Angiotensin II Converting Enzyme (ACE2) Activity Assay Kit (Fluorometric) *rev 10/19*

(Catalog # K897-100; 100 assays, Store kit at -20°C)

I. Introduction:

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. BioVision's ACE2 Activity Assay Kit kit will help the research progress in this field. This kit utilizes the ability of an active ACE2 to cleave a synthetic MCA based peptide substrate to release a free fluorophore. The released MCA can be easily quantified using a fluorescence microplate reader. We also provide an ACE2 specific inhibitor that can differentiate the ACE2 activity from other proteolytic activity.The kit can detect as low as 0.4 mU. Our assay kit is simple and can be used in a high-throughput format.

MCA-ACE2 Substrate $\xrightarrow{\textbf{ACE2}}$ Cleaved ACE2 Substrate + MCA (Ex/Em= 320/420 nm)

II. Applications:

- Detection of ACE2 activity in tissue/cell lysates
- Determination of enzymatic activity of purified ACE2

III. Sample Type:

- Animal tissues: Lung, heart, kidney
- Overexpressed ACE2 in cell lysates

IV. Kit Contents:

Components	K897-100	Cap Code	Part Number
ACE2 Assay Buffer	25 ml	WM	K897-100-1
ACE2 Dilution Buffer	1.5 ml	Clear	K897-100-2
ACE2 Lysis Buffer	50 ml	NM	K897-100-3
ACE2 Positive Control	5 µl	Green	K897-100-4
ACE2 Substrate	200 µl	Brown	K897-100-5
ACE2 Inhibitor (22 mM)	50 µl	Blue	K897-100-6
MCA-Standard (1 mM)	15 µl	Yellow	K897-100-7

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Multi-well fluorescence microplate reader.
- BCA Protein Assay Kit - Reducing Agent Compatible (BioVision Cat# **K818, K819** or equivalent).

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the assay**.**

- **ACE2 Assay Buffer, ACE2 Lysis Buffer & ACE2 Dilution Buffer**: Ready to use. Store at 4°C or -20°C. Bring to room temperature (RT) before use.
- **ACE2 Positive Control**: Store at -20°C. Before use, add 95 µl of ACE2 Dilution Buffer to the ACE2 Positive Control vial. Avoid multiple freeze/thaw of the enzyme. Use within 3 months.
- **ACE2 Substrate**: Ready to use. Store at -20°C. Thaw before use.
- **ACE2 Inhibitor**: Store at -20°C. Bring the ACE2 Inhibitor and the ACE2 assay buffer to room temperature before use. Add 170 µl ACE2 Assay Buffer to the ACE2 Inhibitor vial, mix properly at RT. Avoid multiple freeze/thaw of the inhibitor. Use within 3 months.
- **MCA Standard (1 mM)**: Store at -20°C. Thaw before use.

VII. ACE2 Activity Assay Protocol:

1. **Sample Preparation:** Homogenize tissue (~100 mg) or pelleted cells (1-2 x 10^6) with 400 µl ACE2 Lysis Buffer using a Dounce homogenizer, keep on ice for 10 min. Vortex gently for 10 s, and keep on ice for another 5 min. Centrifuge the homogenate at 16,000 x g, 4°C for 10 min. Discard the pellet.

   Protein concentration measurement: Transfer the clarified supernatant to a clean pre-chilled tube and keep on ice. Measure the amount of protein in the lysate or purified enzyme using BCA Protein Assay Kit, Reducing Agent Compatible (BioVision Cat # K818, K819 or equivalent).
2. **Assay Procedure**: 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. For Background Control (BC), add same volume of lysis buffer. For Positive Control (PC), add 2 µl of the diluted ACE2 Positive Control into desired well(s). For Negative Control (NC), add 2 µl of the diluted ACE2 Inhibitor to the wells containing Sample and/or ACE2 Positive Control. Adjust the volume of S, BC, NC and PC to 50 µl/well with ACE2 Assay Buffer. Mix well, incubate for 15 min. at RT.

   Notes: We recommend using the tissue/cell homogenate immediately to measure the ACE2 activity. If desired, snap freeze the sample lysate and store at -80°C. Unused diluted ACE2 Positive Control can be stored at -20°C in small aliquots.

3. **MCA-Standard Curve Preparation:** Prepare 25 µM solution of MCA-Standard by diluting 5 µl of 1 mM MCA-Standard with 195 µl of ACE2 Assay Buffer. Add 0, 2, 4, 6, 8 and 10 µl of 25 µM MCA-Standard into a series of wells in a 96-well plate and adjust the final volume to 100 µl/well with ACE2 Assay Buffer. This will generate 0, 50, 100, 150, 200 and 250 pmol/well of MCA Standard respectively. Mix well and measure the fluorescence (Ex/Em = 320/420 nm) in an end point mode.
4. **MCA Substrate Mix:** Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of the Substrate Mix:

   48 µl ACE2 Assay Buffer
   2 µl ACE2 Substrate

   Mix & add 50 µl of ACE2 Substrate Mix into each of S, BC, PC and NC wells. Mix well.

