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Carbonic Anhydrase (CA) Activity Assay Kit

Catalog No.: BC00068

Size: 100T

If you have any questions or need further help during experiment, please don't hesitate to contact us through the following methods:

✉Email (Sale) order@enkilife.com

✉Email (Techsupport) techsupport@enkilife.com

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Shelf life: Please refer to the label on the outer package.

Techsupport: In order to provide you with better service, please inform us the lot number on the label of the outer package.

Basic Information

Product Name	Carbonic Anhydrase (CA) Activity Assay Kit
Detection Methods	Colorimetric
Sample type	Tissue, cell, bacteria , fluid samples
Detection Type	Enzyme activity
Detection instrument and wavelength	Microplate reader (405 nm)
Range	0.625-10U/mL
Sensitivity	0.217U/mL

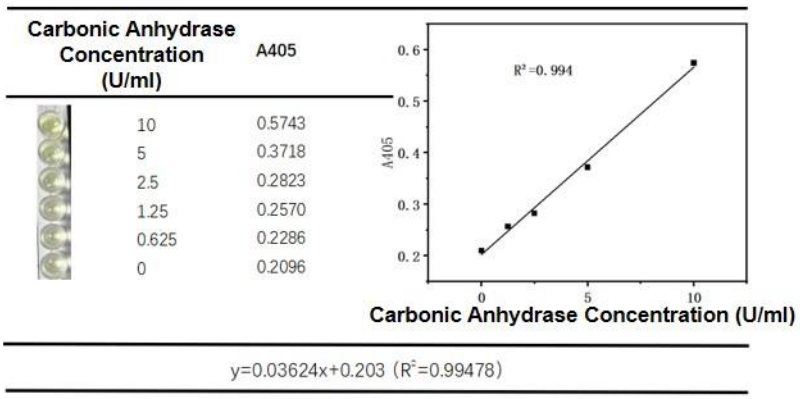
Product Introduction

Carbonic anhydrase (CA) is a metalloenzyme with Zn^{2+} as the active center. It can be used to efficiently catalyze the reversible hydration reaction of CO^2: $CO_2 + H_2O \leftrightharpoons HCO_3^- + H^+$. The catalytic rate can reach 107 times that under natural conditions, making it one of the fastest enzymes known to date.

Detection principle

Carbonic anhydrase can catalyze the reaction of p-nitrophenyl acetate to produce light yellow p-nitrophenol. The activity of carbonic anhydrase can be reflected by detecting the rate of increase of absorbance at 405 nm.

The following standard curve is for reference only:



Product Composition

Serial Number	Product Name	Packing Specifications (100T)	Storage
Reagent 1	Extract	15 mL	-20℃, can be stored at room
Reagent 2	Enzyme solution	1mL/tube, 2 tubes	-20℃, store at 4°C after
Reagent 3	Substrate working	3 mL	-20℃
Consumables 1	96-well ELISA plate	1 plate	RT
Consumables 2	96-well membrane	2 pieces	RT

Storage Conditions

The unopened kit can be stored at -20℃ for 12 months.

Preparation before the experiment

Sample processing

1. Tissue: Homogenize in an ice bath at a ratio of 1:5-10 (tissue mass (g): extract volume (mL) (it is recommended to weigh about 0.1g tissue and add 1mL extract). Centrifuge at 8000g for 10min at 4℃, take the supernatant, and place on ice for testing.
2. Bacteria or cultured cells: first collect bacteria or cells into a centrifuge tube and discard the supernatant after centrifugation; according to the number of bacteria or cells (104): the volume of extract (mL) is 500~1000:1 (it is recommended to add 5 million bacteria or cells to 1mL of extract), ultrasonically disrupt bacteria or cells (ice bath, power 200W, ultrasonic for 3s, interval 10s, repeat 30 times), 8000g, 4℃ centrifuge for 10min, take the supernatant, and place on ice for testing.
3. Liquid: Measure directly. (If the solution is turbid, centrifuge and take the supernatant before measuring.)

· Preparation of the kit

1. Before testing, the reagents in the kit are equilibrated to room temperature, and double distilled water is required.
2. Dilution of enzyme solutions of different concentrations: Mix reagent 2 and reagent 1 at a ratio of 1:99 to prepare reagent 2 working solution. Dilute the reagent 2 working solution with reagent 1 in half to different concentrations such as 10, 5, 2.5, 1.25, 0.625, 0.15625, and 0 U/ml.
3. Preparation of reagent 3 working solution: Before use, mix reagent 3: double distilled water in a

ratio of 3:22 to prepare reagent 3 working solution, and prepare it before use.

Operation process

1. Standard tube: Take 100 μL of enzyme solution standards of different concentrations and add them to the ELISA plate.
2. Assay tube: Take 100 μL of the sample to be tested and add it to the ELISA plate.
3. Quickly add 150 μL of Reagent III working solution to each well in step (1).
4. Preheat the microplate reader at 37°C for 3 min, set the wavelength at 405 nm, measure the OD value to obtain A_1, incubate at 37°C for 20 min, measure the OD value again to obtain A_2, and take the difference between the two absorbances, $\Delta A = A_2 - A_1$.

The operation table is as follows:

	Standard well	Determination well
Standard (μL)	100	--
Sample to be tested (μL)	--	100
Reagent 3 working solution (μL)	150	150
Preheat the microplate reader at 37°C for 3 min, set the wavelength at 405 nm, measure the OD value to obtain A_1, incubate at 37°C for 20 min, measure the OD value again to obtain A_2, and take the difference between the two absorbances, $\Delta A = A_2 - A_1$		

Result calculation

1. To calculate the absolute activity of carbonic anhydrase , a standard curve can be drawn using different concentrations of carbonic anhydrase standards and the corresponding absorbance values, and the activity of carbonic anhydrase in the sample can be calculated using the corresponding formula of the standard curve .
2. carbonic anhydrase standard curve was drawn with the difference of the absorbance (OD405) of the two tests as the ordinate and the carbonic anhydrase activity (U /mL) as the abscissa . At the same time, the formula of the trend line was calculated.
3. A4 05 nm = k × carbonic anhydrase activity unit (U) + b, the slope k and intercept b of the trend line are calculated by Excel and other software. The carbonic anhydrase activity in the sample is calculated according to the above formula.

Actual absorbance of sample (OD4 05) $\Delta A = A_2 - A_1$

Carbonic anhydrase activity unit (U) in the detection system = (OD4 05 -b)/k

Carbonic anhydrase activity in the sample (U/ml) = Carbonic anhydrase activity unit in the detection system (U) / detection sample volume

Notes

1. If A1 is greater than 0.5 or ∆A is greater than 1, dilute the sample with distilled water or shorten the 37°C enzymatic reaction time. If ∆A is less than 0.02, increase the sample volume or extend the 37°C enzymatic reaction time .
2. The test kit is for research use only and must not be used for clinical diagnosis or treatment, used in medicine or food, or stored in ordinary residences.
3. Please read the instructions carefully and adjust the instrument before the experiment, and conduct the experiment strictly in accordance with the instructions.
4. The detection range of the kit is not equivalent to the concentration range of the analyte in the sample. If the concentration of the analyte in the sample is too high or too low, please dilute or concentrate the sample appropriately.