CA / Carbonic Anhydrase Activity Assay Kit

ARG83557 CA / Carbonic Anhydrase Activity Assay Kit can be used to measure CA / Carbonic Anhydrase in Serum, Plasma, Tissue extracts, Cell lysate, Cell culture media, other biological fluids

Catalog number: ARG83557

Package: 96 wells

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PRINCIPLE OF THE ASSAY

ARG83557 CA / Carbonic Anhydrase Activity Assay Kit provides a simple and sensitive method for monitoring Carbonic Anhydrase activity in various samples. The enzyme catalyzed reaction products p-nitrophenol can be measured at a colorimetric readout at 405 nm.

MATERIALS PROVIDED & STORAGE INFORMATION

Store Standard and Positive Control at -20 °C, all other component at 2-8°C. Use the kit before expiration date.

Component	Quantity	Storage
Microplate	1 X 96-well plate	
Standard (2 µmol/mL)	1 ml	-20 °C, keep in dark
Substrate	2 vial (lyophilized)	4 °C, keep in dark
Substrate Diluent	0.3 ml	4 °C
Reaction Buffer	25 ml	4 °C
Positive Control	1 vial (lyophilized)	-20 °C
Assay Buffer	4 X 30 ml	4 °C
Plate sealer	3 adhesive strips	

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes and pipette tips
- Deionized or distilled water

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store Standard and Positive Control at-20 °C, all other component at 2-8°C. Use the kit before expiration date.
- Briefly spin down the reagents before use.
- It is highly recommended that the standards and samples be assayed in at least duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell and Bacteria - Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation. Mix and sonicate (with power 20%, sonicate 3s, interval 10s, repeat 30 times) with 1 ml Assay buffer per 5 × 10^6 cell or bacteria. Centrifuged at 10,000g 4 °C for 20 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Tissue - 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.

Liquid samples - Detect directly.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at-20°C up to 1 month or-80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

- **Substrate:** Briefly centrifuge prior to opening. Dissolve each substrate vial in **0.15 ml** Substrate Diluent before use. Make sure the Substrate is dissolved completely and mixed thoroughly before use. Keep in dark and store at -20 °C for 7 days or 4°C for 12 hours
- **Substrate Diluent:** Thaw Substrate Diluent at room temperature before use.
- **Substrate Working Solution:** Prepare ready-to-use Substrate Working Solution by mixing Reaction Buffer and Substrate at a ratio of 187:3 (v/v). Vortex thoroughly until no precipitate is visible and use immediately.
- **Positive Control:** Reconstitute the **Positive Control** with **1 ml** of Assay Buffer. Allow the **Positive Control** keep on bench for few minutes. Make sure the **Positive Control** is dissolved completely and mixed thoroughly before use. The **Positive Control** is stable for up to 4 weeks at -80°C. Mix well before use.
- **Standard**: Completely dissolved and briefly centrifuge prior to opening.

Perform 2-fold serial dilutions of the top standard solution using Reaction Buffer to make the standard curve. The concentration of standard curve could be 2 µmol/mL, 1 µmol/mL, 0.5 µmol/mL, 0.25 µmol/mL, 0.125 µmol/mL, 0.063 µmol/mL, 0.031 µmol/mL, 0.016 µmol/mL. Keep in dark and store at -20 °C for 1 month or 4°C for 3 days.

Note: Divide into small aliquots to avoid repeated freeze-thaw cycles.

ASSAY PROCEDURE

Standards and samples should be assayed in at least duplicates.

1. Add **190 µl** Substrate Working Solution into **Sample**, **Control** and **Positive Control wells**.
2. Add **180 µl** Reaction Buffer into **Standard** and **Blank wells**.
3. Add **10 µl** Distilled water into each **Control wells**.
4. Add **20 µl** Distilled water into each **Blank wells**.
5. Add **20 µl** Standard into each **Standard wells**.
6. Add **10 µl** Positive Control into each **Positive Control wells**.
7. Add **10 µl** Sample into **Sample wells.**
8. Mix well. Put in the oven, **incubate** at 37 °C for **5 min.** Read the OD **at 405 nm.**

Summary of CA / Carbonic Anhydrase Activity Assay Kit Procedure

Reagent	Sample	Control	Positive Control	Standard	Blank
Substrate Working Solution	190 µl	190 µl	190 µl		
Reaction Buffer				180 µl	180 µl
Distilled water	-	10 µl	-	-	20 µl
Standard	-	-	-	20 µl	-
Positive Control	-	-	10 µl	-	-
Sample	10 µl	-	-	-	-
Mix well. Put in the oven, **incubate** at 37 °C for **5 min.** Read the OD **at 405 nm.**					

Note:

1. Reagents must be added sequentially and should not be premixed prior to addition.

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 samples into the reaction system; or increase the reaction time; if the enzyme activity is higher, please dilute the sample, or decrease the reaction time.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of samples, standard and blank.

a.) Definition: One unit of Carbonic Anhydrase activity is defined as the enzyme generates 1 nmol of p-nitrophenol per minute.

$C_{Standard}$: the concentration of standard, 2 µmol/mL =2000 nmol/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.

According to the slope of the standard curve

Activity =

{[(ODSample- ODControl) / - Intercept] / Slope × T} x n (U/mL)

Calculate the initial activity according to sample preparation procedure.

b.) Calculation:

Formula:

a). According to the protein concentration

CA (U/mg) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times (C_{Protein} \times V_{Sample}) \times T]$

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

b). According to the weight

CA (U/mg) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times (V_{Sample} \times W / V_{assay}) \times T]$

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

c). According to the quantity of cell or bacteria

CA ($U/10^4$) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) (V_{Sample} \times N / V_{assay}) \times T]$

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

4. According to the volume

CA (U/mg) =

$(C_{Standard} \times V_{Standard}) \times (OD_{Sample} - OD_{Control}) / [(OD_{Standard} - OD_{Blank}) \times V_{Sample} \times \times T]$

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

Assay range: 0.016- 2 µmol/mL

Sensitivity: 0.016 µmol/mL