



## **TECHNICAL MANUAL**

# **Angiotensin Converting Enzyme 1 (ACE1) Inhibitor Screening Kit**

- **SKU CODE:** MAES0494
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

## 1. Intended use

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

## 2. Detection principle

Angiotensin Converting Enzyme 1 (ACE1), also known as angiotensin converting enzyme (ACE), kininase II, and peptidyl-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.

## 3. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	25 mL x 1 vial	50 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 2	Enzyme Reagent	Powder x 1 vial	Powder x 2 vials	-20 °C, 12 months, shading light
Reagent 3	Substrate	0.11 mL x 1 vial	0.22 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 4	10 mmol/L Enalapril	0.2 mL x 1 vial	0.2 mL x 1 vial	-20 °C, 12 months, shading light
	Black Microplate	96 wells	96 wells	No requirement
	Plate Sealer	2 pieces	2 pieces	

## 4. Materials prepared by users

### Instruments:

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

## 5. 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  $\mu\text{L}$  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  $\mu\text{L}$  of enzyme working solution (mix well 50  $\mu\text{L}$  of buffer solution and 10  $\mu\text{L}$  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  $\mu\text{L}$  of working solution (mix well 250  $\mu\text{L}$  of buffer solution and 5  $\mu\text{L}$  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.)

## 6. 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%.

## 7. Operating steps

1. Blank well: Add 70  $\mu\text{L}$  of buffer solution into blank wells. Total enzyme well: Add 10  $\mu\text{L}$  of buffer solution into total enzyme wells. Positive control well: Add 10  $\mu\text{L}$  of enalapril working solution into positive control wells. Sample well: Add 10  $\mu\text{L}$  of sample into the sample wells.

2. Add 60  $\mu\text{L}$  of enzyme working solution into total enzyme wells, sample wells and positive control wells.
3. Incubate at 37  $^{\circ}\text{C}$  for 20 min.
4. Add 100  $\mu\text{L}$  of working solution into each well.
5. Mix fully for 5 s and incubate at 37  $^{\circ}\text{C}$  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.

## 8. Calculation

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

[Note]

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

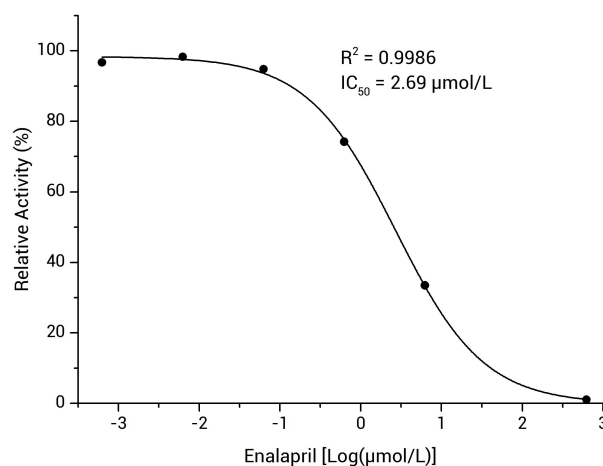
$F_{\text{sample}}$ : The fluorescence intensity of sample well.

$F_{\text{blank}}$ : The fluorescence intensity of blank well.

## 9. Appendix I Performance Characteristics

Inhibition curve

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



## 10. 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!**

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