ACE Kit - WST (100 tests)

Technical Manual

Technical Manual (Japanese Version) is available at http://www.dojindo.co.jp/manual/a502.pdf

Angiotensin-converting enzyme (ACE) is one of the key elements responsible for vasopressure action. ACE converts angiotensin-Ⅰ to angiotensin-Ⅱ, a potent vasopressor, in the rennin-angiotensin system, and additionally contributes increasing blood pressure through inactivating bradykinin, a strong antihypertensive peptide. Recently, various functional foods are getting attention due to those ACE inhibition activity.

ACE activity is conventionally determined by the UV reading of the hippuric acid produced from synthetic substrate, Hyppuryl-His-Leu. Because the assay process is, however, complicated and requires organic solvent, a simple and safe modified method has been developed.

The colorimetric detection system in the ACE Kit-WST can determine the amount of 3-Hyroxybutylic acid (3HB) generated from 3-Hydroxybutylyl-Gly-Gly-Gly with the enzyme method. Since the kit is designed for 96-well microplate assays, it is suitable for multiple sample measurements. Since no organic solvent extraction is required, ACE Kit-WST assay is safe and simple, and gives highly reproducible data.

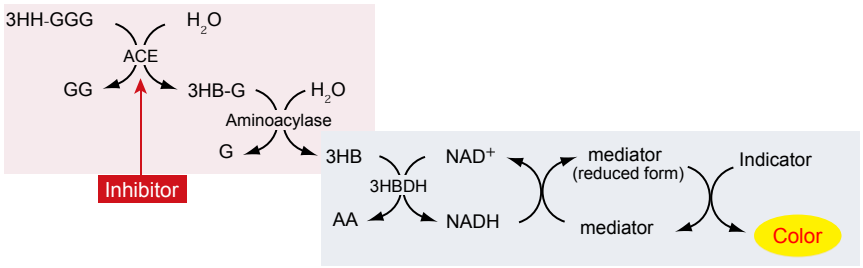

Fig.1 Principle of the ACE inhibitory activity assay using ACE Kit - WST

Contents of the Kit

- Substrate buffer	1 ml x 2	- Enzyme A	x 2
- Enzyme B	x 2	- Enzyme C	x 2
- Coenzyme	x 2	- Indicator solution	5 ml x 2

Storage

Store the kit at 0-5°C. The kit is stable for 6 months.

Precaution

- Several kit components are in glass vials. Please handle with care.
- The kit contains two sets. One set is sufficient for 50 tests.
- Multiple measurements such as triplicate are recommended for accurate data.
- 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 %.
- If the sample solution is acidic, adjust the pH of the sample solution at 5 or higher prior to use for the measurement.
- 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.
- If the sample solution contains insoluble materials, remove with centrifuge or filtration prior to use for the measurement.

Required Equipment and Materials

- Microplate reader (450 nm filter)
- 2-20 µl, 20-200 µl, 100-1000 µl pipettes
- Multi-channel pipette
- 96-well microplate
- Incubator
- Disposable syringe (1 ml)

Preparation of Working Solution

Enzyme working solution :

Dissolve Enzyme B with 2 ml of deionized water to prepare Enzyme B solution. Then add 1.5 ml of Enzyme B solution to Enzyme A to prepare Enzyme working solution.

* Enzyme A and B vials are capped under vacuum pressure. Add deionized water or solution through a rubber septum with a syringe, and then remove the septum.

* The Enzyme working solution is stable at -20°C for 2 weeks. If store in a refrigerator, stable for 3 days.

Indicator working solution :

Dissolve Enzyme C and Coenzyme with 3 ml of deionized water each. Add 2.8 ml of Enzyme C solution and 2.8 ml of Coenzyme solution to Indicator solution to prepare Indicator working solution.

* Enzyme C and Coenzyme vials are capped under vacuum pressure. Add deionized water through a rubber septum with a syringe, and then remove the septum.

* The Indicator working solution is stable at -20°C for 2 weeks. If store in a refrigerator, stable for 3 days.

