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

Catalog No：E-BC-D016

Specification：48T/96T

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em = 320 nm/420 nm)

Elabscience® Angiotensin Converting Enzyme 1 (ACE1) Inhibitor Screening 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
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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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Intended use

This kit is used to screen samples of compounds acting on angiotensin converting enzyme 1 (ACE1) inhibitors.

Detection principle

Angiotensin Converting Enzyme 1(ACE1), also known as angiotensin converting enzyme (ACE), kininase II, and peptidy-carboxylpeptidase, is an important component of the renin-angiotensin system (RAS). In the RAS system, angiotensin is converted into angiotensin II. Angiotensin II can cause strong contractions of vascular smooth muscle, increasing peripheral vascular resistance and thereby leading to elevated blood pressure. Excessively high ACE1 activity can directly result in elevated blood pressure. ACE1 inhibitors can achieve the purpose of lowering blood pressure by inhibiting ACE1 activity.

The detection principle of this kit: ACE1 catalyzes the decomposition of the substrate, releasing fluorescent products. After adding the inhibitor, the generation of fluorescent substances will be inhibited, and the effect of the inhibitor is determined based on the degree of inhibition.

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 shading light
Reagent 2	Enzyme Reagent	Powder × 1 vial	Powder × 2 vials	-20°C, 12 months shading light
Reagent 3	Substrate	0.11 mL × 1 vial	0.22 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	10 mmol/L Enalapril	0.2 mL × 1 vial	0.2 mL × 1 vial	-20°C, 12 months shading light
	Black Microplate	96 wells		No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

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:

Fluorescence microplate reader (Ex/Em=320 nm/420 nm), Incubator

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of enzyme solution：

Dissolve one vial of enzyme reagent with 550 μL of double distilled water, mix well to dissolve. Store at -20°C for 7 days.

③ The preparation of enzyme working solution：

For each well, prepare 60 μL of enzyme working solution (mix well 50 μL of buffer solution and 10 μL of enzyme solution). The enzyme working solution should be prepared on spot and used up within 8 h.

④ The preparation of working solution：

Before testing, please prepare sufficient working solution according to the test wells. For example, prepare 255 μL of working solution (mix well 250 μL of buffer solution and 5 μL of substrate). The working solution should be prepared on spot and used up within 8 h.

⑤ The preparation of enalapril working solution：

The concentration of enalapril is 10 mmol/ L. When using the enalapril working solution, it should be diluted to the required concentration with double distilled water. (This reagent is an ACE1 inhibitor. As a positive control, the determination of the inhibition rate can be used as a reference.)

The key points of the assay

It is recommended to dilute the sample with double distilled water. If the water solubility of the sample is poor, DMSO can be prepared into a high-concentration solution and then diluted with double distilled water. The content of DMSO in the reaction system should be less than 5%.

Operating steps

① Blank well: Add 70 μL of buffer solution into blank wells.

Total enzyme well: Add 10 μL of buffer solution into total enzyme wells.

Positive control well: Add 10 μL of enalapril working solution into positive control wells.

Sample well: Add 10 μL of sample into the sample wells.

② Add 60 μL of enzyme working solution into total enzyme wells, sample wells and positive control wells.

③ Incubate at 37ºC for 20 min.

④ Add 100 μL of working solution into each well.

④ Mix fully for 5 s and incubate at 37ºC for 10 min. Measure the fluorescence intensity of each well at the excitation wavelength of 320 nm and the emission wavelength of 420 nm, as F.

Calculation

$$\text{Inhibition Rate (\%)} = (F_{total} - F_{sample}) \div (F_{total} - F_{blank}) \times 100\%$$

[Note]

F_{total}: The fluorescence intensity of total enzyme well.

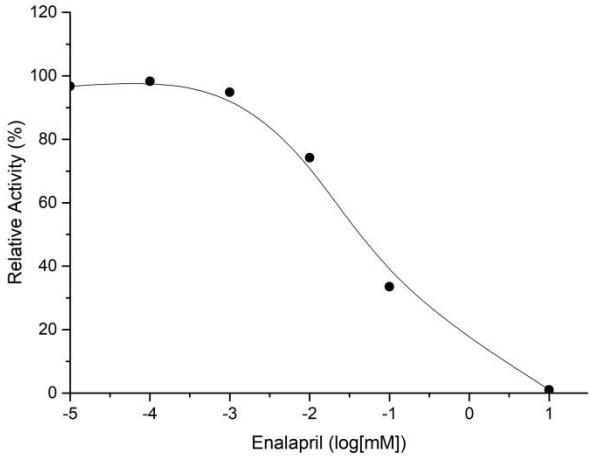
F_{sample}: The fluorescence intensity of sample well.

F_{blank}: The fluorescence intensity of blank well.

Appendix I Performance Characteristics

Inhibition curve

Effect diagram of angiotensin converting enzyme 1 (ACE1) inhibitor screening kit for detecting ACE1 inhibitor enalapril.



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.