



# **Carbonic Anhydrase Microplate Assay Kit User Manual**

**Catalog # CAK1288**

(Version 1.1A)

Detection and Quantification of Carbonic Anhydrase (CA) Activity in  
Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, Other  
biological fluids Samples.

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

|   |   |
|---|---|
| I. INTRODUCTION.....                          | 2 |
| II. KIT COMPONENTS.....                       | 3 |
| III. MATERIALS REQUIRED BUT NOT PROVIDED..... | 3 |
| IV. SAMPLE PREPARATION.....                   | 4 |
| V. ASSAY PROCEDURE.....                       | 5 |
| VI. CALCULATION.....                          | 6 |
| VII. TYPICAL DATA.....                        | 7 |
| VIII. TECHNICAL SUPPORT.....                  | 7 |
| IX. NOTES.....                                | 7 |

## 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 CO<sub>2</sub>. 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 Anhydrase Microplate Assay Kit provides a simple and sensitive method for monitoring Carbonic Anhydrase activity in various samples. The enzyme catalysed reaction products p-nitrophenol can be measured at a colorimetric readout at 405 nm.

## II. KIT COMPONENTS

| Component             | Volume     | Storage            |
|-----------------------|------------|--------------------|
| 96-Well Microplate    | 1 plate    |                    |
| Assay Buffer          | 30 ml x 4  | 4 °C               |
| Reaction Buffer       | 20 ml x 1  | 4 °C               |
| Substrate             | Powder x 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      | Powder x 1 | -20 °C             |
| Plate Adhesive Strips | 3 Strips   |                    |
| Technical Manual      | 1 Manual   |                    |

### Note:

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

**Positive Control:** add 1 ml Assay Buffer to 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 ml Assay 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

Weigh out 0.1 g tissue, homogenize with 1 ml Assay 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 into the microplate:

| Reagent  | Sample      | Control     | Standard    | Blank       | Positive Control |
|--|-------------|-------------|-------------|-------------|------------------|
| Reaction Buffer  | 180 $\mu$ l | 180 $\mu$ l | 180 $\mu$ l | 180 $\mu$ l | 180 $\mu$ l      |
| Substrate  | 10 $\mu$ l  | 10 $\mu$ l  | --          | --          | 10 $\mu$ l       |
| Distilled water  | --          | 10 $\mu$ l  | --          | 20 $\mu$ l  | --               |
| Standard   | --          | --          | 20 $\mu$ l  | --          | --               |
| Positive Control   | --          | --          | --          | --          | 10 $\mu$ l       |
| Sample   | 10 $\mu$ l  | --          | --          | --          | --               |
| 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 as the enzyme generates 1 nmol of p-nitrophenol per minute.

### 1. According to the protein concentration of sample

$$\begin{aligned} \text{CA (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (C_{\text{Protein}} \\ &\quad \times V_{\text{Sample}}) / T \\ &= 800 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / C_{\text{Protein}} \end{aligned}$$

### 2. According to the weight of sample

$$\begin{aligned} \text{CA (U/g)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \times \\ &\quad W / V_{\text{Assay}}) / T \\ &= 800 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / W \end{aligned}$$

### 3. According to the quantity of cells or bacteria

$$\begin{aligned} \text{CA (U/10}^4\text{)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / (V_{\text{Sample}} \\ &\quad \times N / V_{\text{Assay}}) / T \\ &= 800 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / N \end{aligned}$$

### 4. According to the volume of sample

$$\begin{aligned} \text{CA (U/mg)} &= (C_{\text{Standard}} \times V_{\text{Standard}}) \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) / V_{\text{Sample}} / \\ &\quad T \\ &= 800 \times (\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}) / (\text{OD}_{\text{Standard}} - \text{OD}_{\text{Blank}}) \end{aligned}$$

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

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

W: the weight of sample, g;

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

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

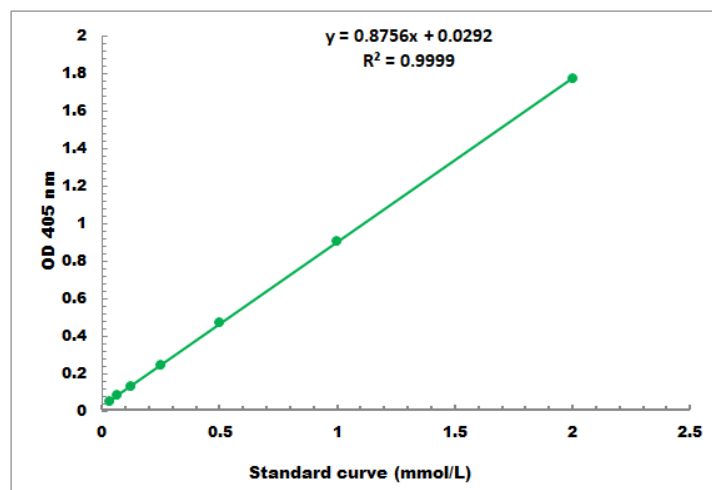
$V_{\text{Sample}}$ : the volume of sample, 0.01 ml;

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

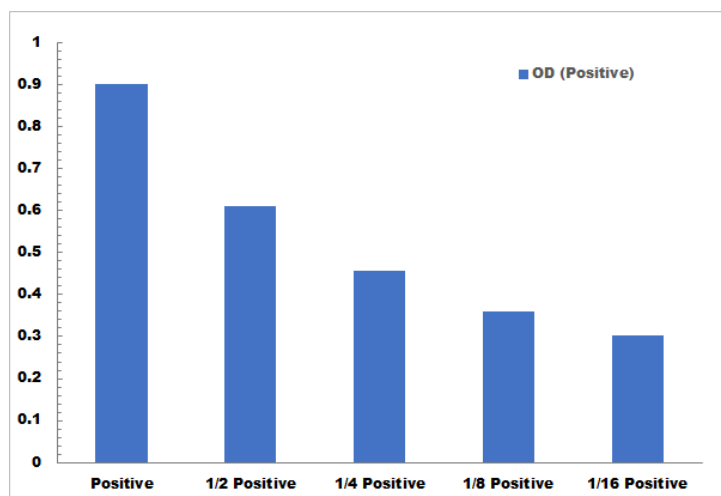
T: the reaction time, 5 minutes.

## VII. TYPICAL DATA

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



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



Positive Control reaction in 96-well plate assay with decreasing the concentration

## VIII. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to [www.cohesionbio.com](http://www.cohesionbio.com) or contact us at [techsupport@cohesionbio.com](mailto:techsupport@cohesionbio.com)

## IX. NOTES