Version 2 Last updated 6 November 2023

ab239703
ACE Assay Kit (Angiotensin I Converting Enzyme)

For the detection of ACE1 activity in tissue/cell lysates.

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

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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1. Overview

ACE Assay Kit (Angiotensin I Converting Enzyme) (ab239703) utilizes an active ACE1 to cleave a synthetic *o*-aminobenzoyl peptide (Abz-based peptide) substrate to release a fluorophore. The released Abz can be easily quantified using a fluorescence microplate reader. This assay kit is simple, rapid and can detect ACE activity as low as 10 mU in biological samples, such as lung, heart and kidney tissue, and serum and plasma. It can also be used to determine the enzymatic activity of purified ACE1.

2. Protocol Summary

Prepare samples and determine protein concentration.

Add samples and controls to the wells and adjust volume to 50 µL/well.

Prepare standard curve.

Prepare substrate mix and add 50 µL to each sample and positive control well.

Measure fluorescence (Ex/Em = 330/430 nm) in a kinetic mode for 1-2 hr at 37°C.

Choose two time points (T1 & T2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU1 and RFU2). Calculate ΔRFU/ΔT.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
ACE1 Assay Buffer	20 mL	-20°C
ACE1 Lysis Buffer	40 mL	-20°C
ACE1 Dilution Buffer	1 mL	-20°C
ACE1 Positive control	5 µL	-20°C
ACE1 Substrate	300 µL	-20°C
Abz Standard/Abz-Standard (1 mM)	100 µL	-20°C

5. Materials Required, Not Supplied

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

- 96-well white plate with flat bottom.
- Multi-well fluorescence microplate reader.
- ab207003 - BCA protein assay kit reducing agent compatible (microplate) or ab207004 - BCA protein assay kit reducing agent compatible (test tube)

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 ACE1 Assay Buffer:

Ready to use. Store at -20°C. Bring to room temperature (RT) before use.

6.2 ACE1 Lysis Buffer:

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

6.3 ACE1 Dilution Buffer:

Ready to use. Store at -20°C. Bring to room temperature (RT) before use.

6.4 ACE1 Positive control:

Store at -20°C. Avoid multiple freeze/thaw of the enzyme. Use within 6 months. Unused diluted ACE1 Positive Control can be stored at -20°C in small aliquots.

6.5 ACE1 Substrate:

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

6.6 Abz Standard/Abz-Standard (1 mM):

Ready to use. Store at -20°C.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Prepare 100 µM solution of Abz Standard/Abz-Standard by diluting 50 µL of Abz Standard/1 mM Abz-Standard with 450 µL of ACE1 Assay Buffer.

7.2 Add 0, 2, 4, 6, 8, and 10 µl of 100 µM Abz Standard/Abz-Standard into a series of wells in a 96-well plate and adjust the final volume to100 µL/well with ACE1 Assay Buffer to generate 0, 200, 400, 600, 800 and 1000 pmol/well of Abz Standard/Abz-Standard respectively.

7.3 Mix well and measure the fluorescence (Ex/Em = 330/430 nm) in an end point mode.

Standard #	Abz Standard/100 µM Abz-Standard (µL)	ACE1 Assay Buffer (µL)	Abz Standard/Abz-Standard /well
1	10	90	1000 pmol
2	8	92	800 pmol
3	6	94	600 pmol
4	4	96	400 pmol
5	2	98	200 pmol
6	0	100	0 pmol

8. Sample Preparation

8.1 Homogenize tissue (~100 mg) or pelleted cells (1-2 x 10^6) with 400 µL ACE1 Lysis Buffer using dounce homogenizer, keep on ice for 10 min.

8.2 Vortex gently for 10 s, and keep on ice for another 5 min.

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

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

8.5 Measure the amount of protein in the lysate or purified enzyme using ab207003 - BCA protein assay kit reducing agent compatible (microplate) or ab207004 - BCA protein assay kit reducing agent compatible (test tube).

Δ Note:

- We recommend using the tissue/cell homogenate immediately to measure the ACE1 activity. If desired, snap freeze the lysate and store at -80°C.
- Tissue or cell lysates of more than 2 µg of total protein/well might suppress the enzymatic activity of ACE1 with the provided substrate. In that case, dilute the sample with ACE1 Lysis Buffer and use 3-5 different amounts of the diluted lysate per well.
- Plasma or serum samples can be used directly in the assay. The volume used per well will need to be optimized by the user.
- Protein content should be measured.

9. Assay Procedure

9.1 Add 1-10 µL of lysate (maximum up to 2 µg of protein) into desired well(s) in a 96-well plate. If necessary, dilute the lysate or enzyme with ACE1 Lysis buffer.

9.2 For Background Control, add lysis buffer.

9.3 For Positive Control, add 95 µL of ACE1 Dilution Buffer to the ACE1 Positive control vial and use 10 µL of the diluted ACE1 Solution into desired well(s).

