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

Catalog No：E-BC-D001

Specification：96T

Measuring instrument：Fluorescence Microplate Reader(Ex/Em=325nm/395nm)

Elabscience®Angiotensin Converting Enzyme 2 (ACE2) 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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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 I converting enzyme 2 (ACE2) inhibitors.

Detection principle

Angiotensin converting enzyme (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. Adding inhibitors can inhibit the fluorescence value, and the inhibition ability of inhibitors can be determined by the fluorescence value.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL ×1 vial	-20ºC, 12 months
Reagent 2	Enzyme Reagent	0.15 mL ×1 vial	-20ºC, 12 months shading light
Reagent 3	Substrate	0.08 mL ×1 vial	-20ºC, 12 months shading light
Reagent 4	Inhibitors	Powder ×1 vial	-20ºC, 12 months shading light
	Black Microplate		No requirement
	Plate Sealer		

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=325 nm/395 nm)

Reagents:

DMSO

Reagent preparation

① Keep enzyme reagent on ice during use. Equilibrate other reagents to room temperature before use.

② The preparation of enzyme working solution：

For each well, prepare 45 uL of enzyme working solution (mix well 1 μL of enzyme reagent and 44 μL of buffer solution). The enzyme solution should be prepared on spot. Store at 2-8^{o}C for 1 day.

③ The preparation of substrate working solution：

Before testing, please prepare substrate working solution according to the test wells. For example, prepare 500 μL of substrate working solution (mix well 5 μL of substrate and 495 μL of buffer solution). The substrate working solution should be prepared on spot. Store at 2-8^{o}C for 1 day.

④ The preparation of 10mM inhibitor：

Dissolve one vial of inhibitors with 1.65 mL of DMSO. Mix well to dissolve. The prepared solution can be divided into smaller packages and stored at -20^{o}C for 1 week. (This specific inhibitor of ACE2 can be used according to experimental requirements.）

The key points of the assay

① The reagent preparation should be done with shading light, and enzyme reagent should be placed on ice for use.

② Enzyme reagent should be centrifuged for a few seconds before use and it should be stored at -20ºC after use. Mix enzyme working solution fully with vortex mixer for a few seconds, and the prepared enzyme working solution should be placed on ice box for use.

③ After adding sample, it is recommend to mix fully with microplate reader.

④ 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

① Blank well: add 5 μL of sample solvent into the blank wells.
Control well: add 5 μL of sample solvent into the control wells.
Sample well: add 5 μL of sample into the sample wells.

② Add 45 μL of buffer solution into blank wells; Add 45 μL of enzyme working solution into control wells and sample wells.

③ Add 50 μL of substrate working solution into each wells.

④ Incubate at 37ºC for 30 min. Measure the fluorescence intensity of each well at the excitation wavelength of 325 nm and the emission wavelength of 395 nm.

Calculation

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

[Note]

F_{sample}: The fluorescence intensity of sample well, when the sample has inhibitory activity, the fluorescence value is lower than the fluorescence value of the control well.

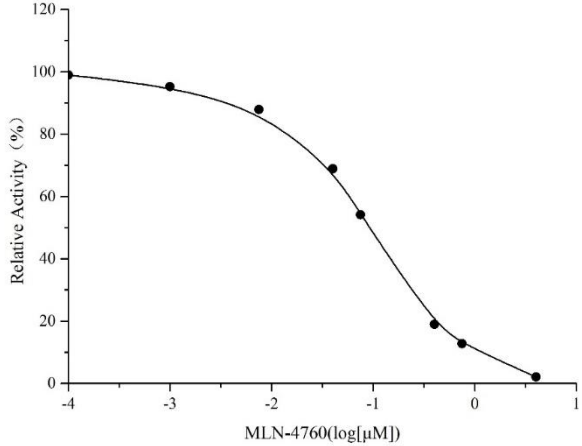
$F_{control}$: The fluorescence intensity of control well, equivalent to 100% enzyme activity.

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

Appendix I Performance Characteristics

Inhibition curve

Effect diagram of angiotensin converting enzyme 2 (ACE2) inhibitor screening kit for detecting ACE2 inhibitor MLN-4760



Appendix II Example Analysis

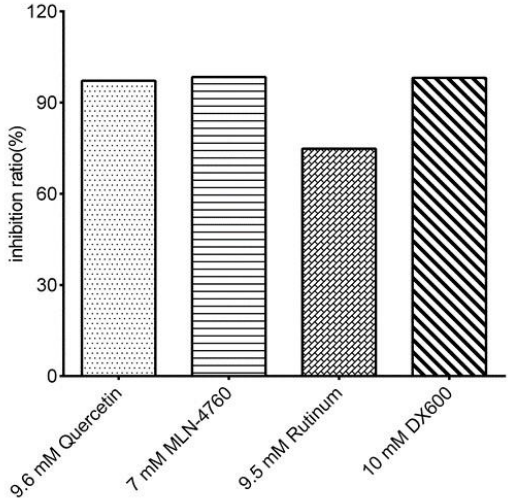
Example analysis:

For quercetin (the concentration is 9.6 mmol/L), and carry the assay according to the operation steps. The results are as follows:

the fluorescence value of the control ($F_{control}$) is 5977.31, the fluorescence value of the sample (F_{sample}) is 347.16, the fluorescence value of the blank (F_{blank}) is 183.18,and the calculation result is:

Inhibition Rate (%) = (5977.31 –347.16) ÷ (5977.31 -183.18) × 100% = 97.17%

Detect quercetin samples dissolved in DMSO (the concentration is 9.6 mmoL/L), MLN-4760 samples dissolved in DMSO (the concentration is 7 mmoL/L), rutinum samples dissolved in DMSO (the concentration is 9.5 mmoL/L) and DX600 samples dissolved in DMSO (the concentration is10 mmoL/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.