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Carbonic Anhydrase (CA) Inhibitor Screening Kit (Colorimetric)

12/16

(Catalog # K473-100; 100 assays; Store at -20°C)

I. Introduction:

Carbonic anhydrases (CA; EC: 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. make it an important drug target. CA inhibition in different organs can result in different effects. Inhibition of CA helps in different disease treatment procedures; for example, it can help in the treatment of Glaucoma, urine alkalization, managing epilepsy, sleep apnea, acute mountain sickness etc. BioVision's CA Inhibitor Screening Kit can be used to screen for potent inhibitors of CA activity. It utilizes the esterase activity of an active CA on an ester substrate which releases a chromogenic product. 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 decrease of absorbance. This assay kit is simple and can be used to identify and characterize CA inhibitors in a high-throughput format. Here, we have used the specific inhibitor Acetazolamide, a potent inhibitor of CA I, CA II, CA IV, CA IX, CA XII etc. except CA III.

Substrate $\xrightarrow{\text{Carbonic Anhydrase}}$ Product (Absorbance: 405 nm)

Substrate $\xrightarrow[\text{+ CA inhibitor}]{\text{Carbonic Anhydrase}}$ Decrease in absorbance (Absorbance: 405 nm)

II. Applications:

- Screening/characterizing inhibitors/ligands of CA

III. Kit Contents:

Components	K473-100	Cap Code	Part Number
CA Assay Buffer	20 ml	WM	K473-100-1
CA Dilution buffer	1.5 ml	Clear	K473-100-2
CA Enzyme	1 vial	Green	K473-100-3
CA Substrate	500 µl	Brown	K473-100-4
CA Inhibitor (2 mM Acetazolamide)	200 µl	Blue	K473-100-5

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well Absorbance microplate reader

V. Storage Conditions and Reagent Preparation:

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

- **CA Assay Buffer and CA Dilution Buffer:** Store at -20 °C or 4 °C. Bring to room temperature before use.
- **CA Enzyme:** Store at -20°C. Reconstitute by adding 500 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°C. Thaw and aliquot before use. Avoid multiple freeze thaw.
- **CA Inhibitor:** Ready to use. Store at -20°C. Thaw before use. Avoid multiple freeze/thaw of the inhibitor.

VI. CA Inhibitor Screening Protocol:

1. **CA Enzyme Working Solution Preparation**: To each well (Enzyme Control-EC, Sample-S, Inhibitor Control-IC and Solvent Control-SC, Background control -BC), add:

	BC	EC	S/SC/IC
CA Assay Buffer	85 µl	90 µl	80 µl
CA Enzyme	----	5 µl	5 µl

Mix well and add each mix to the corresponding wells.

2. **Screening Compounds, Inhibitor Control & Enzyme Control Preparations:** Dissolve candidate inhibitors at 10X highest final test concentration using preferred solvent (eg. DMSO). Add 10 µl test inhibitors (S, BC) or inhibitor solvent (SC). For Inhibitor Control (IC), add 10 µl CA Inhibitor into IC well(s), and BC well. Incubate at room temperature (RT) for 10 min.

   Note: High solvent concentration might affect the enzymatic activity. Prepare parallel well(s) as Solvent Control to test the effect of the solvent on enzyme activity (same as EC in presence of final solvent concentration). We recommend that every test compound is run alongside with its own background control as it may affect the signal from the probe and result in false negative result.

3. **CA Substrate:** Add and mix 5 µl of CA Substrate into BC, EC, S, SC and IC wells. Mix well.
4. **Measurement:** Measure absorbance at 405 nm in a kinetic mode for 1 hr at room temperature.
5. **Calculations**: Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the Absorbance (Ab_1 and Ab_2). Calculate the slope for all samples, ΔAbsorbance/Δt**.**

$$\%\ \textbf{Relative activity} = \frac{\Delta\ \textbf{Ab of S}}{\Delta \textbf{Ab of EC}} \times 100$$

$$\%\ \textbf{Relative Inhibition} = \frac{\Delta\ \textbf{Ab of EC} - \Delta\ \textbf{Ab of S}}{\Delta \textbf{Ab of EC}} \times 100$$

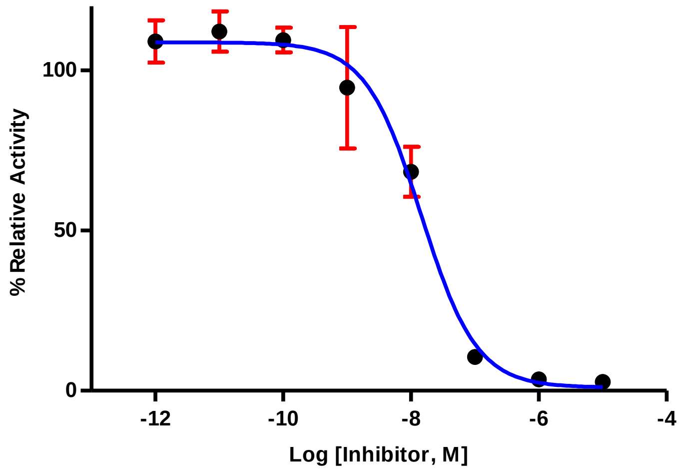

Figure: Inhibition of CA activity by CA Inhibitor (Acetazolamide), IC_{50} = 16.3 ± 2 nM (n = 3). Assay was performed following the kit protocol.

VII. 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)

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