ab283387 – Carbonic Anhydrase (CA) Inhibitor Screening Kit (Colorimetric)

For the screening of Carbonic Anhydrase 1/CA inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283387

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer 16	25 mL	-20°C
Dilution Buffer VI	1.5 mL	-20°C
CA Enzyme II	1 vial	-20°C
CA Substrate	500 µl	-20°C
CA Inhibitor II	200 µl	-20°C

PLEASE NOTE: Assay Buffer 16 was previously labelled as Assay Buffer XVI and CA1 Assay Buffer. 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:

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

Reagent Preparation

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

Assay Buffer 16 and Dilution Buffer VI: Store at -20 °C or 4 °C. Bring to room temperature before use.

CA Enzyme II: Store at -20°C. Reconstitute by adding 500 µl Dilution Buffer VI/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 II: Ready to use. Store at -20°C. Thaw before use. Avoid multiple freeze/thaw of the inhibitor.

Assay Protocol

Screening Compounds, Inhibitor Control & Background Control preparations:

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

	BC	EC	S/SC/IC
Assay Buffer 16	85 µL	90 µL	80 µL
CA Enzyme II	--	5 µL	5 µL

Mix well and add each mix to the corresponding wells

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.*

CA Substrate: Add and mix 5 µl of CA Substrate into BC, EC, S, SC and IC wells. Mix well.

Measurement

Measure absorbance at 405 nm in a kinetic mode for 1 hr at room temperature.

Calculation:

Choose two time points (t1 & t2) in the linear range of the plot and obtain the corresponding values for the Absorbance (Ab1 and Ab2). Calculate the slope for all samples, ΔAbsorbance/ Δt.

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

$$\% \text{ Relative activity} = \frac{\Delta Ab\ of\ EC - \Delta Ab\ of\ S}{\Delta Ab\ of\ (EC)} \times 100$$

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)