Technical Bulletin

Carbonic Anhydrase (CA) Activity Assay Kit (Colorimetric)

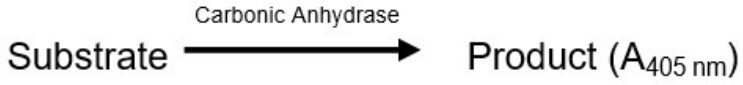
Catalog Number MAK404

Product Description

Carbonic anhydrases (CA) 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. make carbonic anhydrases an important drug target. Characteristic increase or decrease of CA activity is observed in different physiological conditions and diseases such as anemia, thalassemia, hypothyroidism, hyperthyroidism, and in several cases of lung and liver diseases and leukemia.

The Carbonic Anhydrase (CA) Activity Assay Kit can be used to measure CA activity in biological samples. The assay utilizes the esterase activity of active CA on an ester substrate which releases a chromophore. The released product can be easily quantified spectrophotometrically. In the presence of a CA specific inhibitor, the enzyme activity is blocked, which results in a decrease of absorbance. The assay kit is simple and suitable for high-throughput applications. To measure specific CA activity, the CA inhibitor Acetazolamide is included. Acetazolamide is a potent inhibitor of CA I, CA II, CA IV, CA IX, CA XII, etc., but does not inhibit CA III.

The kit is suitable for the detection of carbonic anhydrase activity in hemolysates and serum, as well as the determination of enzymatic activity of purified CA.



Components

The kit is sufficient for 100 colorimetric assays in 96-well plates.

- CA Assay Buffer 40 mL
  Catalog Number MAK404A
- CA Dilution Buffer 1.5 mL
  Catalog Number MAK404B
- CA Positive Control 1 vial
  Catalog Number MAK404C
- CA Substrate 500 µL
  Catalog Number MAK404D
- CA Inhibitor 200 µL
  (20 mM Acetazolamide)
  Catalog Number MAK404E
- Nitrophenol Standard (2 mM) 400 µL
  Catalog Number MAK404F

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (including multichannel pipettor)
- 96 well clear flat-bottom plate. Cell culture or tissue culture treated plates are **not** recommended.
- Spectrophotometric multiwell plate reader
- Refrigerated microcentrifuge capable of RCF ≥15,000 × *g*
- Trizma® base (Catalog Number T1503 or equivalent)
- Sodium chloride (NaCl) (Catalog Number S9888 or equivalent)
- Hemoglobin Assay Kit (optional, only required for hemolysate CA determination) (Catalog Number MAK115 or equivalent)

Precautions and Disclaimer

For Research Use Only. Not for use in diagnostic procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store kit at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials at low speed prior to opening.

CA Assay Buffer and CA Dilution Buffer: Store at -20 °C or 2-8 °C. Bring to room temperature prior to use.

CA Positive Control: Store at -20 °C. Reconstitute vial with 50 µL of CA Dilution Buffer. Aliquot and store at -20 °C. Avoid repeated freeze/thaw cycles. Use within one month after reconstitution.

CA Substrate: Ready to use. Thaw and aliquot before first use. Store aliquots at -20 °C. Avoid repeated freeze/thaw cycles.

CA Inhibitor: Ready to use. Thaw and aliquot before first use. Store aliquots at -20 °C. Avoid repeated freeze/thaw cycles.

Tris Buffered Saline (TBS): Prepare 1 mM Tris using Trizma® base (not included). Adjust pH to pH 8.0 at 25 °C. Add 200 mM NaCl (not included).

Lyse Buffer: Prepare 1 mM Tris using Trizma® base (not included). Adjust pH to pH 8.0 at 25 °C.

Procedure

All samples and standards should be run in duplicate.

Sample Preparation

Serum

1. Dilute serum 10× with CA Assay Buffer.
2. Add 1-10 µL (in duplicate) of diluted serum into desired wells in a 96-well plate. For unknown samples, test several doses to ensure the readings are within the Standard Curve range.
3. Adjust the total volume of each Sample well to 95 µL with CA Assay Buffer. Mix well.

Hemolysate

1. Collect blood in a heparinized tube.
2. Centrifuge at 3000 × *g* for 1 minute to separate red blood cells from plasma.
3. Wash 50 µL red blood cells twice with 100 µL of **ice-cold Tris Buffered Saline (TBS)**.
4. Centrifuge at 3000 × *g* for 5 minutes at 4 °C.
5. Lyse red blood cells with 150 µL of **ice-cold Lyse Buffer** and place the sample on ice for 10 minutes.
6. Complete lysis by placing samples at -80 °C for 15 minutes.

7. Centrifuge at 15000 × *g* for 15 minutes to remove the cellular debris.
8. Collect the supernatant (hemolysate).
9. Determine hemolysate hemoglobin concentration in g /µL using Hemoglobin Assay Kit (Catalog Number MAK115) or preferred method.
10. Dilute hemolysate 10× with CA Assay Buffer.
11. Add 1-10 µL (in duplicate) into desired wells in a 96-well plate. For unknown samples, test several doses to ensure the readings are within the Standard Curve range.
12. Adjust the total volume of each Sample well to 95 µL with CA Assay Buffer. Mix well.

