High-throughput Carbonic Anhydrase Activity and Inhibitor Screening Assays

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Introduction

Carbonic Anhydrase (CA) is a zinc based metalloprotease present in at least 14 different isoforms in mammals. It catalyzes the reversible hydration of carbon dioxide to form bicarbonate and proton, thus playing an important role in pH and CO_2 homeostasis. Changes in CA activity are associated with various diseases such as - glaucoma, type II diabetes mellitus, lung, liver diseases etc. As CA is also an important target for cancer therapy, a robust and sensitive high-throughput carbonic anhydrase activity and inhibitor screening assays are very important tools.

Carbonic anhydrase activity or carbon dioxide hydration rate is widely measured by using potentiometer or a stopped-flow instrument as reflected by the change in pH over time. However, this method is not suitable for high-throughput assay. Consequently, in BioVision Inc. we have launched the first high throughput Carbonic Anhydrase Activity Assay Kit, detecting the esterase activity of this enzyme. This kit utilizes the zinc-hydroxide mechanism for carbon dioxide hydration and hydrolysis of an ester substrate resulting in the release of a chromophore signal that is proportional to the enzyme activity.

CA Hydration and Esterase Activity

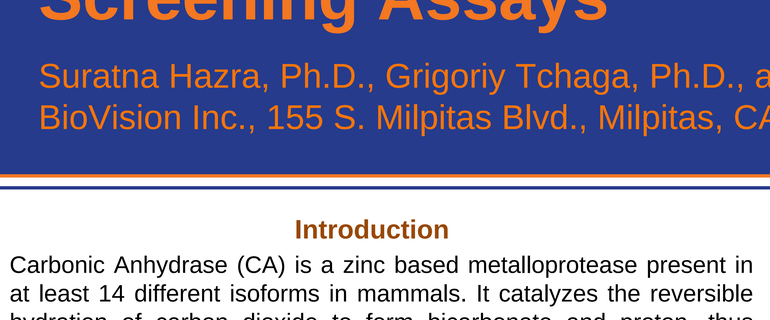


Figure 1. Catalytic Cycle for Human CA II Catalyzed (a) Hydration of CO2 to HCO3 and H+ and (b) Ester Hydrolysis to Carboxylic Acid and Alkoxide

CA Assay Scheme

Substrate —(Carbonic Anhydrase)→ **Product (Absorbance: 405 nm)**

Substrate —(Carbonic Anhydrase; CA inhibitor)→ **Decrease in Absorbance (Absorbance : 405 nm)**

A Plate Based Spectrophotometric Assay to Measure CA Activity with Intra and Inter Assay Variability

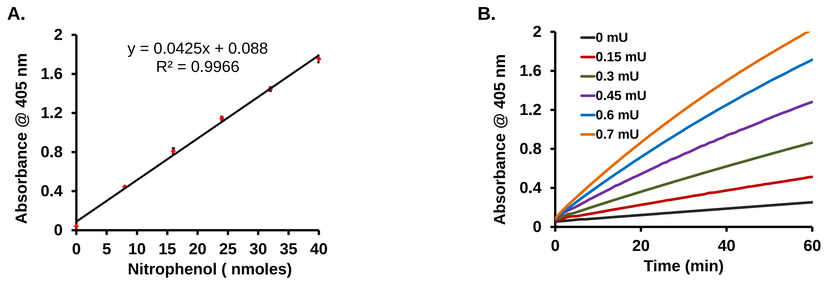
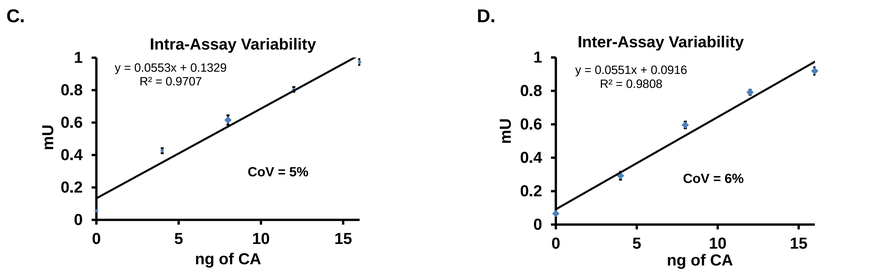


Figure 2. A) Nitrophenol-Standard Curve (8-40 nmole), error bars indicate SD (n=3). **B) Kinetic activity curves** using different amounts of CA Positive Control in the assay .



