Carbonic Anhydrase Activity Assay Kit

Note: Take two or three different samples for prediction before test.

Operation Equipment: Spectrophotometer/Microplate reader

Cat No: BC5445

Size:100T/96S

Components:

Extract solution:120 mL×1. Storage at 2-8℃.

Reagent I: 20 mL×1. Storage at 2-8℃.

Reagent II: powder×2. Storage at -20℃; Before use, take a bottle of Reagent II, add 200μL acetone to shake and dissolve, the reagent can be divided and stored for 1 week in -20℃, avoid repeated freeze-thaw;

Reagent II working solution: before use according to the ratio of reagent II: distilled water = 20μL: 480μL (12T) to mix and prepare into reagent II working solution. Reagents are prepared at the time of use.

Standard: 1 mL×1. Storage at 2-8℃. 5 μmol/mL phenol standard solution. Before use, take 100 μL of 5 μmol/mL phenol standard solution in an EP tube, add 700 μL of distilled water and mix thoroughly to make 0.625 μmol/mL of phenol standard solution.

Product Description:

Carbonic anhydrase (CA, EC4.2.1.1) is a metalloenzyme with Zn^{2+} as the active center, which can efficiently catalyze the reversible hydration reaction of CO^2: $CO_2+H_2O \leftrightharpoons HCO_3^-+H^+$ at a rate 10^7 times higher than that under natural conditions, which is one of the fastest known enzymes. It is one of the fastest known enzymes.

Carbonic anhydrase can catalyze the reaction of p-nitrophenyl acetate to form p-nitrophenol, and the rate of increase of absorbance value at 405 nm reflects the activity of carbonic anhydrase.

Reagents and Equipment Required but Not Provided:

Spectrophotometer/microplate reader, centrifuge, water bath/incubator, adjustable pipette, Micro glass cuvettes/96 well plates , mortar/homogenizer/cell sonicator, ice and distilled water..

Procedure

I. Extraction of crude enzyme solution:

a. Tissue

The ratio of tissue mass (g): the volume of Extract solution (mL) is 1: 5~10 (it is suggested to take about 0.1 g of tissue and add 1mL of Extract solution), ice-bath homogenate. Centrifuge at 8000 g for 10 minutes at 4℃, take the supernatant and placed on the ice for test.

b. Bacteria or cell

The ratio of bacteria/cell amount (10^4): the volume of Extract solution (mL) is 500~1000:1(it is suggested to take about 5 million bacteria/cells and add 1 mL of Extract solution). Bacteria/cell is split by ultrasonic (placed on ice, 200 W, work time 3 s， interval 10 s， repeat 30 times). Centrifuge at 8000g

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for 10 minutes at 4℃, take the supernatant and placed on the ice for test.

c. Serum (plasma) sample: Detect sample directly. (If the solution is turbid, centrifuge to take the supernatant and then measure)

II. Determination procedure

a. Preheat the spectrophotometer/microplate reader 30 minutes, adjust wavelength to 405 nm, the spectrophotometer set zero with distilled water.

b. Standard operate

(1) Determination of standard tube: Add 20μL of standard solution and 180μL of reagent I to the micro glass cuvettes/96 well plates, mix thoroughly and then measure the absorbance value at 405nm, and record it as As.

(2) Determination of standard blank tube: Add 20μL distilled water and 180μL of reagent I to the micro glass cuvettes/96 well plates, mix thoroughly and then measure the absorbance value at 405nm, and record as Ab.

(3) Calculate ΔAs= As- Ab. (Standard tube and standard blank tube should be done only 1-2 times.).

c. Then operate according to the following table.