Positive Control (PC)

Add 10 µL of CA Positive Control and 85 µL of CA Assay Buffer into desired well(s). Mix well.

Background Control (BC)

Add 95 µL of CA Assay Buffer into desired well(s).

Negative Control (NC)

Add 2 µL of CA Inhibitor (20 mM Acetazolamide) into desired well(s). Add the same volume of sample as used in Sample well(s) (1-10 µL). Adjust the total volume to 95 µL with CA Assay Buffer. Mix well.

Alternatively, add 2 µL of CA Inhibitor (20 mM Acetazolamide) into desired well(s). Add 10 µL of CA Positive Control and 83 µL of CA Assay Buffer. Mix well.

Standard Curve Preparation

Prior to use, thaw Nitrophenol Standard (2 mM) to room temperature. Prepare Nitrophenol Standards according to Table 1.

Table 1.
Preparation of Nitrophenol Standards

Well	2 mM Nitro-phenol	CA Assay Buffer	Nitrophenol (nmol/well)
1	0 µL	100 µL	0
2	4 µL	96 µL	8
3	8 µL	92 µL	16
4	12 µL	88 µL	24
5	16 µL	84 µL	32
6	20 µL	80 µL	40

Mix well and measure the Standards at 405 nm (A_{405}) in end-point mode.

Assay Reaction

1. Incubate all Sample, Positive Control, Background Control, and Negative Control wells for 15 minutes at room temperature.

2. Add 5 µL of CA Substrate into each Sample, Positive Control, Background Control, and Negative Control wells. Mix well. Do **not** add Substrate Mix to the Nitrophenol Standard wells.

Measurement

Measure absorbance at 405 nm (A_{405}) in kinetic mode for 1 hour at room temperature.

Results

1. Choose two time points (T_1 and T_2) in the linear range of the plot and obtain the corresponding values for the absorbance (A_1 and A_2).
2. Calculate $\Delta A/\Delta T$.
3. Plot the Nitrophenol Standard Curve and obtain the slope of the curve (ΔA/nmol).

4. If the Background Control (BC) reading is significant, subtract the Background Control reading from Sample reading(s).
5. To calculate the Specific CA activity of Sample, subtract ΔA of Negative Control (ΔA_{NC}) from Sample (ΔA_S).
6. Calculate CA Activity:

Serum Specific CA Activity (mU/mL) =

$$\frac{B \times D \times 1000}{\Delta T \times V}$$

Hemolysate Specific CA Activity (mU/g Hemoglobin) =

$$\frac{B \times D}{\Delta T \times V \times P}$$

where:

B = Released Nitrophenol in sample based on standard curve slope (nmol)

D = Dilution Factor (D=1 when Samples are undiluted)

1000 = 1 ml ≡ 1000 µL

ΔT = Reaction time (minutes)

V = Sample volume (µL)

P = Hemolysate hemoglobin concentration (g /µL)

Unit Definition: One unit of CA activity is the amount of enzyme that catalyzes the release of 1 µmol of nitrophenol per minute from the substrate under the assay conditions at 25 °C.

Figure 1.
Typical Nitrophenol Standard Curve. (8-40 nmol), error bars indicate SD (n=3).

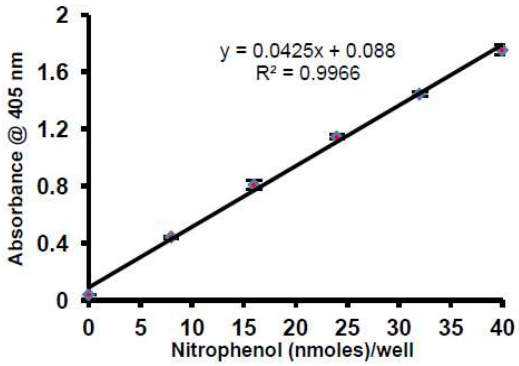


Figure 2.
Kinetic activity curves using different amounts of CA Positive Control in the assay.

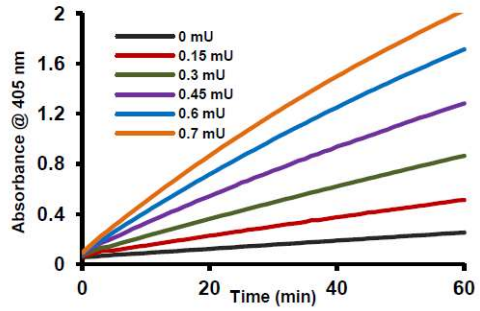
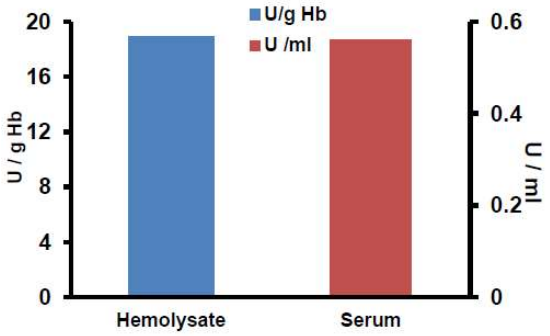


Figure 3.
Specific CA activity. Hemolysate and serum were measured after diluting 10× and then 2 µL of the diluted samples were used for this assay.



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