Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

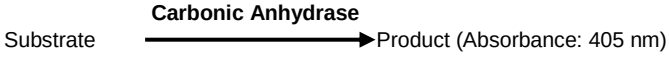
Carbonic Anhydrase Activity Assay Kit (Colorimetric)

rev 12/20

(Catalog # K472-100; 100 assays, Store kit at -20 °C)

I. 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. **BioVision's CA Activity Assay Kit** 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. This assay kit is simple and can be used to measure CA activity in biological samples like hemolysate, serum and purified CA activity, CA activity in biological samples like hemolysate and serum in a high-throughput format. To measure the Specific CA activity we have used the specific inhibitor Acetazolamide, which is a potent inhibitor of CA I, CA II, CA IV, CA IX, CA XII etc. except CA III.



II. Applications:

- Detection of CA activity in hemolysates and serum.
- Determination of enzymatic activity of purified CA.

III. Sample Type:

- Hemolysate, serum
- Purified CA

IV. Kit Contents:

Components	K472-100	Cap Code	Part Number
CA Assay Buffer	40 ml	NM	K472-100-1
CA Dilution Buffer	1.5 ml	Clear	K472-100-2
CA Positive Control	1 vial	Green	K472-100-3
CA Substrate	500 µl	Brown	K472-100-4
CA Inhibitor (20 mM Acetazolamide)	200 µl	Blue	K472-100-5
Nitrophenol Standard (2 mM)	400 µl	Yellow	K472-100-6

V. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. Clear plates are required for this assay.
- Multi-well absorbance microplate reader.

VI. Storage Conditions and Reagent Preparation:

Store kit at -20 °C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the assay**.**

- **CA Assay Buffer and CA Dilution Buffer:** Store at -20 °C or 4 °C. Bring to room temperature (RT) before use
- **CA Positive Control**: Store at -20 °C. Reconstitute by adding 50 µl CA Dilution buffer per tube before use and aliquot. Once reconstituted, use within one month. Avoid multiple freeze thaws.
- **CA Substrate**: Ready to use. Store at -20 ºC. Thaw and aliquot before use. Avoid multiple freeze thaw cycles.
- **CA Inhibitor**: Ready to use. Store at -20 °C. Thaw before use. Avoid multiple freeze thaw of the inhibitor.
- **Nitrophenol Standard**: Store at -20 °C.

VII. CA Activity Assay Protocol:

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

   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 ºC. 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 ºC for 15 min. After lysis, centrifuge at 15000 g for 15 min to remove the cellular debris. Collect the supernatant (hemolysate) for immediate assay.

2. **Nitrophenol Standard Curve Preparation:** Before use, thaw Nitrophenol Standard. 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. This will generate 0, 8, 16, 24, 32 and 40 nmol/well of Nitrophenol Standard respectively. Mix well and measure the absorbance at 405 nm in an end-point mode.

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

3. **CA Substrate Mix:** Add and mix 5 µl of CA Substrate into BC, S, NC and PC wells. Mix well.
   Note: Don't add Substrate Mix to the Nitrophenol Standard wells.
4. **Measurement:** Measure absorbance at 405 nm in a kinetic mode for 1 hr. at RT. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the absorbance (A_1 and A_2). Calculate ΔA/Δt.
5. **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 (ΔA_{NC}) from Sample (ΔA_S).

$$\textbf{Serum Specific CA Activity} = \frac{\mathbf{B} * \mathbf{D} * \mathbf{1000}}{\Delta \mathbf{t} * \mathbf{V}} \left(\frac{\mathbf{mU}}{\mathbf{ml}}\right)$$

$$\textbf{Hemolysate Specific CA Activity} = \frac{\mathbf{B} * \mathbf{D}}{\Delta \mathbf{t} * \mathbf{V} * \mathbf{P}} \left(\frac{\mathbf{mU}}{\mathbf{g\ Hemoglobin}}\right)$$

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 = $\Delta A_S - \Delta A_{NC}$
Where Hb concentration is determined by Hemoglobin Colorimetric Assay Kit (K219)

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 ºC.

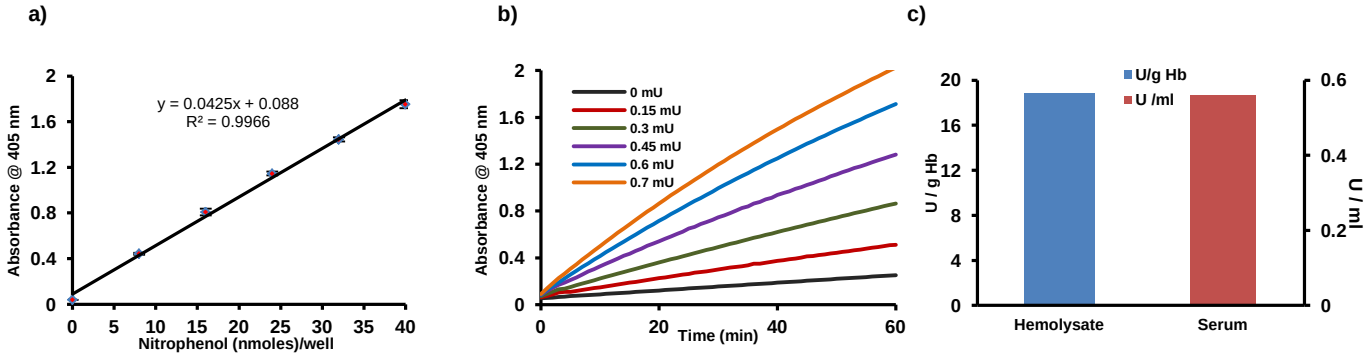


Figure: (a) Nitrophenol-Standard Curve (8-40 nmol), error bars indicate SD (n=3). **(b) Kinetic activity curves** using different amounts of CA Positive Control in the assay. **(c) Specific CA activity** in hemolysate and serum was measured after diluting 10X and then 2 µl of the diluted sample was used for the assay.

VIII. RELATED PRODUCTS:

Carbonic Anhydrase 3, human recombinant (7833)
Carbonic anhydrase-1, human recombinant (P1048)
Carbonic anhydrase-8, human recombinant (P1047)
E. coli Recombinant Carbonic anhydrase (P1049)
Human CellExp™ Carbonic Anhydrase 10/CA10, human recombinant (7485)
Human CellExp™ Carbonic Anhydrase 2/CA2, human recombinant (7479)
Human CellExp™ Carbonic Anhydrase 4/CA4, human recombinant (7484)
Human CellExp™ Carbonic Anhydrase 9/CA9, human recombinant (7478)
Human Recombinant Carbonic anhydrase 2 (6390)
Carbonic Anhydrase (CA) Inhibitor Screening kit (Colorimetric) (K473)
Hemoglobin Colorimetric Assay kit (K219)

FOR RESEARCH USE ONLY! Not to be used on humans.