9.4 Adjust the volume of Samples, Background Control & Positive Control to 50 µL /well with ACE1 Assay Buffer.

9.5 Prepare the ACE1 Substrate Mix: Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of the Substrate Mix:

	Substrate Mix
ACE1 Assay Buffer	47 µL
ACE1 Substrate	3 µL

9.6 Mix & add 50 µL of ACE1 Substrate Mix into each sample, and Positive Control well. Mix well. Do not add to standard wells or background control wells.

9.7 Measure fluorescence (Ex/Em = 330/430 nm) in a kinetic mode for 1-2 hr at 37°C.

9.8 Choose two time points (T1 & T2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU1 and RFU2). Calculate $\Delta RFU/\Delta T$.

10. Data Analysis

10.1 Subtract 0 Standard reading from all readings. Plot the Abz Standard/Abz-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 reading.

$$\text{Sample ACE1 Activity} = B \times D / (\Delta T \times P) = \text{pmol/min/mg} = \text{mU/mg}$$

Where:

B = Abz in sample calculated from ΔRFU and Standard Curve

ΔT = Reaction time (min)

P = Sample used in the reaction well (in mg)

D = Sample dilution factor (D=1 when samples are undiluted)

Unit Definition: One unit of ACE1 activity is the amount of enzyme that catalyzes the release of 1 nmol of Abz per min from the substrate under the assay conditions at 37°C.

11. Typical Data

Typical data provided **for demonstration purposes only**.

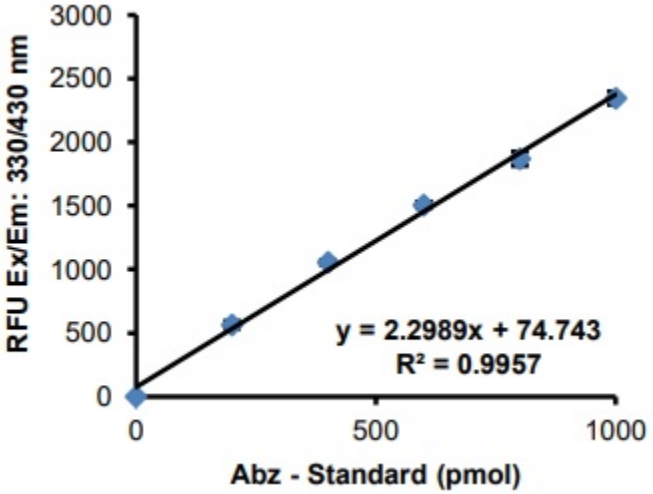

Figure 1. Abz Standard/Abz-Standard Curve (0-1000 pmol), error bars indicate SD (n=3).

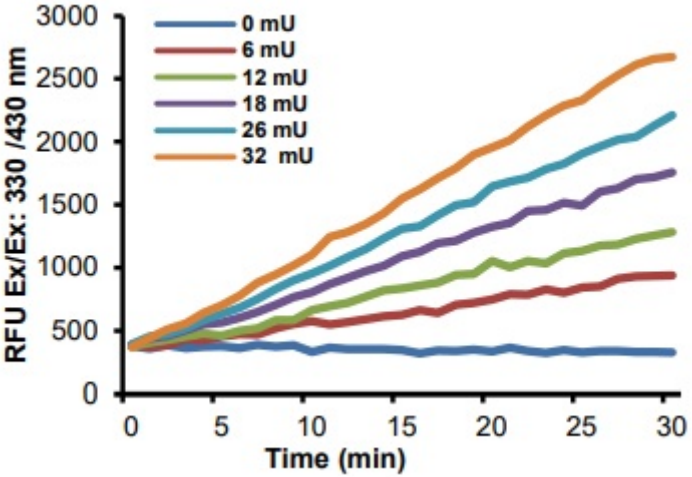

Figure 2. Kinetic activity curves using different amounts of ACE1 Positive Control in the assay.

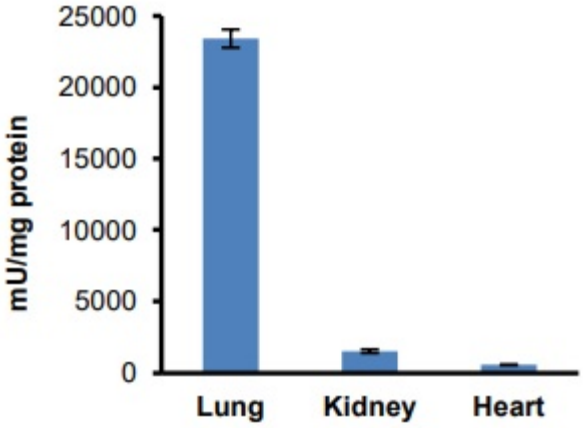

Figure 3. ACE1 activity was measured for different types of rat tissue samples (lung, heart and kidney; 0.6 µg each). Assays were performed following the kit protocol.

12. Notes

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