Reagent name（μL）	Test tube(T)	Control tube(C)
sample	20	-
Extract solution	-	20
Reagent I	140	140
Reagent II working solution	40	40

Add the above reagents sequentially to the micro glass cuvette/96-well plate according to the spiking table, mix thoroughly and measure the absorbance value A1 at 405nm for 10s, then quickly place it in a 37℃ water bath or constant temperature incubator for 5min (the enzyme marker has a temperature control function to adjust the temperature to 37℃), take out and dry it quickly to measure the absorbance value A2 at 5min10s. Calculate At= A2t- A1t, Ac= A2c - A1c, ΔA =At-Ac. (Control tube should be done only 1-2 times.)

III. Calculation formula

(1) Calculated by Protein concentration:

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 μmol p-nitrophenol per mg protein of tissue per minute at 37℃ .

Carbonic anhydrase (U/mg prot) ==Cs×ΔA÷ΔAs×Vs÷（Vs×Cpr）÷T×F=0.125×ΔA÷ΔAs÷Cpr×F

(2) Calculated by sample weight

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 μmol p-nitrophenol per gram of tissue per minute at 37℃ .

Carbonic anhydrase (U/g mass) =Cs×ΔA÷ΔAs×Vs÷（Vs÷Ve×W）÷T×F=0.125×ΔA÷ΔAs÷W×F

(3)Calculated by the volume of culture medium

Unit definition: One unit of enzyme activity is defined as the amount of enzyme that catalytic production of 1 μmol p-nitrophenol every milliliter per minute at 37℃.

Carbonic anhydrase (U/mL) = Cs×ΔA÷ΔAs× Vs÷Vs÷T×F=0.125×ΔA÷ΔAs×F

Cs：Standard concentration，0.625μmol/mL;

Vs: Sample volume in the reaction system,0.02mL;

V_e: The volume of extract, 1 mL;

T: Reaction time, 5 minutes

Cpr: Sample protein concentration, mg/mL;

W: Sample weight, g;

Note:

1. If A1 is greater than 0.5 or ΔA is greater than 1, dilute the sample with distilled water or shorten the enzymatic reaction time at 37°C; ΔA is less than 0.02, increase the sample volume or extend the enzymatic reaction time at 37°C. Note the simultaneous modification of the calculation formula when calculating

Experimental example:

1. Weigh 0.1029g of mouse liver tissue, add the extract for ice bath homogenization, centrifuge the supernatant, dilute the supernatant 40 times and operate according to the procedure, measure with a 96-well plate to calculate At = A2t - A1t = 0.809-0.182=0.627, Ac = A2c - A1c = 0.155-0.115=0.040, ΔA=At-Ac=0.587, ΔAs=As-Ab=0.627-0.047=0.58, brought into the equation to calculate.

   CA activity (U/g mass) = 0.125 × ΔA ÷ ΔAs ÷ W × F = 49.177 U/g mass

2, weigh 0.1058g of lettuce leaf tissue, added to the extract for ice bath homogenization, centrifuged the supernatant, diluted 2 times with distilled water and operated according to the procedure, measured with a 96-well plate to calculate At = A2t - A1t= 0.408 - 0.155 = 0.253, Ac= A2c - A1c = 0.155 - 0.115 = 0.040, ΔA =At-Ac=0.213, ΔAs=As-Ab=0.627-0.047=0.58, brought into the equation to calculate.

   CA activity (U/g mass) = 0.125 × ΔA ÷ ΔAs ÷ W × F = 0.868 U/g mass

3. 20 μL of rabbit serum was aspirated, diluted 2 times and operated according to the procedure, and measured in a 96-well plate to calculate At = A2t - A1t= 1.097 - 0.251 = 0.846, Ac= A2c - A1c = 0.155 - 0.115 = 0.040, ΔA = At - Ac = 0.806, ΔAs = As - Ab = 0.627-0.047=0.58, brought into the equation to calculate.

   CA activity (U/mL) = 0.125 × ΔA ÷ ΔA standard × F = 0.347 U/mL.

Related products:

BC0440/BC0445 Ribulose 1, 5-bisphosphate carboxylase/oxygenase(Rubisco) Assay Kit

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