KAMIYA BIOMEDICAL COMPANY

# ACE Inhibition Screening Kit

**For the measurement of ACE inhibitory activity**

Cat. No. KT-534

**For Research Use Only. Not for Use in Diagnostic Procedures.**

# PRODUCT INFORMATION

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## PRODUCT
The **K-*ASSAY*®** ACE Inhibition Screening Kit is for the measurement of ACE inhibitory activity.

## BACKGROUND
Angiotensin-converting enzyme (ACE) is one of the vasopressor principle. ACE converts angiotensin I to angiotensin II, that has a vasopressor action, in the renin-angiotensin system and also inactivates bradykinin that is an antihypertensive peptide. Recently, modification of the conventional ACE inhibition assay procedure has been requested because of the use of harmful organic solvent such as ethyl acetate for the extraction of hippuric acid cleaved from Hippuryl-His-Leu by ACE and its complicated procedure.

## PRINCIPLE
The **K-*ASSAY*®** ACE Inhibition Screening Kit is a simple and convenient kit to measure the ACE inhibitory activity. 3-Hydroxybutyryl-Gly-Gly-Gly (3HB-GGG) is utilized as a substrate for ACE, and the amount of cleaved 3-hydroxybutyric acid (3HB) from 3HB-GGG is measured by the enzymatic method. Neither organic solvent nor extraction procedure is required through the whole procedure. In addition, since the **K-*ASSAY*®** ACE Inhibition Screening Kit is optimized for 96-well microplate assay, a large number of samples can be tested at once.

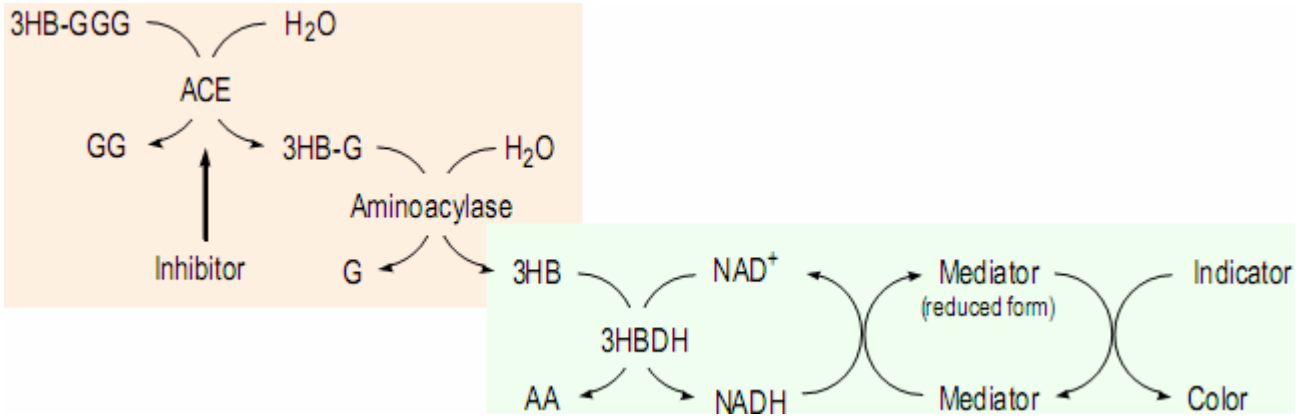


Figure 1. Principle of ACE Inhibitory activity assay using the **K-*ASSAY*®** ACE Inhibition Screening Kit

## COMPONENTS
- Substrate Buffer, 2 vials
- Enzyme A, 2 vials
- Enzyme B, 2 vials
- Enzyme C, 2 vials
- Coenzyme, 2 vials
- Indicator solution, 2 vials

## MATERIALS OR EQUIPMENT REQUIRED BUT NOT PROVIDED
- Microplate reader (450 nm filter).
- 96-well microplate.
- 2 - 20 µL, 20 - 200 µL & 100 - 1,000 µL pipettes and a multi-channel pipette.
- Incubator
- Disposable syringe (1 mL)

## PREPARATION OF WORKING SOLUTION

### A. Preparation of Enzyme working solution

- Add 2 mL of de-ionized water to enzyme B.
- Add 1.5 mL of Enzyme B solution to Enzyme A to prepare Enzyme working solution.
  *Since inside of each vial of Enzyme A and Enzyme B is under reduced pressure, add de-ionized water or Enzyme B solution to the vial with a syringe in order to avoid the dispersal of powder. Please do not open the cap at the beginning.
  *The Enzyme working solution is stable at -20℃ for 2 weeks. If stored in a refrigerator, stable for 3 days.

### B. Preparation of Indicator working solution

- Add 3 mL of de-ionized water to each vial of Enzyme C and Coenzyme.
- Add 2.8 mL of Enzyme C solution and Coenzyme solution to Indicator solution to prepare Indicator working solution.
  *Since inside of the vial of Enzyme C and Coenzyme is under reduced pressure, add de-ionized water to the vial with a syringe in order to avoid the dispersal of powder. Please do not open the cap at the beginning.
  *The Indicator working solution is stable at -20℃ for 2 weeks. If stored in a refrigerator, stable for 3 days.

