



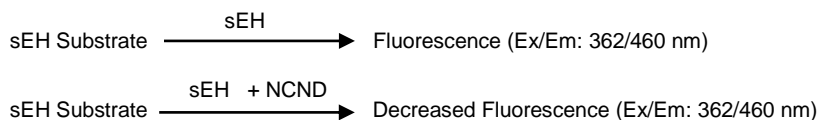
Soluble Epoxide Hydrolase Inhibitor Screening Kit (Fluorometric)

rev 03/19

(Catalog # K480-100; 100 assays; Store at -20°C)

I. Introduction:

Soluble Epoxide Hydrolase (EC 3.3.2.10) is a cytosolic enzyme with highest expression in the liver but present ubiquitously in several organs including the kidney, pancreatic islets, pituitary gland, lymphoid tissues, muscles, and the gastrointestinal tract. It catalyzes the hydrolysis of epoxyeicosatrienoic acids (EETs), i.e., epoxides derived from cytochrome P-450 mediated metabolism of arachidonic acid, to form vicinal diols. The EETs cause vasodilation and have anti-inflammatory actions. Since the EETs are hydrolyzed to diols through soluble epoxide hydrolase, the inhibition of this enzyme is likely to enhance the beneficial properties of EETs. This makes soluble epoxide hydrolase a very useful target for cardiovascular and pain medicine. BioVision's Soluble Epoxide Hydrolase Inhibitor Screening Kit is a micro-plate based fluorometric kit for screening sEH inhibitors. Its assay principle is based on the hydrolysis of a non-fluorescent substrate to a fluorescent product by sEH. It uses *N*-Cyclohexyl-*N*-dodecylurea (NCND), a well-known sEH inhibitor, as positive control. The kit provides a simple and fast method to screen/ characterize potential sEH inhibitors.



II. Applications:

- Screening of potential inhibitors of soluble epoxide hydrolase

III. Kit Contents:

| Components | K480-100 | Cap Code | Part Number |
|------------------|----------|----------|-------------|
| sEH Assay Buffer | 25 ml | WM | K480-100-1 |
| sEH Substrate | 200 µl | Blue | K480-100-2 |
| NCND (5 mM) | 100 µl | Orange | K480-100-3 |
| Human sEH Enzyme | 1 vial | Green | K480-100-4 |

IV. User Supplied Reagents and Equipment:

- 96-well white/clear plate with flat bottom
- Multi-well spectrophotometer
- DMSO or appropriate solvent to dissolve test compound.

V. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C, protected from light. Briefly centrifuge small vials before opening. Read entire protocol before performing the assay. Components are stable for at least three months.

- **sEH Assay Buffer:** Warm to room temperature before use.
- **sEH Substrate:** Aliquot and store at -20°C in the dark. Thaw sEH substrate at room temperature before use. **DO NOT EXPOSE sEH SUBSTRATE TO LIGHT.**
- **NCND:** Store at -20°C in the dark. Place on ice before use and dilute in sEH Assay Buffer at 1:5 to obtain 1 mM solution.
- **sEH Enzyme:** Lyophilized enzyme is stable for 12 months at -20°C. Reconstitute in 220 µl sEH Assay Buffer. Aliquot and store at -20°C. Reconstituted enzyme is stable for at least 3 months.

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

VI. sEH Inhibitor Screening Assay Protocol:

1. Test compound preparation: Dissolve the test compound in appropriate solvent. Prepare 100 X stock solution in the solvent and dilute it with sEH 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 sEH Assay Buffer at 1:10 to obtain 10 µM (10X). Add 10 µl test compound to each well of the clear 96 well plate. For "Solvent Control", dilute the solvent used for test compound preparation in sEH Assay Buffer at 1:10 and add 10 µl in to solvent control wells. For "Inhibitor Control", add 10 µl of the prepared 1 mM NCND, the provided sEH inhibitor. Bring up the volume to 40 µl to each well by adding 30 µl of sEH Assay Buffer. For "Enzyme Control" well, add 40 µl sEH Assay Buffer and for "Background Control" add 80 µl sEH Assay Buffer to desired wells.

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

| | Reaction Mix |
|------------------|--------------|
| sEH Assay Buffer | 38 µl |
| sEH Enzyme | 2 µl |

Mix well and add Reaction Mix to wells containing the Enzyme Control, Inhibitor Control, Solvent Control and Test Compounds. 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**

3. sEH Substrate Mix: Prepare enough substrate mix for number of reaction to be performed. For each well prepare 20 µl Substrate Mix containing:

