ab283399 – ENO1 Inhibitor Screening Kit (Colorimetric)

For the screening of ENO1 inhibitors.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283399

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
Enolase Assay Buffer	25 mL	-20°C
Red Probe	0.2 mL	-20°C
Enolase Substrate	1 vial	-20°C
Enolase Converter	1 vial	-20°C
Enolase Developer	1 vial	-20°C
Enolase 1	15 µl	-20°C
Enolase Inhibitor Control	1 vial	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Multi-well spectrophotometer (ELISA reader)
- 96-well clear plate with flat bottom.

Reagent Preparation

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

Enolase Assay Buffer: Warm to room temperature (RT) before use.

Enolase Substrate, Enolase Converter and Enolase Developer: Reconstitute each vial with 220 µl Enolase Assay Buffer. Aliquot and store at -20°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.

Enolase 1: Ready to use. Store at -20°C.

Enolase Inhibitor Control: Reconstitute with 100 µl dH_2O. Aliquot and store at -20°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.

Assay Protocol

Screening Compounds, Inhibitor Control and Background Control preparations:

1. Dissolve candidate inhibitors into an appropriate solvent at highest concentration to be tested.
2. Dilute to 2X desired test concentration with Enolase Assay Buffer.
3. Add 50 µl diluted candidate inhibitor or Enolase Assay Buffer into desired wells for Sample Screen [S], and Enzyme Control [EC] (no inhibitor) respectively.
4. For Inhibitor Control (IC), dilute Inhibitor Control 50 times by adding 10 µl Inhibitor Control to 490 µl Enolase Assay Buffer.
5. Add 50 µl of diluted Inhibitor Control into desired well(s).

Δ Note: *Solvents used to solubilize the inhibitors might affect the enzymatic activity. Prepare a [SC] to test the effect of solvent on the enzymatic activity.*

Enolase Enzyme Preparation:

1. Dilute Enolase 1:50 with Enolase Assay Buffer (i.e. dilute 2 µl of Enolase into 98 µl Assay buffer, mix well). Prepare enough Diluted Enolase Enzyme for the number of assays to be performed.
2. Add 5 µl diluted Enolase Enzyme into Candidate compounds, Enzyme Control Inhibitor and Solvent Control.
3. Incubate for 5 minutes at 25°C.

Δ Note: *Discard the unused diluted Enolase.*

Substrate Solution Preparation:

1. Make enough reagents for the number of assays to be performed. For each well, prepare 45 µl of Substrate solution containing:

Reaction Mix	Volume
Enolase Assay Buffer	37 µl
Enolase Substrate	2 µl
Enolase Converter	2 µl
Enolase Developer	2 µl
Red Probe	2 µl

2. Mix and add 45 µl of Substrate solution into each well. Mix well with gentle shaking.

Measurement

Measure OD 570 nm in kinetic mode for 5-30 minutes at 25°C. Choose two time points (t1 and t2) in the linear range of the plot and obtain the corresponding values for the OD 570 nm (OD1 and OD2).

Calculation:

1. Calculate the slope for all samples, including Enzyme Control (EC) as 100%, by dividing the net ΔOD (= OD2 - OD1) value by the time Δt (= t2 - t1).
2. Calculate % relative inhibition as follows:

$$\%\ \text{Relative inhibition} = \frac{slope\ of\ EC - slope\ of\ S}{slope\ of\ EC} \times 100$$

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

Where Slope of EC is the slope of Enzyme Control Slope of S is the slope of Sample Screen.

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)