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

Catalog No：E-BC-F054

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Fluorescence Microplate reader

(Ex/Em = 325 nm/395 nm)

Detection range：0.028–10.47 U/L

Elabscience® Angiotensin I Converting Enzyme 2（ACE2）Activity Fluorescence Assay Kit

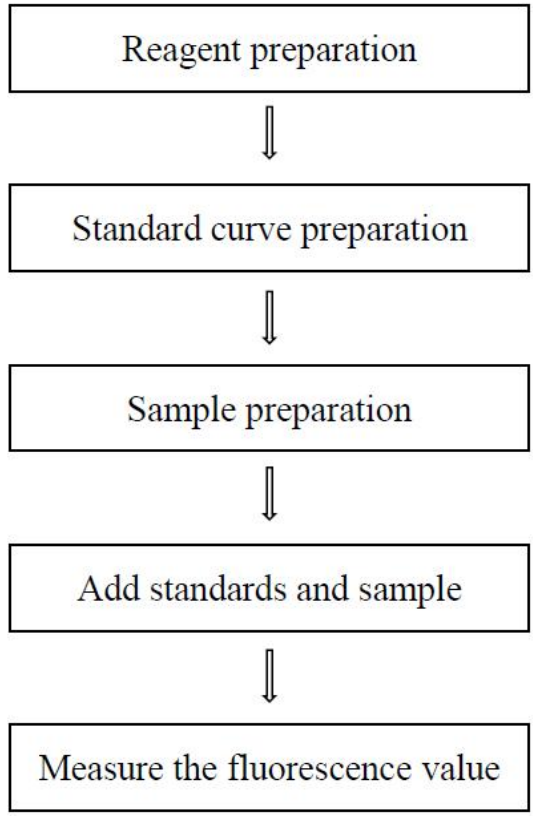
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

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.

Table of contents

Assay summary



Intended use

This kit can be used to measure angiotensin converting enzyme 2 (ACE2) activity in serum, plasma, animal tissue and cell samples.

Detection principle

Angiotensin converting enzyme 2 (ACE2) is an important component of the renin angiotensin system (RAS). ACE2 is a negative regulatory factor of RAS, which can balance multiple functions of ACE. By regulating angiotensin II, ACE2 can cleave angiotensin II into Ang1-7, to protect heart and relax blood vessels. It is also one of the key active receptors in the field of pharmaceutical science research. The principle of this kit is that ACE2 catalyzes the decomposition of substrates, releasing fluorescent products. The higher the fluorescence value, the higher the ACE2 activity in the sample. The ACE2 enzyme activity in the sample can be calculated by the standard curve.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	25 mL × 1 vial	50 mL × 1 vial	-20°C, 12 months
Reagent 2	Extraction Solution	40 mL × 1 vials	40 mL × 2 vials	-20°C, 12 months
Reagent 3	Substrate	0.1 mL × 1 vial	0.2 mL × 1 vial	-20°C, 12months, shading light
Reagent 4	Standard	0.3 mL × 1 vial	0.6 mL × 1 vial	-20°C, 12months, shading light
	Black 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:

Fluorescent Microplate reader (Ex/Em = 325 nm/393 nm)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all the reagents to room temperature before use.

② The preparation of substrate working solution：

Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 201 μL of substrate working solution (mix well 1 μL of substrate and 200 μL of buffer solution). Store at 2-8°C for 1 day.

③ The preparation of 100 μmol/L standard：

Dilute 20 μL of standard with 1980 μL of buffer solution. Store at 2-8°C for 2 days.

④ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 100 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 100, 80, 70, 60, 50, 40, 20, 0 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**20**	**40**	**50**	**60**	**70**	**80**	**100**
100 μmol/L standard (μL)	0	10	20	25	30	35	40	50
Buffer solution (μL)	50	40	30	25	20	15	10	0

Sample preparation

① Sample preparation

Serum (plasma) and urine samples: detect directly. If the sample is turbidity, centrifuge at 4°C 10000×g for 10 min, then take the supernatant for detection.

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 extraction solution 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).

Cells (adherent or suspension) samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Wash cells with normal saline (0.9% NaCl).

③ Homogenize 1×10^6 cells in 200 μL extraction solution with a ultrasonic cell disruptor at 4°C.

④ Centrifuge at 10000×g for 10 minutes 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
10% Mouse kidney tissue homogenization	2-8
10% Mouse lung tissue homogenization	1-5
10% Mouse heart tissue homogenization	1
10% Mouse liver tissue homogenization	1
1×10^6 293 cell	1
1×10^6 HL-60 cell	1-2
1×10^6 Hela cell	1-2

Note: The diluent is buffer solution. For the dilution of other sample types, please do pretest to confirm the dilution factor

The key points of the assay

① Substrate and substrate working solution should be protected from light during use and preparation.

② Choose appropriate dilution ratio according to the activity of ACE2 enzyme in different samples. The diluent is buffer solution.

③ When preparing standards with different concentrations, it can be properly mixed well.

④ After adding sample, it is recommend to mix well with microplate reader.

⑤ The reaction will start immediately after adding substrate. It is recommended to use the multichannel pipeter when the number of samples is large.

Operating steps

The measurement of samples

① Standard well: add 10 μL of standards with different concentrations to the corresponding well.

Sample well: add 10 μL of sample to the corresponding well.

② Add 90 μL of buffer solution into standard wells;

Add 90 μL of substrate working solution into sample wells.

