MA-0158

Angiotensin II Converting Enzyme Activity Assay

(100 wells, Fluorometric, Ex/Em = 320/420 nm; Store at -20°C)

Introduction:

Angiotensin II converting enzyme (ACE2) is part of the renin-angiotensin system (RAS). It plays a pivotal role in both blood pressure regulation and entry of coronavirus. It cleaves Angiotensin II converting it into Angiotensin 1-7. Whereas Angiotensin II is a vasoconstritor, Angiotensi 1-7 is a vasodilator, so the action of ACE-2 can exert significant control over blood pressure. ACE2 is a known receptor of human coronaviruses, such as SARS. It is a cell-surface protein, expressed in vascular endothelial cells of lung, kidney and heart. ACE2 is a possible therapeutic target for cardiovascular and coronavirus-induced diseases. AkrivisBio's ACE2 Activity Assay is a simple sensitive means of monitoring ACE-2 activity. We utilize a synthetic peptide substrate which releases MCA upon ACE2 based cleavage. A specific ACE-2 inhibitor is included allowing for differentiation of ACE2 activity from other proteolytic activities which may recognize the peptide substrate.

Assay Principle:

1 – ACE-2 cleaves the peptide substrate at the Pro-Lys bond, separating MCA from the quenching effect of DNP which is attached to the other end of the molecule.
2 – Free MCA fluoresces strongly at Excitation 320 nm; Emission 420 nm.

Assay Components:

Assay Buffer	25 ml	WM	MA-0158A
Enzyme Dilution Buffer	1.5 ml	Clear	MA-0158B
Cell Lysis Buffer	50 ml	NM	MA-0158C
ACE2 Positive Control	5 µl	Green	MA-0158D
Peptide Substrate	200 µl	Brown	MA-0158E
DX600 Inhibitor	50 µl	Blue	MA-0158F
MCA Standard (10 µM)	15 µl	Yellow	MA-0158G

Storage and Handling Considerations:

Store unopened assay at -20°C. Centrifuge all small vials briefly before opening the vials. Bring all assay components to room temperature before use.
Assay Buffer, Cell Lysis Buffer & Enzyme Dilution Buffer: Ready to use as supplied. Store at 4°C.
ACE2 Positive Control: Dilute to the working concentration by adding 195 µl of Enzyme Dilution Buffer to the ACE2 Positive Control. Aliquot into convenient portions and store at -20° to avoid multiple freeze/thaw cycles.
Peptide Substrate: Ready to use as supplied. Store at -20°C.
DX600 Inhibitor: Store at -20°C. Add 170 µl ACE2 Assay Buffer to the DX600 Inhibitor and mix well.
MCA Standard: Ready to use as supplied. Store at -20°C.

Assay Protocol:

1. MCA-Standard Curve: Transfer 0 – 5 – 10 – 15 – 20 – 25 µl of 10 µM MCA Standard into a series of wells in a 96-well plate and adjust the well volumes to 100 µl with Assay Buffer, giving 0, 50, 100, 150, 200 and 250 pmol of MCA respectively. Measure fluorescence (Ex/Em = 320/420 nm) in end point mode.

2. Sample Preparation: Homogenize tissue (10 mg) or cells (10^6) with 200 µl Cell Lysis Buffer. Place on ice for 10 minutes, vortex gently for 10 seconds then place back on ice for an additional 5 minutes. Centrifuge at 16,000 x g, 4°C for 10 minutes and transfer the clear supernatant to a fresh tube.
Optional protein concentration measurement: Determine the protein concentration in the lysate or purified enzyme using Nanodrop or comparable spectrophotometer or a BCA Protein Assay.

3. Samples: Add 1-5 µl of clear supernatant into wells of a 96-well plate.

4. Positive Control: Add 1-2 µl of the diluted ACE2 Positive Control into desired well(s). Optionally dilute a portion 5-10X and add a larger, more controllable volume

5. Negative Control: Add 2 µl of the diluted DX600 Inhibitor to he wells containing Sample and/or ACE2 Positive Control. Adjust the volume of all Sample, Positive Control and Negative Control wells to 50 µl with Assay Buffer. Allow inhibitor binding to occur for 15 minutes at RT.

Note: Enzyme activity is labile. If samples cannot be assayed immediately, the should be snap frozen and held at -80°C until measurement is possible.

6. Initiate Reaction: Each Sample, Positive and Negative Control well will require 50 µl of Rection Mix. Prepare sufficient reagent for the number of wells being run, containing:

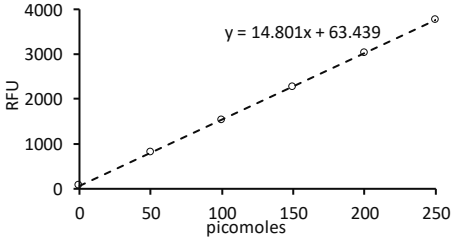
Assay Buffer	48 µl
Peptide Substrate	2 µl

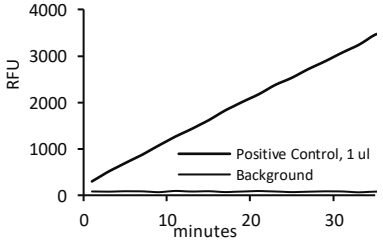
Mix and add 50 µl of Reaction Mix to each Sample, Positive and Negative Control well.

4. Measurement: Measure fluorescence (Ex/Em = 320/420 nm) in kinetic mode for 30 minutes to 2 hours at RT, depending upon the amount of enzyme activity observed. The enzyme activity should be linear at least until about 200-250 pmoles of substrate have been hydrolyzed. Beyond that, decreasing substrate concentration will give a noticeable bend to the enzyme activity.

5. Typical Results:

pmole Standard	Standard Raw Values RFU	Background Corrected Values RFU
0	66.8	0
50	810.4	743.7
100	1536.8	1469.9
150	2269.2	2202.4
200	3028.2	2961.5
250	3769.9	3703.2





6. Calculation: Subtract the zero Standard from all standard, sample, Positive and negative Control readings. Plot the Standard Curve and obtain the slope of the curve (ΔRFU/pmol). This defines the sensitivity of the system. Determine the slope of the various Samples and Controls (RFU/minute). Divide the Sample and Control well slopes by the slope of the Standard curve to convert to picomoles per minute. To convert back to activity in the original samples, divide the pmol/minute in the well by the volume of sample added to the well, giving pmole/min/µl of sample. Multiply that by the total volume of the clear supernatant recovered in step 2 above, then divide by the amount of tissue or # of cells used per sample.

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