### C. Preparation of Sample solution

- Dilute sample solution with de-ionized water.
  - Dilution ratio: 1 (without dilution), 1/5, $1/5^2$, $1/5^3$, $1/5^4$. $1/5^5$, $1/5^6$

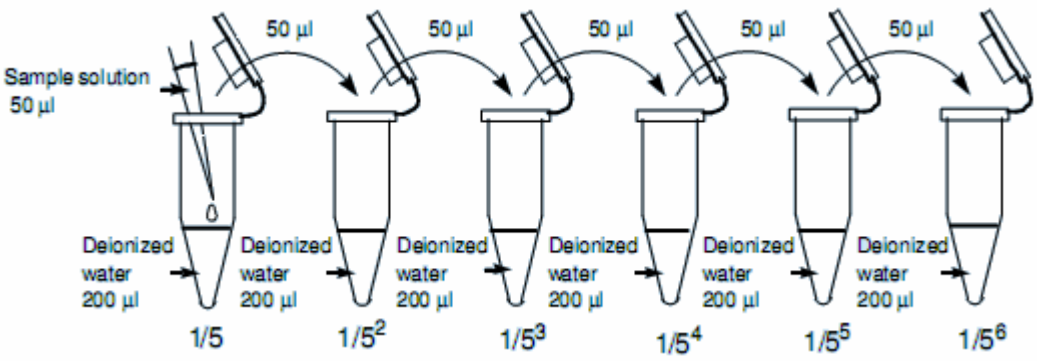


Figure 2. Preparation of sample solution

## PROTOCOL

See table and Figure 3.

1. Add 20 µL of sample solution (sample) or de-ionized water (blank 1, blank 2) to each well.
2. Add 20 µL of Substrate buffer to each well
3. Add 20 µL of de-ionized water to each “blank 2” well.
4. Add 20 µL of Enzyme working solution to each sample and “blank 1” well.
   *Since 3-Hyroxybutyric acid (3HB) will be produced immediately after the addition of the Enzyme working solution to a well, use a multi-channel pipette to avoid the reaction time lag between each well.
5. Incubate the plate at 37℃ for 60 min.
6. Add 200 µL of Indicator working solution to each well.
7. Incubate the plate at room temperature for 10 min.
8. Read the absorbance at 450 nm with a microplate reader.
9. Calculate the ACE inhibitory activity (inhibition rate %) using the following equation:

$$\textbf{ACE inhibitory activity (inhibition rate \%)} = [(A_{blank1} - A_{sample}) / (A_{blank1} - A_{blank2})\} \times 100$$

Blank 1 : positive control (without ACE inhibition), blank 2: reagent blank

*if the color of the sample solution is strong, subtract the absorbance of the sample blank, that is prepared by sample solution (20 µL) and de-ionized water (240 µL), from that of the sample ($A_{sample}$) per each sample.

**Table. Amount of each solution for sample, blank 1, and blank 2**

| | Sample | Blank 1 | Blank 2 |
|---|---|---|---|
| Sample solution | 20 µL | - | - |
| De-ionized water | - | 20 µL | 40 µL |
| Substrate buffer | 20 µL | 20 µL | 20 µL |
| Enzyme working solution | 20 µL | 20 µL | - |
| Indicator working solution | 200 µL | 200 µL | 200 µL |

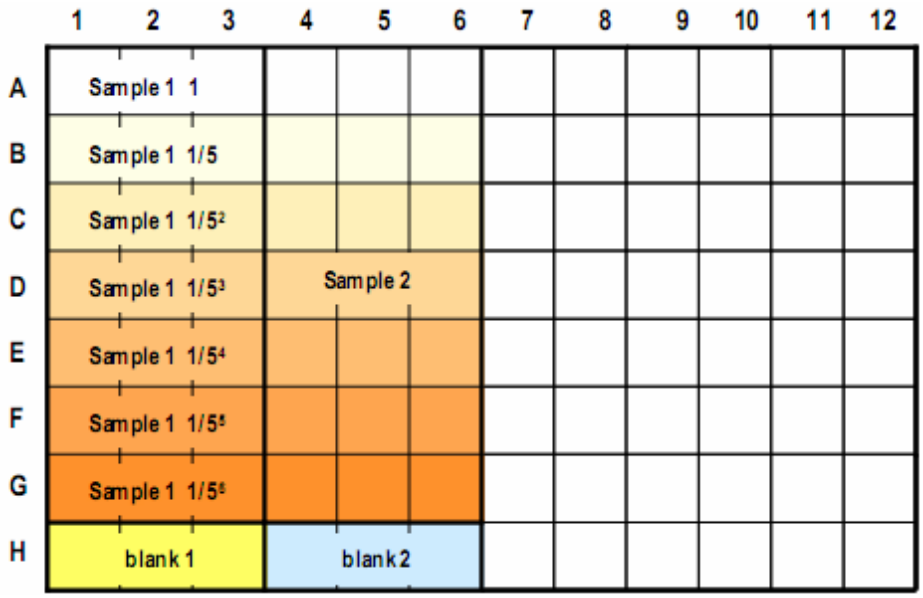


Figure 3. Example of sample and blank arrangement on a 96-well plate

## NOTES

1. This kit contains glass vials. Please handle them carefully.
2. Two vials of each component are included in this kit. One vial of each corresponds to 50 tests.
3. For the highest accuracy, we recommend that each sample be assayed in triplicate.
4. If the water solubility of the sample is low, use DMSO or ethanol to dissolve. Then, dilute the solution with an appropriate buffer. The final concentration of organic solvent should be lower than 1%.
5. If the sample solution is acidic, adjust the pH of the sample solution at 5 or higher prior to use for the measurement.
6. Ascorbic acid may interfere with the assay. The concentration of the ascorbic acid in the sample solution should be lower than 0.01%w/v.
7. If the sample solution contains insoluble materials, remove with centrifuge or filtration prior to use for the measurement.

## STORAGE

Store the kit at 4°C. This kit is stable at 4°C for six months in unopened condition.

**FOR RESEARCH USE ONLY**
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