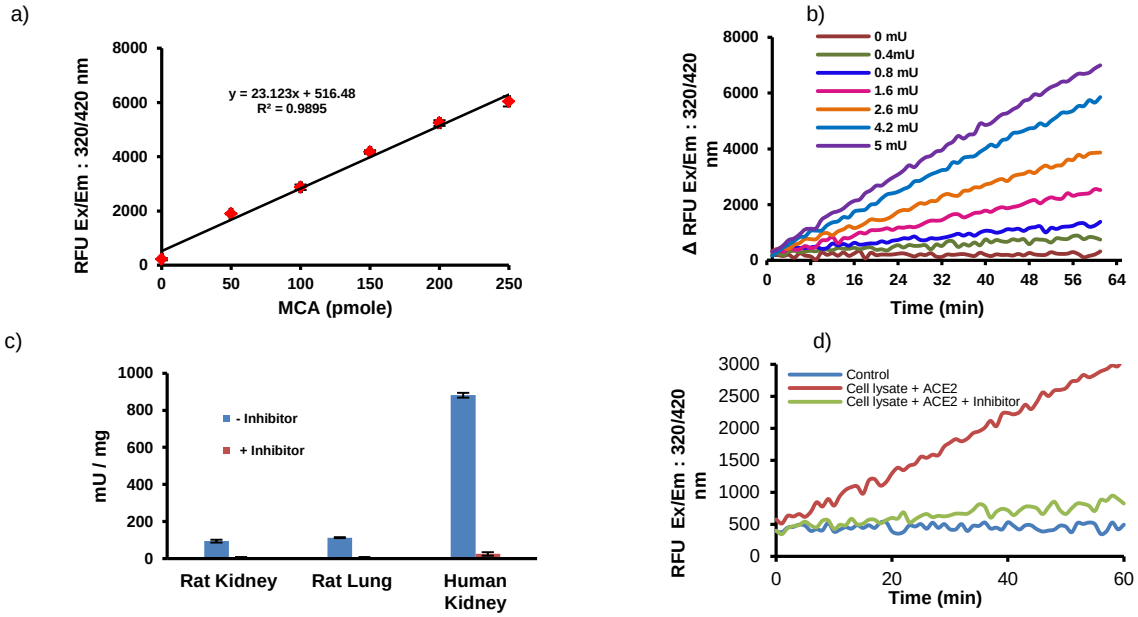
   Note: Don't add Substrate Mix to the Standard wells.
4. **Measurement:** Measure fluorescence (Ex/Em = 320/420 nm) in a kinetic mode for 30 mins to 2 hr at RT. Choose any two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 and RFU_2). Calculate ΔRFU/ΔT.
5. **Calculation**: Subtract 0 Standard reading from all readings. Plot the MCA-Standard Curve and obtain the slope of the curve (ΔRFU/pmol). If Sample Background Control reading is significant then subtract the Background Control reading from Sample readings. To calculate the specific ACE2 activity of Sample, subtract ΔRFU of Negative Control (ΔRFU_{NC}) from Sample (ΔRFU_S).

$$\textbf{Sample ACE2 Activity} = \mathbf{B \times D / (\Delta T * P)} = \mathbf{pmol/min/mg} = \mathbf{mU/mg}$$

Where:

B = Released MCA in Sample based on the Std. curve slope (pmol)
ΔT = Reaction time (t_2-t_1 in min)
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 min from the substrate under the assay conditions at RT.



Figures: (a) MCA Standard Curve (0-300 pmol), error bars indicate SD (n=3). (b) Kinetic activity curves using different amounts of ACE2 Positive Control in the assay. (c) 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. d) Spiked ACE2 activity and inhibition measured in HEK293 cell lysate (total protein: 37 µg). Assays were performed following the kit protocol

VIII. RELATED PRODUCTS:

ACE antibody (CT) (6703)
Angiotensin II, human (4917)
AGT antibody (NT) (6709)
ACE2 (human) ELISA Kit (K4918)
Angiotensin I converting enzyme (ACE1) Inhibitor Screening kit (K228)
Angiotensin II converting enzyme (ACE2) Inhibitor Screening Kit (K310)
Renin Activity Fluorometric assay kit (K800)
Renin Inhibitor Screening Kit (Fluorometric) (K799)
Angiotensin I converting enzyme (ACE1) Activity assay kit (K227)

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