（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K003-M

Specification：48T(46 samples)/ 96T(94 samples)

Measuring instrument：Microplate reader (340 nm)

Detection range：25-620 U/L

Elabscience®Angiotensin-converting Enzyme (ACE1) Activity Colorimetric Assay Kit

This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tel: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

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

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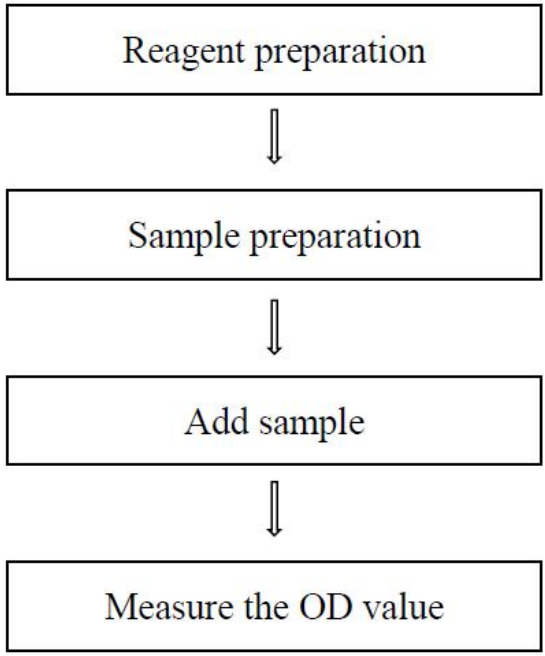
Assay summary



Intended use

This kit can be used to measure angiotensin converting enzyme (ACE1) activity in serum (plasma) and animal tissue samples.

Detection principle

N-(3-(2-furyl) acryloyl)-L-phenylalanyl-glycyl-glycine (FAPGG) have the maximum absorption peak at 340 nm. ACE1 catalyzes hydrolysis of FAPGG substrate to furanyl phenylalanine (FAP) and glycylglycine (GG), and the absorbance at 340 nm will be decreased. The activity of ACE1 can be calculated indirectly by measuring the decrease in absorbance at 340 nm.

Kit components & storage

Item	Component	Size 1 (48 T)	Size 2 (96 T)	Storage
Reagent 1	Working Solution	10 mL ×1 vial	20 mL × 1 vial	2-8°C, 12 months
	UV-Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		

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. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Microplate reader (340 nm)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

Equilibrate all the reagents to 25℃ before use.

Sample preparation

① Sample preparation

Serum and plasma: detect directly.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Wash tissue in cold normal saline (0.9% NaCl).

③ Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

④ Centrifuge at 10000×g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep it on ice for detection.

⑤ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② Dilution of sample

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 (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

The key points of the assay

① Reaction time and operation time must be strictly controlled.

② It's better to measure no more than 8 sample wells at same time.

Operating steps

① Blank well: add 20 μL of double distilled water to the well.
Sample well: add 20 μL of sample to the well.

② Add 180 μL of working solution to the the well.

③ Mix fully with microplate reader for 3 s. Incubate at 37°C for 90 s, and measure the OD value at 340 nm with microplate reader, as A_1. Incubate at 37°C for 5 min, and measure the OD value at 340 nm with microplate reader, as A_2, $\Delta A = A_1 - A_2$.

Calculation

The sample:

1. Serum (plasma) sample:

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

$$\underset{\text{U/L}}{\text{ACE1 activity}} = \left(\frac{\Delta A_{sample}}{\Delta T} - \frac{\Delta A_{blank}}{\Delta T}\right) \times \frac{1000}{\varepsilon \times d} \times \frac{V_{total}}{V_{sample}} \times f$$

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.

$$\underset{\text{U/gprot}}{\text{ACE1 activity}} = \left(\frac{\Delta A_{sample}}{\Delta T} - \frac{\Delta A_{blank}}{\Delta T}\right) \times \frac{1000}{\varepsilon \times d} \times \frac{V_{total}}{V_{sample}} \div C_{pr} \times f$$

[Note]

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

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

ΔT: The reaction time, 5 min.

1000: 1 mmol = 1000 μmol.

V_{total}: The total volume of reaction system, 0.2 mL.

V_{sample}: The volume of sample added into the reaction system, 0.02 mL.

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

f: Dilution factor of sample before tested.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	100.0	250.0	500.0
%CV	3.1	2.6	1.8

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	100.0	250.0	500.0
%CV	5.2	4.5	2.9

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 99.7%.

	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	100.0	250.0	500.0
Observed Conc. (U/L)	95.00	255.00	510.00
Recovery rate (%)	95.0	102.0	102.0

Sensitivity

The analytical sensitivity of the assay is 25 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Appendix II Example Analysis

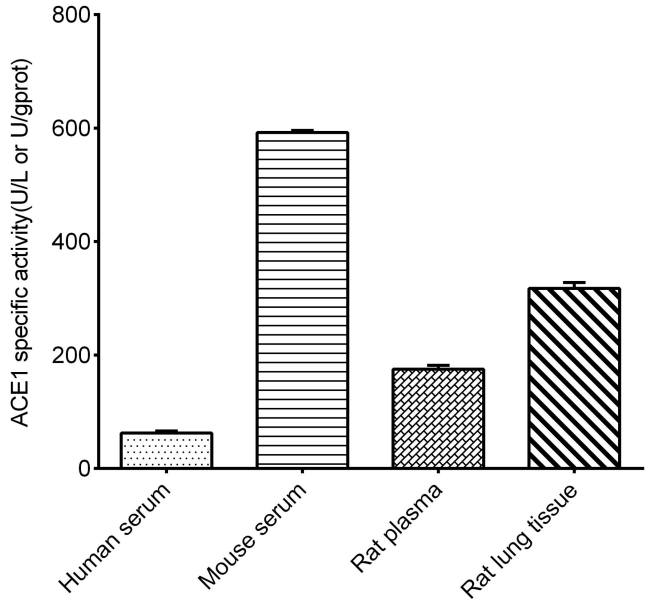
Example analysis：

Take 20 μL mouse serum sample into the well, and carry the assay according to the operation steps. The results are as follows:

The A_1 value of the blank well is 1.465, the A_2 value of the blank well is 1.465, the A_1 value of the sample well is 1.538, the A_2 value of the sample well is 1.419, and the calculation result is:

$$\text{ACE1 activity (U/L)} = \left(\frac{1.538 - 1.419}{5} - \frac{1.465 - 1.465}{5}\right) \times \frac{1000}{0.8 \times 0.5} \times \frac{0.2}{0.02} \times 1 = 595 \text{ U/L}$$

Detect human serum, mouse serum, rat plasma and 10% rat lung tissue homogenate (the concentration of protein is 5.03 gprot/L, dilute for 4 times) according to the protocol, the result is as follows：



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.