Carbonic Anhydrase Activity Assay Kit

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

Operation Equipment: Spectrophotometer

Cat No: BC5440

Size:50T/48S

Components:

Extract solution:60 mL×1. Storage at 2-8℃.

Reagent I: 50 mL×1. Storage at 2-8℃.

Reagent II: powder×2. Storage at -20℃; Before use, take a bottle of Reagent II, add 320μL 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 = 40μL: 960μL (5T) to mix and prepare into reagent II working solution.

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 1500 μL of distilled water and mix thoroughly to make 0.3125 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, centrifuge, water bath/incubator, adjustable pipette, 1mL glass cuvette, 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 30 minutes, adjust wavelength to 405 nm, set zero with distilled water.

b. Standard operate

(1) Determination of standard tube: Add 100μL of standard solution and 900μL of reagent I to the cuvette, mix thoroughly and then measure the absorbance value at 405nm, and record it as As.

(2) Determination of standard blank tube: Add 100μL of distilled water and 900μL of reagent I to the cuvette, 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	100	-
Extract solution	-	100
Reagent I	700	700
Reagent II working solution	200	200

Add the above reagents in 1mL glass cuvette in order according to the spiking table, immediately mix thoroughly and measure the absorbance value A1 at 405nm for 10s, quickly place the reaction in a 37°C water bath or constant temperature incubator for 5min, take out and dry 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°C .

Carbonic anhydrase (U/mg prot) ==Cs×ΔA÷ΔAs×Vs÷（Vs×Cpr）÷T×F=0.0625×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°C .

Carbonic anhydrase (U/g mass) =Cs×ΔA÷ΔAs×Vs÷（Vs÷Ve×W）÷T×F=0.0625×ΔA÷ΔAs÷W×F

(4) 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=0.0625×ΔA÷ΔAs×F

Cs：Standard concentration，0.3125μmol/mL;
Vs: Sample volume in the reaction system,0.1mL;

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 160 times and operate according to the procedure, measure with a 1mL glass cuvette to calculate At = A2t- A1t = 0.599-0.169=0.43, Ac = A2c- A1c = 0.266-0.13= 0.136, ΔA =At-Ac=0.294, ΔAs=As-Ab=0.434-0.003=0.431, brought into the equation to calculate.
   CA activity (U/g mass) = 0.0625 × ΔA ÷ ΔAs ÷ W × F = 66.29 U/g mass
2. Weigh 0.1058g of baby lettuce leaf tissue, add the extract for ice bath homogenization, centrifuge and take the supernatant, dilute the supernatant 4 times and operate according to the procedure, measure with a 1mL glass cuvette to calculate At= A2t - A1t = 0.424 - 0.158 = 0.266, Ac = A2c - A1c = 0.266 - 0.13 = 0.136, ΔA =At-Ac=0.13, ΔAs=As-Ab=0.434-0.003=0.431, brought into the equation to calculate.
   CA activity (U/g mass) = 0.0625 × ΔA ÷ ΔAs ÷ W × F = 0.713 U/g mass
3. 100 μL of rabbit serum was aspirated, the serum was diluted 8 times with distilled water0 and then operated according to the procedure, measured with a 1 mL glass cuvette to calculate At = A2t-A1t = 0.739-0.197=0.542, Ac = A2c-A1c = 0.266-0.13=0.136, ΔA = A t-Ac = 0.406, ΔAs = As - Ab= 0.434 - 0.003 = 0.431, brought into the equation to calculate.
   CA activity (U/mL) = 0.0625 × ΔA ÷ ΔAs × F = 0.471 U/mL.

Related products:

BC0440/BC0445	Ribulose 1, 5-bisphosphate carboxylase/oxygenase(Rubisco) Assay Kit
BC3310/BC3315	Phosphoenolpyruvate Carboxykinase (PEPCK) Activity Assay Kit