ab284550 – Carbonic Anhydrase (CA) Activity Assay Kit (Colorimetric)

For the detection of CA activity in hemolysates and serum.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab284550

Introduction

Carbonic anhydrases (CA) (4.2.1.1) are zinc enzymes present in both prokaryotes and eukaryotes. They efficiently catalyze the reversible hydration of CO_2 to bicarbonate. Their important patho-physiological roles in respiration, pH and CO_2 homeostasis, secretion, gluconeogenesis, ureagenesis etc. makes it an important drug target. Characteristic increase and decrease of CA Activity is observed in different physiological conditions and diseases such asanemia, Thalassemia, Hypothyroidism, Hyperthyroidism, in several cases of lung and liver diseases and Leukemia. Carbonic Anhydrase (CA) Activity Assay Kit (ab284550 - K472) can be used to measure CA activity in biological samples like serum and hemolysates. The assay utilizes the esterase activity of an active CA on an ester substrate which releases a chromophore. The released product can be easily quantified using an absorbance microplate reader. In the presence of a CA specific inhibitor, the enzyme loses its activity which results in a decrease of absorbance.

Sample Types

- Hemolysate, serum
- Purified CA

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
CA Assay Buffer	40 mL	-20°C
Dilution Buffer VI	1.5 mL	-20°C
CA Enzyme I	1 vial	-20°C
CA Substrate	500 µl	-20°C
CA Inhibitor I	200 µl	-20°C
Nitrophenol Standard	400 µl	-20°C

PLEASE NOTE: Dilution Buffer VI was previously labelled as CA Dilution Buffer, and CA Inhibitor I as CA Inhibitor (20 mM Acetazolamide), and CA Enzyme I as CA Positive Control. The composition has not changed.

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Multi-well absorbance microplate reader.
- 96-well clear plate with flat bottom.

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

CA Assay Buffer and Dilution Buffer VI: Warm to room temperature (RT) before use. Store at -20°C.

CA Enzyme I: Store at -20°C. Reconstitute by adding 50 µl of Dilution Buffer VI before use and aliquot. Once reconstituted, use within one month. Avoid multiple freeze thaws.

CA Substrate and CA Inhibitor I: Ready to use. Store at -20 °C. Thaw/aliquot before use. Avoid multiple freeze thaw cycles

Nitrophenol Standard: Store at -20 °C.

Assay Protocol

Sample preparations:

1. Dilute Serum/hemolysate (see Note) 10X with CA Assay Buffer. Then, use this diluted serum/hemolysate directly in your experiment.
2. For Sample (S), add 1-10 µl (in duplicates) of diluted serum/ hemolysate into desired well(s) in a 96-well plate.
3. For Background Control (BC), add same volume of CA assay buffer.
4. For Positive control (PC), add 10 µl of the CA Enzyme I into two desired well(s).
5. For Negative Control (NC), add 2 µl of the CA Inhibitor I into one of the wells containing Sample and/or CA Enzyme I, mix properly.
6. Adjust the volume of S, BC, NC and PC to 95 µl/well with CA Assay Buffer. Incubate for 15 min at RT.

Δ Note: Hemolysate preparation protocol: Collect blood in heparinized tube, centrifuged at 3000 x g for 1 min to separate RBCs from plasma. Wash 50 µl RBCs twice with 2 volumes of ice-cold saline solution (1 mM Tris, pH 8.0, 200 mM NaCl; not provided) and then collect sample by centrifugation at 3000 x g for 5 min at 4 °C. Then, lyse RBCs in 3 volumes of ice-cold buffer (1 mM Tris, pH 8.0; not provided), and place the sample on ice for 10 min. Finally, complete lysis by placing samples at -80 °C for 15 min. After lysis, centrifuge at 15000 g for 15 min to remove the cellular debris. Collect the supernatant (hemolysate) for immediate assay.

Nitrophenol Standard Curve Preparation:

1. Before use, thaw Nitrophenol Standard.
2. Add 0, 4, 8, 12, 16 and 20 µl of 2 mM Nitrophenol Standard into a series of wells in a clear 96-well plate and adjust the final volume to 100 µl/ well with CA Assay Buffer.
3. This will generate 0, 8, 16, 24, 32 and 40 nmol/well of Nitrophenol Standard respectively.
4. Mix well and measure the absorbance at 405 nm in an end-point mode.

Δ Note: For Unknown Samples, we suggest testing several dilutions to ensure the readings are within the Standard Curve range.

CA Substrate Preparation:

1. Add and mix 5 µl of CA Substrate into BC, S, NC, and PC wells. Mix well.

Δ Note: Don't add Substrate Mix to the Nitrophenol Standard wells.

Measurement

Measure absorbance at 405 nm in a kinetic mode for 1 hr. at RT. Choose two time points (t1 & t2) in the linear range of the plot and obtain the corresponding values for the absorbance (A1 and A2). Calculate ΔA/Δt.

Calculation

- Plot the Nitrophenol Standard Curve and obtain the slope of the curve (ΔA/nmol).
- If substrate background control reading is significant then subtract the background control reading from sample reading.
- To calculate the Specific CA activity of sample, subtract ΔA of Negative Control (ΔANC) from Sample (ΔAS).

$$\text{Serum Specific CA Activity} = \frac{\boldsymbol{B \times D \times 1000}}{\boldsymbol{\Delta t \times V}} \left(\frac{\boldsymbol{mU}}{\boldsymbol{ml}}\right)$$

$$\text{Hemolysate Specific CA Activity} = \frac{\boldsymbol{B} \times \boldsymbol{D}}{\boldsymbol{\Delta t} \times \boldsymbol{V} \times \boldsymbol{P}} \left(\frac{\boldsymbol{mU}}{\boldsymbol{g\ Hemoglobin}} \right)$$

Where: **B** = Released Nitrophenol in sample based on the Standard Curve slope (nmol)
D = Dilution Factor (D = 1 when samples are undiluted)
1000 = 1 ml = 1000 µl
Δt = Reaction time (min)
V = Sample volume (µl)
P = Hemolysate concentration (g Hemoglobin/µl)

Specific CA Activity = ΔAS - ΔANC
Where Hb concentration is determined by Hemoglobin Assay Kit (Colorimetric) (Cat. No. ab234046)
Unit Definition: One unit of CA activity is the amount of enzyme that catalyzes the release of 1 µmol of nitrophenol per min from the substrate under the assay conditions at 25 °C.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)