Preparation of Sample Solution

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

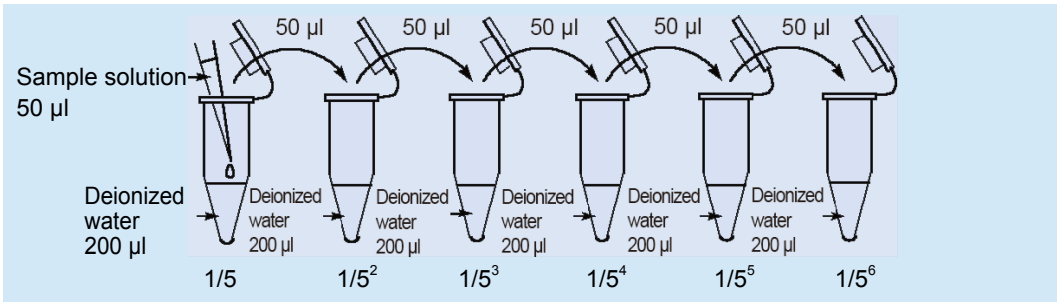

Fig. 2 Preparation of sample solutions

General Procedure for the Assay

See Table and Fig. 3
1) Add 20 µl of sample solution to a sample well and 20 µl of deionized water to blank 1 and blank 2 wells.
2) Add 20 µl of Substrate buffer to each well.
3) Add 20 µl of deionized water to blank 2 well.
4) Add 20 µl of Enzyme working solution to each sample well and blank 1 well.
 * Since the enzymatic reaction starts immediately after the addition of the Enzyme working solution, use a multi-channel pipette to minimize the well-to-well time lag.
5) Incubate at 37°C for 1 hour.
6) Add 200 µl of Indicator working solution to each well.
7) Incubate at room temperature for 10 minutes.
8) Read the absorbance at 450 nm with a microplate reader.
9) ACE inhibitory activity can be calculated by the following equation.

$$\text{ACE inhibitory activity (inhibition rate \%)} = [(A_{\text{blank 1}} - A_{\text{sample}}) / (A_{\text{blank 1}} - A_{\text{blank 2}})] \times 100$$

Table. Amount of each solution for sample, blank1 and 2.

	Sample	blank 1	blank 2
Sample solution	20 µl	-	-
Deionized 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

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

* Preparation of the sample blank solution (20 µl of sample + 240 µl of deionized water) will be required for the subtraction of the blank absorbance if the sample solution has visible color.

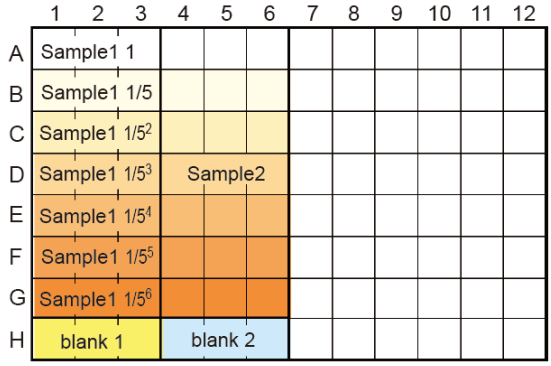

Fig. 3 Example of sample and blank arrangement on a 96-well plate

Determination of IC_{50} (50% inhibitory concentration)

- Prepare an inhibition curve with using the sample concentration for X axis and ACE inhibitory activity for Y axis. Typical inhibition curve is shown in Fig. 4.
- Determine the concentration of the sample solution that gives 50% ACE inhibitory activity as indicated in Fig. 4 .
- Since the total volume of the inhibition assay is 60 µl (first step of the assay), the original sample is diluted 3 times in the reaction. Therefore, the concentration of the sample at 50% inhibition is one third of the concentration determined by the inhibition curve.

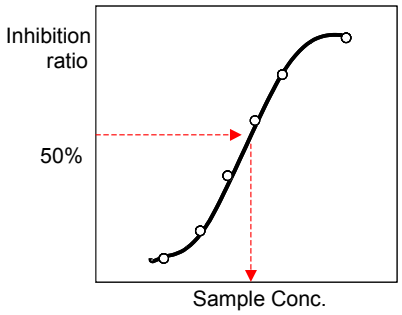

Fig.4 Inhibition curve

References

1. Le Hoang Lam, T. Shimamura, K. Sakaguchi, K. Noguchi, M Ishiyama, Y. Fujimura and H. Ukeda, *Anal. Biochem.*, **2007**, *364*, 104.
2. Le Hoang Lam, T. Shimamura, S. Manabe, M Ishiyama and H. Ukeda, *Anal. Sci.*, **2008**, 24, 1057.

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