Angiotensin Converting Enzyme (ACE) 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: BC5545

Size:100T/96S

Components:

Reagent I: Liquid 110mL×1. Store at 2-8℃.

Reagent II: Liquid 11mL×1. Store at 2-8℃.

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. It is important to detect ACE activity for diagnosis and treatment of lung, liver, thyroid and other organ diseases.

ACE could catalyze FAPGG to form FAP and glycylglycine. FAPGG has a characteristic absorption peak at 340 nm, and ACE 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. 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 split bacteria or 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.

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. Keep Reagent I at 37℃ for 10 min.
3. Add reagents in micro quartz cuvette/96 well UV plate as the following:

Reagent (μL)	Blank tube	Test tube
Sample	-	100
Reagent I	100	-
Reagent II	100	100
Mix thoroughly. Record the initial absorbance A1 at the wavelength of 340 nm for 15s and incubate for 5min at 37℃. Record the absorbance A2 at the wavelength of 340 nm for 5min15s. Calculate $\Delta A_T=A_{T1}-A_{T2}$, $\Delta A_B=A_{B1}-A_{B2}$, $\Delta A=\Delta A_T-\Delta A_B$. Blank tube only be measured once or twice.		

III. ACE activity calculation:

A. micro quartz cuvette:

1. Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes hydrolysis of 1 nmol FAPGG in the reaction system per minute at 37℃ every mg protein.

ACE activity (U/mg prot) $=\Delta A\div(\varepsilon\times d)\times Vrv\times10^9\div(Cpr\times Vs)\div T\times F=527.7\times\Delta A\div Cpr\times F$

2. Sample weight:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes hydrolysis of 1 nmol FAPGG in the reaction system per minute at 37℃ every g sample.

ACE activity (U/g weight) $=\Delta A\div(\varepsilon\times d)\times Vrv\times10^9\div(W\times Vs\div Ve)\div T\times F=527.7\times\Delta A\div W\times F$

3. Cell amount:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes hydrolysis of 1 nmol FAPGG in the reaction system per minute at 37℃ every 10^4 bacteria or cells.

ACE activity (U/10^4 cell) $=\Delta A\div(\varepsilon\times d)\times Vrv\times10^9\div(N\times Vs\div Ve)\div T\times F=527.7\times\Delta A\div N\times F$

4. Liqiud:

Unit definition: One unit of enzyme activity is defined as the amount of enzymes catalyzes hydrolysis of 1 nmol FAPGG in the reaction system per minute at 37℃ every milliliter liqiud sample.

ACE activity (U/mL) $=\Delta A\div(\varepsilon\times d)\times Vrv\times10^9\div Vs\div T\times F=527.7\times\Delta A\times F$

ε: extinction coefficient of FAPGG at 340nm, 758L/mol/cm;

d: Light path of cuvette, 1cm;

Vrv: Total reaction volume, 2×10^{-4}L;

10^9: Unit conversion factor, 1 mol=10^9 nmol;

Cpr: Sample protein concentration, mg/mL;

Vs: Added sample supernatant volume, 0.1 mL;

T: Reaction time, 5 min;

W: Sample weight, g;

Ve: Reagent I volume, 1mL.

N: Cell amount, 10^4 for one unit.

F: Dilution factor.

B. 96 well UV plate:

Modify d-1 cm in the above formula to d-0.6 cm (light path of the cuvette) for calculation.

Note:

1. It is recommended to control reaction time and operation time strictly to ensure accuracy and stability of the experimental results.
2. If A1 is more than 1.6(micro quartz cuvette)/1(96 well UV plate), or ΔA_T is more than 0.4(micro quartz cuvette)/0.3(96 well UV plate)， it is recommended to dilute the sample with Reagent I before determination. And modify the calculation formula.
3. If ΔA_T is less than 0.01, it is recommended to prolong reaction time before determination. And modify the calculation formula.

Experimental example:

1. Take 0.1027g mouse lungs, add 1 mL of Reagent I, grind the homogenate with ice bath. Dilute supernatant 4 times with Reagent I. Then operate according to the determination steps, calculate $\Delta A_T=A_{T2}-A_{T1}=1.4637-1.1426=0.3211$, $\Delta A_B=A_{B1}-A_{B2}=1.0037-1.0021=0.0016$, $\Delta A=\Delta A_T-\Delta A_B=0.3211-0.0016=0.3195$. The result is calculated according to the sample weight:
   ACE activity (U/g weight)= $527.7\times\Delta A\div W\times F$ =6566.705 U/g weight.
2. Take calf serum and dilute twice with Reagent I. Then operate according to the determination steps, calculate $\Delta A_T=A_{T2}-A_{T1}=1.2163-1.1277=0.0886$, $\Delta A_B=A_{B1}-A_{B2}=1.0037-1.0021=0.0016$, $\Delta A=\Delta A_T-\Delta A_C=0.0886-0.0016=0.087$. The result is calculated according to liquid volume:
   ACE activity (U/mL)= $527.7\times\Delta A\times F$ =91.820 U/mL.

References：

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

[2] 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.

[3] 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).

Related Products：

BC5570/BC5575 Angiotensin Converting Enzyme (ACE) Inhibitor Activity Assay Kit