CA activity in mU per ng: C) Intra-assay Variation and Coefficient of variation (n=3). **D)** Inter-assay Variation and Coefficient of Variation (n=3).

Application of Plate Based CA Activity Assay Kit

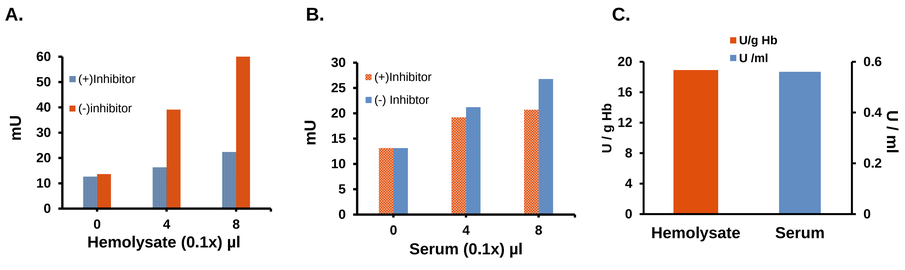


Figure 3. A) CA activity in hemolysate: Difference between the activity in absence and presence of inhibitor accounts for the Specific CA activity in hemolysate. **B) CA activity in serum**: Difference between the activity in absence and presence of inhibitor accounts for the specific CA Activity in serum. **C) Specific CA activity**: in hemolysate and serum were measured after 10x sample dilution with 2 µl of the diluted samples used in the assay.

Spike Recovery CA Activity in Hemolysate and Serum

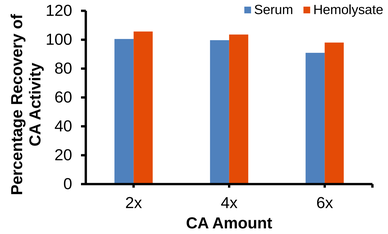


Figure 4. Spike recovery of CA activity in Serum and Hemolysate: Samples were spiked with known amounts of CA and measured using BioVision's CA Activity Assay Kit. The recovery was always ~100%.

IC_{50} Determined by BioVision's CA Inhibition Screening Kit

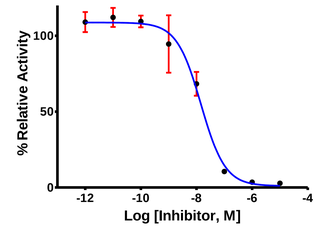


Figure 5. Figure: Inhibition of CA activity by CA Inhibitor (Acetazolamide), IC_{50} = 16.3 ± 2 nM (n = 3). Assay was performed following the kit protocol.

Key Advantages of Carbonic Anhydrase Activity Assay Kit

- **High-throughput homogenous Assay with Simple Protocol:** Direct Spectrophotometric Assay
- **Specific inhibitor is provided to measure specific enzyme activity**
- **Stable reagents**
- **Accurate reliable results:** Reproducible results, 100 % recovery of activity in spiking experiment
- **Versatile sample applications:**

Applications

- **Measurement of CA Activity in Serum and Hemolysate**
- **Measurement of activity of purified CA**
- **Screening / Characterizing ligands/ inhibitors of CA**

Conclusions

BioVision's Carbonic Anhydrase Activity and Inhibitor Screening Assays provide valuable tools for drug discovery and identifying potential modulators of CA Activity in a high-throughput format. The assay kits are commercially available at www.biovision.com Cat # K472-100 and K473-100.