Carbonic Anhydrase Microplate Assay Kit User Manual

Catalog # CAK1288

(Version 1.1A)

Detection and Quantification ofCarbonic Anhydrase (CA)Activityin Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other biological fluidsSamples.

For research use only. Not for diagnostic or therapeutic procedures.

I. INTRODUCTION

Carbonic anhydrase(CA; EC 4.2.1.1) is widespread in nature. In animals it plays an important role in respiration by facilitating the transport of carbon dioxide. In plants, carbonic anhydrases are involved in the photosynthetic fixation of CO2.Mammalian erythrocytes contain two distinct forms of carbonic anhydrase distinguished by differences in their catalytic activities. The enzyme requires zinc for its activity and it has a molecular weight of 30,000.

Carbonic AnhydraseMicroplate Assay Kitprovides a simple and sensitive method for monitoring Carbonic Anhydrase activity in various samples.The enzyme catalysed reaction productsp-nitrophenol can be measured at a colorimetric readout at 405 nm.

II.KIT COMPONENTS

Component	Volume	Storage
96-Well Microplate	1 plate	
Assay Buffer	30mlx 4	4 °C
Reaction Buffer	20 ml x 1	4 °C
Substrate	Powderx 1	4 °C, keep in dark
Substrate Diluent	1 ml x 1	4 °C
Standard (2 mmol/L)	1 ml x 1	4 °C
Positive Control	Powderx 1	-20 °C
Plate Adhesive Strips	3 Strips	
Technical Manual	1 Manual	

Note:

Substrate: add 1 ml SubstrateDiluent to dissolve before use; stored at 4 °C for a month.

Positive Control: add 1 ml Assay Bufferto dissolve before use; stored at -80 °C for a month.

III. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader to read absorbance at 405 nm
2. Distilled water
3. Pipettor, multi-channel pipettor
4. Pipette tips
5. Mortar
6. Centrifuge
7. Timer
8. Ice

IV. SAMPLE PREPARATION

1.For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 mlAssay buffer for 5 $\times 10^6$ cell or bacteria, sonicate (with power 20%, sonicate 3s, interval 10s,repeat 30 times); centrifuged at 10,000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

2.For tissue samples

Weighout 0.1 g tissue, homogenize with 1 mlAssay buffer on ice, centrifuged at 10,000g 4°C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

3.For liquid samples

Detect directly.

V. ASSAY PROCEDURE

Add following reagents intothe microplate:

Reagent	Sample	Control	Standard	Blank	Positive Control
Reaction Buffer	180µl	180µl	180µl	180µl	180µl
Substrate	10 µl	10 µl	--	--	10 µl
Distilled water	--	10 µl	--	20 µl	--
Standard	--	--	20 µl	--	--
Positive Control	--	--	--	--	10 µl
Sample	10 µl	--	--	--	--
Mix, wait for 5 minutes, record absorbance measured at 405 nm.					

Note:

1) Perform 2-fold serial dilutions of the top standards to make the standard curve.

2) For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range. If the enzyme activity is lower, please add more sample into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

3) Reagents must be added step by step, can not be mixed and added together.

VI. CALCULATION

Unit Definition: One unit of Carbonic Anhydrase activity is defined asthe enzyme generates 1nmol of p-nitrophenol per minute.

1. According to the protein concentration of sample

$$CA\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (C_{Protein} \times V_{Sample}) / T$$

$$= 800 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / C_{Protein}$$

2. According to the weight of sample

$$CA\ (U/g) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times W / V_{Assay}) / T$$

$$= 800 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / W$$

3.According to the quantity of cells or bacteria

$$CA\ (U/10^4) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / (V_{Sample} \times N / V_{Assay}) / T$$

$$= 800 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / N$$

4. According to the volume of sample

$$CA\ (U/mg) = (C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank}) / V_{Sample} / T$$

$$= 800 \times (OD_{Sample} - OD_{Control}) / (OD_{Standard} - OD_{Blank})$$

$C_{Standard}$: the concentration of standard, 2 mmol/L = 2000nmol/ml;

$C_{Protein}$: the protein concentration, mg/ml;

W: the weight of sample, g;

N: the quantity of cell or bacteria, N $\times 10^4$;

$V_{Standard}$: the volume of the standard, 0.02 ml;

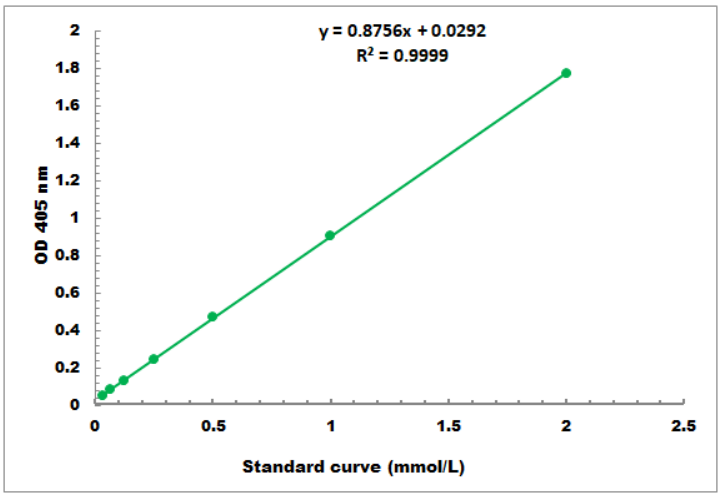
V_{Sample}: the volume of sample, 0.01 ml;

V_{Assay}: the volume of Assay buffer, 1 ml;

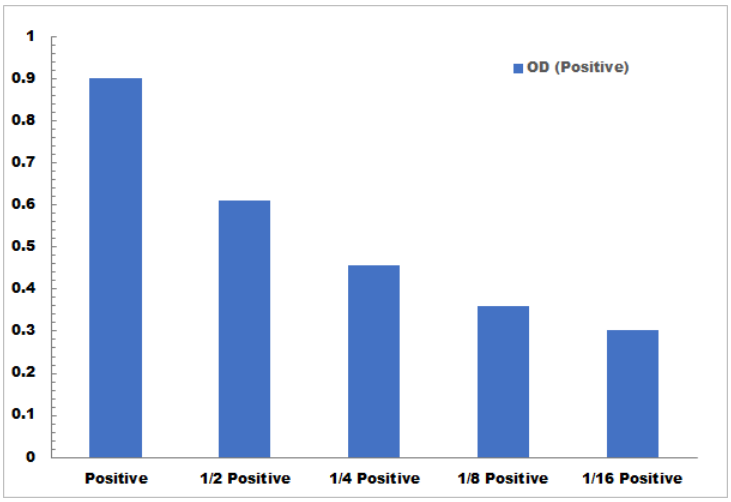
T: the reaction time, 5 minutes.

VII. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



Detection Range: 0.02mmol/L -2mmol/L



Positive Control reaction in 96-well plate assay with decreasing theconcentration

VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online towww.cohesionbio.com or contact us at techsupport@cohesionbio.com

IX. NOTES