TECHNICAL MANUAL

Angiotensin Converting Enzyme (ACE) Activity Assay Kit

- **SKU CODE:** MAES0334
- **SIZE:** 100 Assays
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Intended use

This kit can be used to measure angiotensin-converting enzyme (ACE1) activity in serum (plasma) and animal tissue samples.

2. Detection principle

N-[3-(2-furyl)acryloyl]-L-phenylalanylglycylglycine (FAPGG) has a maximum absorption peak at 340 nm. Angiotensin converting enzyme catalyzes N-(3[2-Furyl]Acryloyl)-Phe-Gly-Gly to produce FAP and GG, resulting in decreased absorbance at 340 nm. The activity of ACE1 can be calculated indirectly by measuring the decrease in absorbance at 340 nm.

3. Kit components & storage

Item	Component	Size 1 (50 Assays)	Size 2 (100 Assays)	Storage
Reagent 1	Working Solution	55 mL × 1 vial	55 mL × 2 vials	2-8°C, 12 months

4. Materials prepared by users

Instruments:

Micropipettor, Vortex mixer, Incubator, Centrifuge, Spectrophotometer (340 nm)

Reagents:

Double distilled water, PBS (0.01 M, pH 7.4)

5. Reagent preparation

Equilibrate all reagents to room temperature before use.

6. Sample preparation

Sample preparation

Serum and plasma: Detect directly. If not detected on the same day, the serum or plasma can be stored at -80°C for one month.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation: 20 mg).

2. Wash tissue in cold PBS (0.01 M, pH 7.4).

3. Homogenize 20 mg tissue in 180 µL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

4. Centrifuge at 10,000 × g for 10 min to remove insoluble material. Collect supernatant and keep it on ice for detection.

5. Meanwhile, determine the protein concentration of supernatant (MAES0177).

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Human serum: 1

Mouse serum: 1

Rat plasma: 1

10% Rat liver tissue homogenate: 1

10% Rat kidney tissue homogenate: 1

10% Rat lung tissue homogenate: 3-5

Note: The diluent is normal saline. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

7. The key points of the assay

1. The temperature and time of incubation should be accurate.
2. If the ACE1 activity is calculated by protein concentration, the protein concentration of the sample needs to be determined separately (MAES0177, MAES0401, MAES0402).

8. Operating steps

1. Blank tube: Add 1000 µL of working solution to the 2 mL EP tube. Sample tube: Add 1000 µL of working solution to the 2 mL EP tube.
2. Blank tube: Add 100 µL of double distilled water to the tube. Sample tube: Add 100 µL of sample to the tube.
3. Mix well and incubate at 37°C for 1.5 min.
4. Set the spectrophotometer to zero with distilled water and measure the OD value at 340 nm with a 0.5 cm optical path cuvette. Then incubate the reaction solution

at 37°C for 5 min and measure the OD value at 340 nm. The OD values at 0 min and 5 min were recorded as A1 and A2, respectively. ΔA = A1 - A2.

9. Calculation

The sample:

1. Serum (plasma) sample:

Definition: The amount of 1 μmol of substrate catalyzed by 1 L of sample per minute at 37°C is defined as 1 unit.

ACE1 activity (U/L) = (ΔA_sample/ΔT - ΔA_blank/ΔT) × 1000 / (ε × d) × V_{total}/V_{sample} × f

2. Tissue sample:

Definition: The amount of 1 μmol of substrate catalyzed by 1 g of tissue protein per minute at 37°C is defined as 1 unit.

ACE1 activity (U/gprot) = (ΔA_sample/ΔT - ΔA_blank/ΔT) × 1000 / (ε × d) × V_{total}/V_{sample} ÷ C_{pr} × f

[Note]

ε: The millimolar extinction coefficient of substrate at 340 nm with 1 cm optical path, 0.8 L/mmol/cm.

d: Optical path of the quartz cuvette, 0.5 cm.

ΔT: The reaction time, 5 min.

1000: 1 mmol = 1000 μmol.

V_{total}: The total volume of reaction system, 1.1 mL.

V_{sample}: The volume of sample added into the reaction system, 0.1 mL.

C_{pr}: Concentration of protein in sample, gprot/L.

f: Dilution factor of sample before tested.

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

Three human serum samples were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Intra-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	35.00	120.00	350.00
%CV	3.2	2.8	3.0

Inter-assay Precision

Three human serum samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	35.00	120.00	350.00
%CV	4.3	4.6	4.3

Recovery

Three samples of high, middle, and low concentrations were tested six times in parallel for each concentration to obtain an average recovery rate of 103%.

Recovery

Standard 1	Standard 2	Standard 3
80	230	600
80.8	241.5	618.0
101	105	103

Sensitivity

The analytical sensitivity of the assay is 27.5 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

11. Appendix II Example Analysis

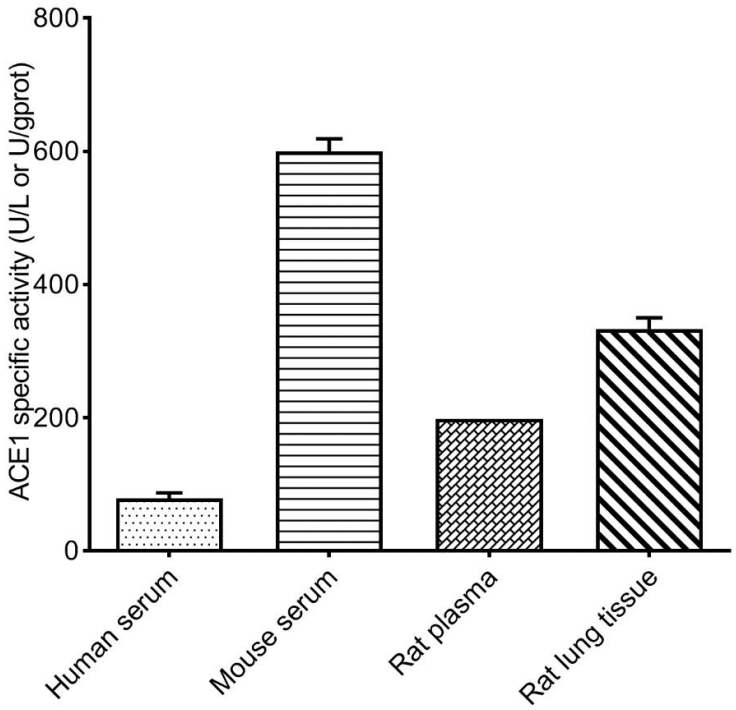
Example analysis:

For rat lung tissue, dilute 10% rat lung tissue homogenate with normal saline 4-fold. Take 100 μL of diluted sample and perform the assay according to the operating steps. The results are as follows:

The average OD value of the sample (A_1) is 1.066, the average OD value of the sample (A_2) is 0.989, the average OD value of the blank (A_1) is 1.037, the average OD value of the blank (A_2) is 1.037, the concentration of protein in sample is 5.03 gprot/L, and the calculation result is:

ACE1 activity (U/gprot) = ((1.066 - 0.989)/5 - (1.037 - 1.037)/5) × 1000/(0.8 × 0.5) × 1.1/0.1 ÷ 5.03 × 4 = 336.78 U/gprot

Detect human serum, mouse serum, rat plasma and 10% rat lung tissue homogenate (the concentration of protein is 5.03 gprot/L diluted 4-fold) according to the protocol. The result is as follows:



12. 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.