③ Measure the fluorescence intensity of each well at the excitation wavelength of 325 nm and the emission wavelength of 395 nm immediately recorded as F_1.

④ Incubate at 37°C for 10 min. Measure the fluorescence intensity of each well at the excitation wavelength of 325 nm and the emission wavelength of 395 nm recorded as F_2.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean F_2 value of the blank (Standard #①) from all standard readings. This is the absoluted F_2 value.

3. Plot the standard curve by using absoluted F_2 value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

Definition: The amount of enzyme required for per liter of serum plasma to catalyze the substrate to produce 1 μmol of product per minute at 37°C is defined as 1 unit.

$$\text{ACE2 activity (U/L)} = (\Delta F_{sample} - b) \div a \div t \times f$$

2. Tissue and cells sample:

Definition: The amount of enzyme required for each gram of sample protein to catalyze the substrate to produce 1 μmol of product per minute at 37°C is defined as 1 unit.

$$\text{ACE2 activity (U/gprot)} = (\Delta F_{sample} - b) \div a \div t \div C_{pr} \times f$$

[Note]

ΔF_{sample}: The absolute fluorescence value of sample, $F_2 - F_1$.

t: the reaction time, 10 min.

f: Dilution factor of sample before tested.

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

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)	0.50	2.60	7.50
%CV	3.7	2.0	1.5

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)	0.50	2.60	7.50
%CV	11.8	9.0	4.4

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%.

	Standard 1	Standard 2	Standard 3
Expected Conc.(μmol/L)	25	55	75
Observed Conc. (μmol/L)	23.8	56.1	75.0
Recovery rate (%)	95	102	100

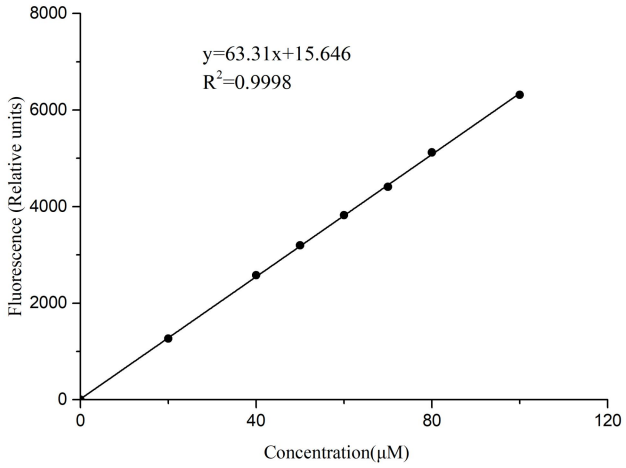
Sensitivity

The analytical sensitivity of the assay is 0.028 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.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only:

Concentration (μmol/L)	**0**	**20**	**40**	**50**	**60**	**70**	**80**	**100**
Fluorescence value (F_2)	165	1443	2863	3423	3960	4571	5263	6494
	175	1432	2631	3315	4024	4588	5320	6481
Average fluorescence value	170	1438	2747	3370	3992	4580	5292	6487
Absoluted fluorescence value	0	1268	2577	3200	3822	4410	5122	6317



Appendix II Example Analysis

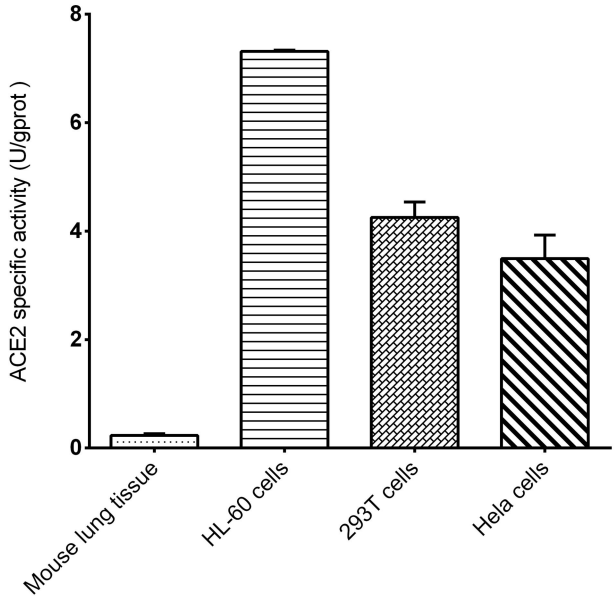
Example analysis:

Take 10% mouse lung tissue homogenate, and carry the assay according to the operation steps. The results are as follows:

standard curve: y = 75.669x + 77.011, the F_1 of the sample well is 329, the F_2 of the sample well is 1120, $\triangle F_{sample}$ = 1120 – 329 = 791, the concentration of protein in sample is 11.25 gprot/L, and the calculation result is:

$$\text{ACE2 activity (U/gprot)} = (1120 - 329 - 77.011) \div 75.669 \div 10 \div 11.5 \times 1 = 0.09 \text{ U/gprot}$$

Detect 10% mouse lung tissue homogenate (the concentration of protein is 9.47 gprot/L, dilution 2.5 times), 1×10^6 HL-60 cell (the concentration of protein is 1.12 gprot/L), 1×10^6 293T cell (the concentration of protein is 0.98 gprot/L) and 1×10^6 Hela cell (the concentration of protein is 1.47 gprot/L) 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.