TECHNICAL MANUAL

ACE2 Fluorometric Inhibitor Screening Kit

- **SKU CODE:** MAES0256
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

This kit is used to screen samples of compounds acting as angiotensin I converting enzyme 2 (ACE2) inhibitors.

2. 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, thereby protecting the heart and relaxing blood vessels. It is also one of the key active receptors in pharmaceutical science research.

The principle of this kit is that ACE2 catalyzes the decomposition of substrates, releasing fluorescent products. Adding inhibitors can suppress the fluorescence value, and the inhibition ability of inhibitors can be determined by measuring the fluorescence value.

3. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20 °C, 12 months
Reagent 2	Enzyme Reagent	0.12 mL × 1 vial	-20 °C, 12 months, shading light
Reagent 3	Substrate	0.08 mL × 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	

4. Materials prepared by users

Instruments:

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

Reagents:

DMSO

5. Reagent preparation

1. Keep enzyme reagent on ice during use. Equilibrate other reagents to room temperature before use.
2. **Preparation of enzyme working solution:** For each well, prepare 45 μL of enzyme working solution (mix well 1 μL of enzyme reagent and 44 μL of buffer solution). The enzyme solution should be prepared fresh. Store at 2-8 °C for 1 day.
3. **Preparation of substrate working solution:** Before testing, prepare substrate working solution according to the number of 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 fresh. Store at 2-8 °C for 1 day.
4. **Preparation of 10 mM inhibitor:** Dissolve one vial of inhibitors with 1.65 mL of DMSO. Mix well to dissolve completely. The prepared solution can be divided into smaller aliquots and stored at -20 °C for 1 week. (This specific inhibitor of ACE2 can be used according to experimental requirements.)

6. The key points of the assay

1. Reagent preparation should be performed in the dark, and enzyme reagent should be kept on ice during use.
2. Enzyme reagent should be centrifuged for a few seconds before use and stored at -20 °C after use. Mix enzyme working solution thoroughly with a vortex mixer for a few seconds, and keep the prepared enzyme working solution on ice during use.
3. After adding samples, it is recommended to mix thoroughly using the microplate reader.
4. The reaction will start immediately after adding substrate. It is recommended to use a multichannel pipette when testing a large number of samples.

7. 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 well.

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.

8. Calculation

Inhibition Rate (%) = ($F_{control}$ - F_{sample}) ÷ ($F_{control}$ - F_{blank}) × 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.

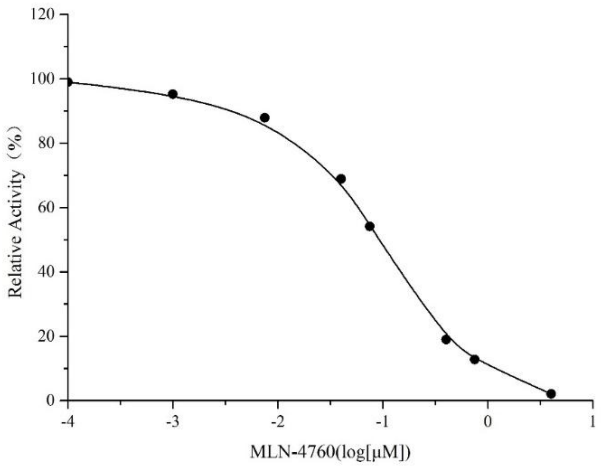
$F_{control}$: The fluorescence intensity of control well, equivalent to 100% enzyme activity.

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

9. Appendix I Performance Characteristics

Inhibition curve

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



10. Appendix II Example Analysis

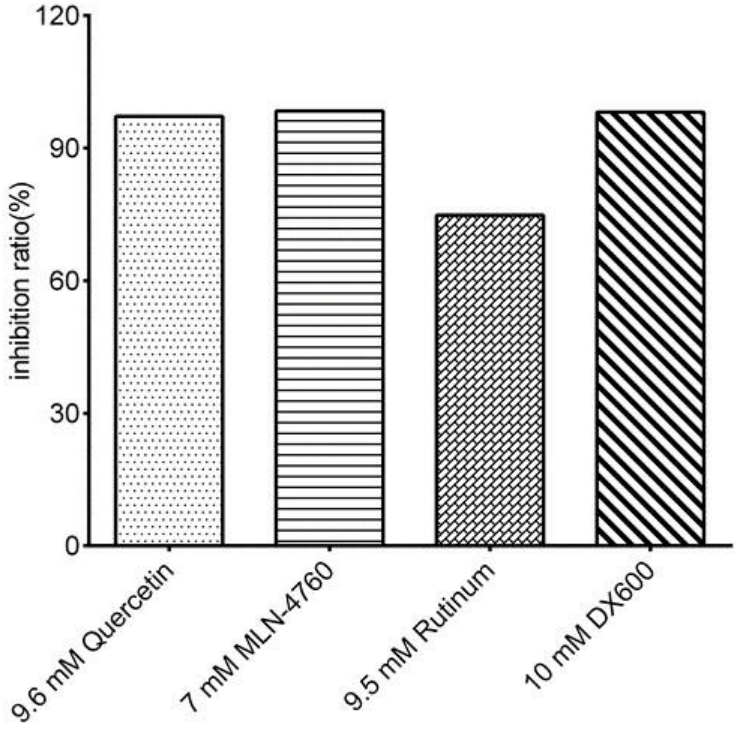
Example analysis:

For quercetin (concentration is 9.6 mmol/L), carrying out the assay according to the operating 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%

Detecting quercetin samples dissolved in DMSO (concentration is 9.6 mmol/L), MLN-4760 samples dissolved in DMSO (concentration is 7 mmol/L), rutinum samples dissolved in DMSO (concentration is 9.5 mmol/L) and DX600 samples dissolved in DMSO (concentration is 10 mmol/L) according to the protocol, the result is as follows:



11. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.
2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.
3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.
5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.
6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.