Version 5b Last updated 12 March 2026

ab273297 Angiotensin II Converting Enzyme (ACE2) Activity Assay Kit (Fluorometric)

View Kit datasheet: https://www.abcam.com/ab273297
(use https://www.abcam.cn/ab273297 for china, or
https://www.abcam.co.jp/ab273297 for Japan)

For the determination of ACE2 activity in cell and tissue lysates and of purified ACE 2 enzyme.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Angiotensin II Converting Enzyme (ACE2) Activity Assay Kit (Fluorometric) (ab273297) 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. The assay kit is simple and can be used in a high-throughput format.

2. Protocol Summary

Prepare lysates as directed and measure protein concentration

Prepare all reagents as directed

Prepare standard curve and measure fluorescence at Ex/Em = 320/420 nm. Calculate slope

Add Positive Control, Samples, Negative Control, Background Control to appropriate wells and adjust volume to 50 µL

Add Substrate Mix (50 µL)

Measure fluorescence (Ex/Em = 320/420 nm) in kinetic mode for 30 minutes to 2 hours at room temperature.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website https://www.abcam.com/en-us.

4. Storage and Stability

Store kit at -20°C in the dark immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
Assay Buffer 11	25 mL	-20°C
ACE2 Dilution Buffer	1.5 mL	-20°C
ACE2 Lysis Buffer	50 mL	-20°C
ACE 2 Enzyme	20 µL	-20°C
ACE2 Substrate	200 µL	-20°C
ACE2 Inhibitor	50 µL	-20°C
MCA Standard	25 µL	-20°C

PLEASE NOTE: Assay Buffer 11 was previously labelled as Assay Buffer XI and ACE2 Assay Buffer, and ACE 2 Enzyme as ACE2 Enzyme and ACE2 Positive Control. The composition has not changed.

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence microplate reader capable of measuring fluorescence at Ex/Em = 320/420 nm
- 96-well black plate with flat bottom
- BCA protein assay (reducing agent compatible)

8. Technical Hints

- **This kit is sold based on number of tests. A "test" simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 **Assay Buffer 11:**

Store at -20 °C. Bring to room temperature before use.

9.2 **ACE2 Dilution Buffer:**

Store at -20 °C. Bring to room temperature before use.

9.3 **ACE2 Lysis Buffer:**

Store at -20 °C. Bring to room temperature before use.

9.4 **ACE 2 Enzyme:**

Store at -20°C. Before use, add 95 µL of ACE2 Dilution Buffer to the ACE 2 Enzyme vial. Avoid multiple freeze/thaw of the enzyme. Use within 3 months.

Δ Note: Unused diluted ACE 2 Enzyme can be stored at -20°C in small aliquots.

9.5 **ACE2 Substrate:**

Ready to use. Store at -20°C. Thaw before use.

9.6 **ACE2 Inhibitor:**

Store at -20°C. Bring the ACE2 Inhibitor and the Assay Buffer 11 to room temperature before use. Add 170 µL Assay Buffer 11 to the ACE2 Inhibitor vial, mix properly at RT. Avoid multiple freeze/thaw of the inhibitor. Use within 3 months.

9.7 **MCA Standard:**

Store at -20°C. Thaw before use.

10. Sample Preparation

10.1 **Tissue/cell lysate preparation:**

10.1.1 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.

10.1.2 Vortex gently for 10 seconds, and keep on ice for another 5 min.

10.1.3 Centrifuge the homogenate at 16,000 x *g*, 4°C for 10 min. Discard the pellet.

10.2 **Protein concentration measurement:**

10.2.1 Transfer the clarified supernatant to a clean pre-chilled tube and keep on ice.

10.2.2 Measure the amount of protein in the lysate or purified enzyme using a BCA Protein Assay Kit that is reducing agent-compatible.

Δ Note: 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.

11. Standard Curve

11.1 Prepare a 25 µM solution of MCA-Standard by diluting 5 µL of 1 mM MCA-Standard with 195 µL of Assay Buffer 11.

11.2 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 Assay Buffer 11. This will generate 0, 50, 100, 150, 200 and 250 pmol/well of MCA Standard respectively.

Standard #	25 µM MCA-Standard (µL)	Assay Buffer 11 (µL)	MCA (pmol/well)
1	10	90	250
2	8	92	200
3	6	94	150
4	4	96	100
5	2	98	50
6	0	100	0

11.3 Mix well and measure the fluorescence (Ex/Em = 320/420 nm) in an end point mode.

12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

12.1.1 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.

12.1.2 For Background Control , add same volume of lysis buffer.

12.1.3 For Positive Control, add 2 µL of the diluted ACE 2 Enzyme into desired well(s).

12.1.4 For Negative Control, add 2 µL of the diluted ACE2 Inhibitor to the wells containing Sample and/or ACE 2 Enzyme.

Δ **Note:** A negative control should be run for each sample to differentiate ACE2 activity from other proteolytic activity in your sample

12.1.5 Adjust the volume of Sample, Background Control, Negative Control and Positive Control to 50 µL/well with Assay Buffer 11. Mix well.

12.1.6 Incubate for 15 min. at room temperature.

MCA substrate mix:

12.1.7 Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of the Substrate Mix:

Component	Volume to add per well
Assay Buffer 11	48 µl
ACE2 Substrate	2 µl

12.1.8 Add 50 µL of ACE2 Substrate Mix into each of Sample, Background Control, Positive Control and Negative Control wells. Mix well.

