Angiotensin Converting Enzyme (ACE) Activity Assay Kit

Catalog No: E-BC-K003-S

Method: Colorimetric method

Specification: 100Assays (Can detect 98 samples without duplication)

Measuring instrument: Spectrophotometer

Sensitivity: 27.5 U/L

Detection range: 27.5- 682 U/L

- Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure angiotensin-converting enzyme (ACE) activity in serum (plasma) and animal tissue samples.

▲Background

Angiotensin-converting enzyme (ACE) is a Cl^- and Zn^{2+}-dependent carboxypeptidase, which catalyzes the hydrolysis of angiotensin I to produce angiotensin II with vasoconstriction effect or hydrolysis of bradykinin with vasoconstriction function to produce Phe-Arg dipeptide. ACE is an important part of the renin-angiotensin system (RAS). RAS is an endocrine system that plays an important role in electrolyte homeostasis, body fluid regulation and cardiovascular control.

▲Detection principle

N-[3-(2-furyl)acryloyl]-L-phenylalanylglycylglycine (FAPGG) have the maximum absorption peak at 340 nm, angiotensin converting enzyme catalyze N-(3[2-Furyl]Acryloyl)-Phe-Gly-Gly to produce FAP and GG, and the absorbance at 340 nm will be decreased. The activity of ACE can be calculated indirectly by measuring the decrease in absorbance at 340 nm.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Working Solution	55 mL×2 vials	2-8°C , 6 months

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.

▲Materials prepared by users

Instruments

Micropipettor, Vortex mixer, Incubator, Centrifuge, Spectrophotometer (340 nm)

Consumptive material

Tips (10 μL, 200 μL, 1000 μL), EP tubes (1.5 mL, 2 mL)

Reagents

Double distilled water, Normal saline

▲Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

▲Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲The key points of the assay

1. The temperature and the time of incubation should be accurate.
2. If the ACE activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (E-BC-K318-M, E-BC-K165-S, E-BC-K168-S).

Pre-assay preparation

▲Reagent preparation

Bring the reagent to room temperature before use.

▲Sample preparation

1. Serum sample:

   Fresh blood was collected and placed at 25°C for 30 min to clot the blood. Centrifuge the sample at 4°C for 15 min at 2000 g, the upper yellowish clear liquid was taken as serum. Place the serum on ice for detection.

2. Plasma sample:

   The fresh blood was added into the test tube containing anticoagulant (heparin is recommended) and mixed upside down. Centrifuge the sample at 4 °C for 10 min at 700~1000 g, the upper yellowish transparent liquid was taken as the plasma, and the middle white interference layer (white blood cells and platelets) could not be absorbed. Place the plasma on ice for detection.

3. Tissue sample:

   Take 0.02-1 g tissue sample, wash with normal saline at 2-8°C . Absorb the water with filter paper and weigh. Then add 9 times the volume of normal saline according to the ratio of Weight (g): Volume (mL) =1:9. Mechanical homogenate the sample in ice water bath. Centrifuge at 10000 g for 10 min, then take the supernatant and preserve it on ice for detection.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (27.5- 682 U/L).

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
Human serum	1
Mouse serum	1
Rat plasma	1
10% Rat liver tissue homogenate	1
10% Rat kidney tissue homogenate	1
10% Rat lung tissue homogenate	3-5

Note: The diluent is normal saline.

Assay protocol	
Ambient temperature	25-30°C
Optimum detection wavelength	340 nm

Instructions for the use of transferpettor:

(1) Equilibrate the pipette tip in that reagent before pipetting each reagent.

(2) Don't add the liquid outside the tips into the reaction system when pipetting each reagent.

▲Operating steps

1. Blank tube: add 1000 μL of working solution to the 2 mL EP tube.
   Sample tube: add 1000 μL of working solution to the 2 mL EP tube.
2. Blank tube: add 100 μL of double distilled water to the the tube.
   Sample tube: add 100 μL of sample to the tube.
3. Mix fully and incubate at 37°C for 1.5 min.
4. Set the spectrophotometer to zero with distilled water and measure the OD value at 340 nm with a 0.5 cm optical path cuvette. Then incubate the reaction solution at 37°C for 5 min and measure the OD value at 340 nm. The OD value of 0 min and 5 min were recorded as A_1 and A_2, respectively. $\Delta A = A_1 - A_2$.

▲Operation table

	Blank tube	Sample tube
Working solution (μL)	1000	1000
Double distilled water (μL)	100	
Sample (μL)		100
Mix fully and incubate at 37°C for 1.5 min. Set the spectrophotometer to zero with distilled water and measure the OD value at 340 nm with a 0.5 cm optical path cuvette. Then incubate the reaction solution at 37°C for 5 min and measure the OD value at 340 nm. The OD value of 0 min and 5 min were recorded as A_1 and A_2, respectively. $\Delta A = A_1 - A_2$.		

▲Calculation

1. Serum (plasma) sample:

Definition: The amount of 1 μmol of substrate catalyzed by 1 L of sample per minute at 37°C is defined as 1 unit.

$$\text{ACE activity (U/L)} = \left(\frac{\Delta A_{sample}}{\Delta T} - \frac{\Delta A_{blank}}{\Delta T}\right) \times \frac{1000}{\varepsilon \times d} \times \frac{V_{total}}{V_{sample}} \times f$$

2. Tissue sample:

Definition: The amount of 1 μmol of substrate catalyzed by 1 g of tissue protein per minute at 37°C is defined as 1 unit.

$$\text{ACE activity (U/gprot)} = \left(\frac{\Delta A_{sample}}{\Delta T} - \frac{\Delta A_{blank}}{\Delta T}\right) \times \frac{1000}{\varepsilon \times d} \times \frac{V_{total}}{V_{sample}} \div C_{pr} \times f$$

Note:

ε: The millimolar extinction coefficient of substrate at 340 nm with 1 cm optical path, 0.8 L/mmol/cm.

d: optical path of the quartz cuvette, 0.5 cm.

ΔT: The reaction time, 5 min.

1000: 1mmol=1000 μmol.

V_{total}: The total volume of reaction system, 1.1 mL.

V_{sample}: The volume of sample added into the reaction system, 0.1 mL.

C_{pr}: concentration of protein in sample, gprot/L.

f: Dilution factor of sample before tested.

▲Notes

1. This kit is for research use only.
2. Instructions should be followed strictly, changes of operation may result in unreliable results.
3. The validity of kit is 6 months.
4. Do not use components from different batches of kit.

Appendix I Performance characteristics

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Detection range	27.5- 682 U/L	Average inter-assay CV (%)	3
Sensitivity	27.5 U/L	Average inter-assay CV (%)	4.4
Average recovery rate (%)	103		

▲Example analysis

For rat lung tissue, dilute 10% rat lung tissue homogenate with normal saline for 4 times, take 100 μL of diluted sample and carry the assay according to the operation table. The results are as follows:

the average OD value of the sample (A_1) is 1.066, the average OD value of the sample (A_2) is 0.989, the average OD value of the blank (A_1) is 1.037, the average OD value of the blank (A_2) is 1.037, the concentration of protein in sample is 5.03 gprot/L, and the calculation result is:

$$\text{ACE activity (U/gprot)} = \left(\frac{1.066 - 0.989}{5} - \frac{1.037 - 1.037}{5}\right) \times \frac{1000}{0.8 \times 0.5} \times \frac{1.1}{0.1} \div 5.03 \times 4$$

$$= 336.78 \text{ U/gprot}$$