PRODUCT INFORMATION & MANUAL

ACE-2 Inhibitor Screening Assay Kit

NBP3-24468

For research use only.
Not for diagnostic or therapeutic procedures.

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ACE-2 Inhibitor Screening Assay Kit

Catalog No: NBP3-24468

Method: Fluorimetric method

Specification: 96T

Instrument: Fluorescence Microplate Reader

Average intra-assay CV (%): 0.8

Average inter-assay CV (%): 10.5

Verage recovery rate (%): 101

▲ This kit is for research use only.
▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
▲ 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 is used to screen samples of compounds acting on angiotensin converting enzyme 2 (ACE2) inhibitor.

▲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. 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	Specification	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20°C, 12 months
Reagent 2	Enzyme Reagent	0.15mL × 1 vial	-20°C, 12 months, shading light
Reagent 3	Substrate	0.08mL × 1 vial	-20°C, 12 months, shading light
Reagent 4	Inhibitors	Powder × 1 vial	-20°C, 12 months, 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.			

▲Materials prepared by users

Instruments

Fluorescence microplate reader (Ex/Em=325 nm/395 nm)

Reagents:

DMSO

⚠ 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 reagent preparation should be done with shading light, and reagent 2 should be placed on ice for use.
2. Reagent 2 should be centrifuged for a few seconds before use and it should be stored at -20°C after use. Mix reagent 2 working solution fully with vortex mixer for a few seconds, and the prepared reagent 2 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.

Pre-assay preparation

▲ Reagent preparation

1. Put reagent 2 on ice box for use and bring other reagents to room temperature before use.
2. Preparation of reagent 2 working solution:
   Dilute reagent 2 with reagent 1 at a ratio of 1:44. Prepare the fresh needed amount before use and the prepared solution can be stored at 2-8°C for 1 day.
3. Preparation of reagent 3 working solution:
   Dilute reagent 3 with reagent 1 at a ratio of 1:99. Prepare the fresh needed amount before use and the prepared solution can be stored at 2-8°Cfor 1 day.
4. Preparation of 10mM inhibitor:
   Dissolve a vial of reagent 4 with 1.65 mL of DMSO and mix fully. The prepared solution can be divided into smaller packages and stored at -20°C for 1 week. （This specific inhibitor of ACE2 can be used according to experimental requirements.）

▲ Sample preparation

It is recommended to use DMSO as the solvent to dissolve compound samples.

▲Detailed operating steps

The measurement of samples

(1) Blank well: Take 5 μL of sample solvent into the blank wells.

Control well: Take 5 μL of sample solvent into the control wells.

Sample well: Take 5 μL of sample into the sample wells .

(2) Add 45 μL of reagent 1 into blank wells; Add 45 μL of reagent 2 working solution into control wells and sample wells.

(3) Add 50 μL of reagent 3 working solution into each wells.

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

▲Summary operation table

	Blank well	Controlwell	Sample well
Sample solvent (μL)	5	5	
Sample (μL)			5
Reagent 1 (μL)	45		
Reagent 2 working solution (μL)		45	45
Reagent 3 working solution (μL)	50	50	45
Incubate at 37°C 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 Data

▲Example analysis

For quercetin (the concentration is 9.6 mmol/L), and carry the assay according to the operation table.

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%