ab283390 – Soluble Epoxide hydrolase Inhibitor Screening Kit (Fluorometric)

For the screening of potential Soluble Epoxide hydrolase inhibitors.
For research use only- not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283390

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
Epoxide hydrolase Assay Buffer	25 ml	-20°C
Epoxide hydrolase Substrate	200 µL	-20°C
Human Epoxide hydrolase Enzyme	1 vial	-20°C
NCND (5 mM)	100 µL	-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
- 96-well white plate with a clear flat bottom.
- DMSO or appropriate solvent to dissolve test compound

Reagent Preparation

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

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

Epoxide hydrolase Substrate: Thaw Epoxide hydrolase substrate at room temperature before use. **DO NOT EXPOSE EPOXIDE HYDROLASE SUBSTRATE TO LIGHT.**

NCND: Store in the dark. Place on ice before use and dilute in Epoxide hydrolase Assay Buffer at 1:5 to obtain 1 mM solution.

Human Epoxide hydrolase Enzyme: Reconstitute in 220 µL Epoxide hydrolase Assay Buffer. Aliquot and store at -20°C. Reconstituted enzyme is stable for at least 3 months.

Δ Note: Keep enzyme and NCND on ice while performing the assay.

Assay Protocol

Test Compound preparation:

1. Dissolve the test compound in appropriate solvent.
2. Prepare 100 X stock solution in the solvent and dilute it with Soluble Epoxide hydrolase Assay Buffer to prepare 10 X. For example , if the concentration to be tested is 1 µM, prepare 100 µM (100 X) stock solution in appropriate solvent and dilute it in Soluble Epoxide hydrolase Assay Buffer at 1:10 to obtain 10 µM (10X). Add 10 µL test compound to each well of the clear 96 well plate.
3. For "Solvent Control", dilute the solvent used for test compound preparation in Soluble Epoxide hydrolase Assay Buffer at 1:10 and add 10 µL into solvent control wells.
4. For "Inhibitor Control", add 10µL of the prepared 1mM NCND, the provided Soluble Epoxide hydrolase inhibitor.
5. Bring up the volume to 40 µL to each well by adding 30 µL of Soluble Epoxide hydrolase Assay Buffer.
6. For "Enzyme Control" well, add 40 µL Soluble Epoxide hydrolase Assay Buffer and for "Background Control" add 80 µL Soluble Epoxide hydrolase Assay Buffer to desired wells.

Reaction mix:

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 40 µL Mix containing:

	Reaction Mix
Epoxide hydrolase Assay Buffer	38 µL
Human Epoxide hydrolase Enzyme	2 µL

2. Mix well and add Reaction Mix to wells containing the Enzyme Control, Inhibitor Control, Solvent Control and Test Compounds.
3. Incubate plate at RT for 10 minutes before adding substrate. Avoid introducing bubbles into the wells.
   DO NOT ADD REACTION MIX TO "BACKGROUND CONTROL" WELL

Substrate Mix:

1. Prepare enough substrate mix for number of reactions to be performed. For each well prepare 20 µL Substrate Mix containing:

	Substrate Mix
Epoxide hydrolase Assay Buffer	18 µL
Human Epoxide hydrolase Enzyme	2 µL

2. Add 20 µL of Soluble Epoxide hydrolase Substrate Mix to each well using a multichannel pipette.

Δ Note: Have the plate reader ready at Ex/Em: 362/460 nm on Kinetic mode set to record fluorescence every 30 seconds at 25°C.

Measurement

Start recording fluorescence at Ex/Em: 362/460 nm after adding the substrate at 30 second intervals for 15-30 minutes.

Calculation:

1. Subtract "background control" RFU values from "enzyme control", "solvent control" and "test compound" RFU values.
2. Obtain Δ RFU for all reactions by subtracting the background subtracted RFU at time t1 from background subtracted RFU at time t2, such that t2 and t1 is within a linear range of the assay.

3. Calculate slope for all reactions, including “enzyme control” by dividing ΔRFU by time Δt (t2 – t1).
4. If “Solvent Control” slope is significantly different from “Enzyme Control” slope, use its values instead of “Enzyme Control” in the calculations shown below.

$$\% \text{ Inhibition} = \frac{(slope\ of\ (enzyme\ control) - slope\ of\ (test\ compound))}{slope\ of\ (enzyme\ control)} \times 100$$

$$\% \text{ Relative activity} = \frac{slope\ of\ (test\ compound)}{slope\ of\ (enzyme\ control)} \times 100$$

Technical Support

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