Angiotensin Converting Enzyme (ACE) Inhibitor Activity Assay Kit

Note: It is necessary to predict 2-3 large difference samples before the formal determination.
Operation Equipment: Spectrophotometer/Microplate reader
Cat No: BC5575
Size: 100T/96S

Components:

Reagent I: Liquid 65mL×2. Store at 2-8℃.

Reagent II: Liquid 17mL×1. Store at 2-8℃.

Reagent III: Powder×2. Store at 2-8℃. Add 50μL distilled water to each Reagent III (about 66T) and mix well before use. It can be stored at -20℃ for four weeks after dispensing to avoid repeated freezing and thawing.

Reagent III working solution: Reagent III and Reagent I are mixed by the ratio of 9μL:1791μL（1.8mL, 12T）to make working solution according to sample numbers.

Reagent IV: Powder×1, 5mg captopril. Store at room temperature. Add 4.6mL distilled water to prepare a solution of 5mmol/L before use. It can be stored at -20℃ for four weeks after dispensing to avoid repeated freezing and thawing.

Product Description:

Angiotensin Converting Enzyme (ACE, EC 3.4.15.1, also called ACE1) is a zinc-containing peptidyl dipeptide hydrolase with a calculated molecular mass of 120-150 kDa. ACE is widely detected in the endothelial cell of organ tissues included lung, brain, kidney. ACE catalyzes the formation of angiotensin I to angiotensin II, which could cause vasoconstriction, promote synthesis and release of adrenocortical hormone aldosterone. ACE inhibitors could reduce the production of angiotensin II and increase bradykinin activity, which is helpful for the treatment of hypertension, cardiac hypertrophy, heart failure and other diseases.

ACE could catalyze FAPGG to form FAP and glycylglycine. ACE inhibitors could reduce the hydrolysis of FAPGG by inhibiting ACE activity. FAPGG has a characteristic absorption peak at 340 nm, and ACE inhibitor activity is calculated by measuring the rate of decrease in absorbance at 340nm.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/Microplate reader, desk centrifuge, balance, transferpettor, constant temperature foster box/water-bath, micro quartz cuvette/96 well UV plate, mortar/homogenizer/cell ultrasonic crusher, ice and distilled water.

Procedure:

I. Sample preparation

1. **Tissue:** According to the proportion of tissue weight (g): Reagent I volume (mL) of 1:5-10 to extract.

It is suggested that 0.1 g of tissue with 1 mL of Reagent I and fully homogenized on ice bath.

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Centrifuge at 12000g for 20 minutes at 4℃ to remove insoluble materials and take the supernatant on ice before testing.

2. **Cells:** Collect cells into the centrifuge tube, after centrifugation discard supernatant. According to the proportion of cells number (10^4): Reagent I volume (mL) of 500-1000-1 to extract. It is suggested that add 1 mL of Reagent I to 5 million of cells. Use ultrasonication to splitor cells (place on ice, ultrasonic power 200W, working time 3 seconds, interval 7 seconds, repeat for 5 minutes). Centrifuge at 12000g for 20 minutes at 4℃ to remove insoluble materials and take the supernatant on ice for testing.
3. **Liquid:** Detect directly. Centrifuge before detecting if there are precipitation in the samples.
4. **Powder:** Dissolve sample with Reagent I to prepare a solution of the appropriate concentration. **Note:** If sample is insoluble in water, it could be dissolved with a small amount of ethanol, and then diluted with Reagent I to ensure that ethanol content is less than 5%.

II. Determination

1. Preheat spectrophotometer/microplate reader for 30 min, adjust the wavelength to 340 nm and set spectrophotometer counter to zero with distilled water.
2. Dilute 5mmol/L captopril solution with distilled water to the required concentration or concentration gradient as Positive tube.
3. Keep Reagent II at 37℃ for 10 min according to sample numbers.
4. Add reagents in micro quartz cuvette/96 well UV plate as the following:

Reagent (μL)	Blank tube1	Blank tube2	Test tube	Positive tube (Optional)
Reagent IV	-	-	-	15
Supernatant	-	-	15	-
Reagent I	15	165	-	-
Reagent II	150	150	150	150
Reagent III working solution	150	-	150	150
Mix thoroughly. Record the initial absorbance A1 at the wavelength of 340 nm for 10s as $A1_{B1}$, $A2_{B1}$, A_{T1}, A_{P1}. Incubate for 30min at 37℃. Record the absorbance A2 at the wavelength of 340 nm for 30min10s as $A1_{B2}$, $A2_{B2}$, A_{T2}, A_{P2}. Calculate $\Delta A_B=(A1_{B1}-A2_{B1})-(A1_{B2}-A2_{B2})$, $\Delta A_T=A_{T1}-A_{T2}$, $\Delta A_P=A_{P1}-A_{P2}$. Blank tube1 and Blank tube2 only be measured once or twice.				

III. ACE inhibitor activity calculation:

1. Inhibition rate calculation:

   Inhibition rate of ACE inhibitor (%) = $(\Delta A_B \times \Delta A_T) \div \Delta A_B \times 100\%$
2. IC_{50} calculation:

IC_{50} is the concentration of ACE inhibitor when inhibition rate is 50%. An appropriate concentration gradient could be prepared for the samples which inhibit ACE actvity. The inhibition curve is plotted with the sample concentration as x-axis and the inhibition rate as y-axis. And the sample concentration could be calculated when inhibition rate is 50%.

Note:

1. It is recommended to control reaction time and operation time strictly to ensure accuracy and stability of the experimental results.
2. If A_{T1} is more than 1.5, or ΔA_T is more than 0.35， it is recommended to dilute the sample with Reagent I before determination. If ΔA_T is less than 0.01, it is recommended to prolong reaction time before determination. And modify the calculation formula.
3. The reaction time and concentration of solutions, extractions, drugs or tissue homogenates should be the same to compare their inhibitory effects on ACE activity.

Experimental example:

1. Take 0.1082g Ginkgo pulp, add 1 mL of Reagent I, grind the homogenate with ice bath. Dilute supernatant 8 times with Reagent I. Then operate according to the determination steps, calculate $\Delta A_B = (A1_{B1}-A2_{B1}) - (A1_{B2}-A2_{B2}) = 0.333$, $\Delta A_T = A_{T1}-A_{T2} = 0.126$. The result is calculated:
   Inhibition rate (%)=（0.333-0.126）÷0.333×100%=62.162%.
2. Take 0.1072g orange leaves, add 1 mL of Reagent I, grind the homogenate with ice bath. Dilute supernatant 8 times with Reagent I. Then operate according to the determination steps, calculate $\Delta A_B = (A1_{B1}-A2_{B1}) - (A1_{B2}-A2_{B2}) = 0.333$, $\Delta A_T = A_{T1}-A_{T2} = 0.173$. The result is calculated:
   Inhibition rate (%)=（0.333-0.173）÷0.333×100%=48.048%.
3. Take 15μL 0.25μmol/L captopril solution and operate according to the determination steps, calculate $\Delta A_B = (A1_{B1}-A2_{B1}) - (A1_{B2}-A2_{B2}) = 0.333$, $\Delta A_T = A_{T1}-A_{T2} = 0.185$. The result is calculated:
   Inhibition rate (%)=（0.333-0.185）÷0.333×100%=44.444%.

References：

[1] Sun S, Xu X, Sun X, et al. Preparation and Identification of ACE Inhibitory Peptides from the Marine Macroalga Ulva intestinalis[J]. Marine Drugs, 2019, 17(3).

[2] Murray B A, Walsh D J, Fitzgerald R J. Modification of the furanacryloyl-L-phenylalanyl glycylglycine assay for determination of angiotensin-I-converting enzyme inhibitory activity[J]. Journal of Biochemical & Biophysical Methods, 2004, 59(2): 127-137.

[3] Gajanan P G, Elavarasan K, Shamasundar B A. Bioactive and functional properties of protein hydrolysates from fish frame processing waste using plant proteases[J]. Environmental Science & Pollution Research, 2016, 23(24): 24901-24911.

Related Products：

BC5540/BC5545 Angiotensin Converting Enzyme (ACE) Activity Assay Kit