assays were performed following the kit protocol.



Related Products

- Angiotensin II Converting Enzyme (ACE2) Inhibitor Screening Kit, Catalog Number MAK378

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