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

12.1.9 Measure fluorescence (Ex/Em = 320/420 nm) in kinetic mode for 30 minutes to 2 hours at room temperature.

13. Calculations

13.1 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).

13.1 Calculate the slope for all samples, $\Delta RFU/\Delta t$.

13.2 Subtract 0 Standard reading from all Standards readings.

13.3 Plot the MCA-Standard Curve and obtain the slope of the curve (ΔRFU/pmol).

13.4 If Sample Background Control reading is significant then subtract the Background Control reading from Sample readings.

13.5 To calculate the specific ACE2 activity of Sample, subtract ΔRFU of Negative Control (ΔRFU_{NC}) from Sample (ΔRFU_S).

$$Sample\ ACE2\ activity\ = \frac{B\ xD}{(\Delta t\ x\ P)}\quad (pmol/min/mg)$$

B = Released MCA in Samples based on the Standard Curve slope (pmol)
Δt = Reaction time ($t_2 - t_1$)
P = Sample used (in mg)
D = Sample dilution factor (D = 1 for undiluted Samples)

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 room temperature.

14. Typical Data

Typical standard curve – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.

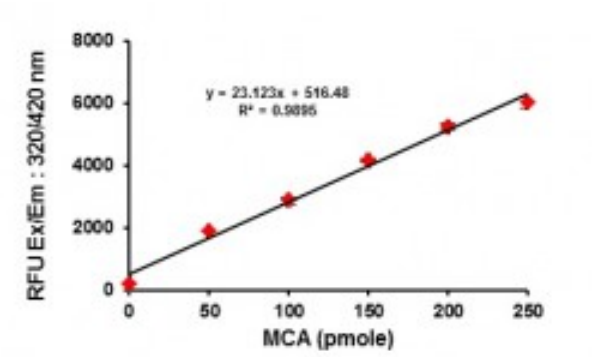

Figure 1. MCA Standard Curve (0-300 pmol), error bars indicate SD (n=3).

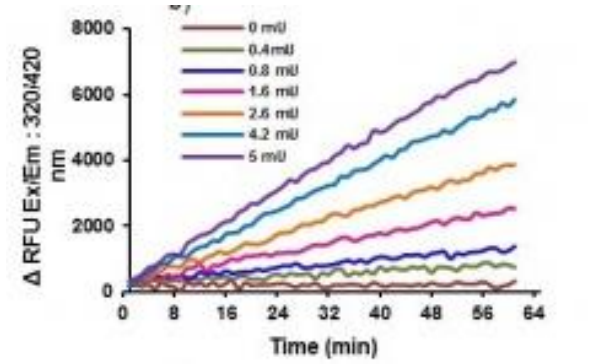

Figure 2. Kinetic activity curves using different amounts of ACE 2 Enzyme in the assay.

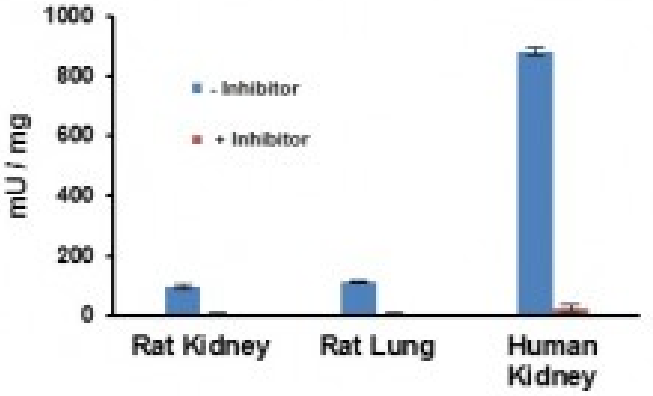

Figure 3. ACE2 activity was measured for different types of rat tissue samples (total protein in lung and kidney; 17 µg and 23 µg respectively), and human kidney tissue sample (10 µg total protein) in presence (+ Inhibitor) and absence (- Inhibitor) of ACE2 Inhibitor.

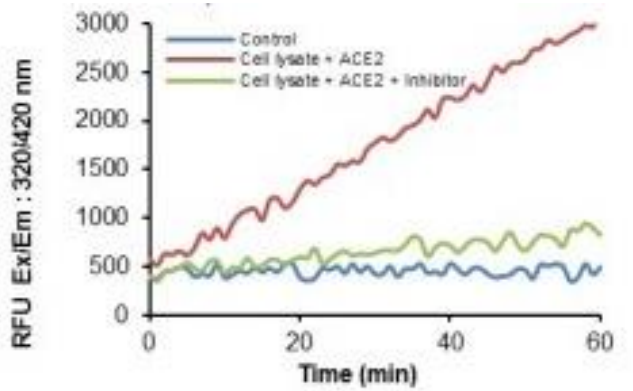

Figure 4 Spiked ACE2 activity and inhibition measured in HEK-293 cell lysate (total protein: 37 µg).

15. FAQ / Troubleshooting

General troubleshooting points are found at https://www.abcam.com/en-us/products/biochemical-assays.

16. Notes

Technical Support

For all technical or commercial enquiries please go to:

https://www.abcam.com/en-us/contact-us

https://www.abcam.cn/contact-us (China)

https://www.abcam.co.jp/contact-us (Japan)