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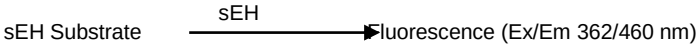
Soluble Epoxide Hydrolase Activity Assay Kit (Fluorometric)

09/17

(Catalog # K477-100; 100 assays; Store at - 20ºC)

I. Introduction:

Soluble Epoxide Hydrolase or sEH (EC 3.3.2.10) is a cytosolic enzyme present ubiquitously in several organs including the liver, 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, and forming vicinal diols. sEH plays a major role in metabolism of endogenous lipids that are implicated in pain and inflammation. BioVision's Soluble Epoxide Hydrolase Activity Assay Kit is a microplate based fluorometric kit for measuring sEH activity in cells and tissues as well as purified protein. It is based on the ability of sEH to hydrolyze a non-fluorescent substrate to a fluorescent product. The kit includes a specific inhibitor for soluble epoxide hydrolase, since the substrate can be hydrolyzed by non-specific hydrolases that are present in cell and tissue lysates. Specific sEH activity can be obtained by subtracting the activity in presence of sEH inhibitor form the total activity.



II. Applications:

Measurement of sEH activity in cell and tissue lysates using a 96-well plate format.

III. Sample Type:

- Cell lysate (eg. HEK-293 or other cell types expected to have high sEH activity)
- Tissue lysate (eg. Liver tissue)
- Recombinant enzyme
- Purified protein

IV. Kit Contents:

Components	K477-100	Cap Code	Part Number
sEH Assay Buffer	25 ml	WM	K477-100-1
sEH Substrate	200 µl	Blue	K477-100-2
sEH Inhibitor 5X	100 µl	Orange	K477-100-3
Fluorescence Standard	1 vial	Yellow	K477-100-4
sEH Positive Control	1 vial	Green	K477-100-5

V. User Supplied Reagents and Equipment:

- 96-well white/clear plate with flat bottom
- Multi-well spectrophotometer
- DMSO

VI. 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 Buffer:** Warm to room temperature before use.
- **sEH Substrate:** Aliquot and store at -20ºC in the dark. Thaw at room temperature before use. ***DO NOT EXPOSE TO LIGHT.***
- **sEH Inhibitor:** Aliquot and store at -20ºC in the dark. Thaw at room temperature before use. Prepare 1X working solution by dissolving in sEH Assay Buffer at 1:5.
- **Florescence Standard:** Reconstitute with 55 µl of DMSO to yield a 5 mM solution. When stored at -20°C, it is stable for 3 freeze/thaw cycles.
- **sEH Positive Control:** Lyophilized enzyme is stable for 12 months at -20ºC. Reconstitute in 50 µl sEH buffer. Aliquot and store at -20ºC. Reconstituted enzyme is stable for at least 3 months.

  Note: *Keep positive control on ice while performing the assay.*

VII. sEH Activity Assay Protocol:

1. **Sample preparation:** Homogenize cells (4 x 10^5 cells) or tissue (10 mg) with 100 µl ice-cold sEH Assay buffer to perform lysis and keep on ice for 10 minutes followed by centrifugation at 10,000 x g for 15 minutes at 4ºC. Collect the supernatant (lysate) and estimate protein concentration using preferred method. *We recommend BCA protein assay kit (BV# K813-2500). Protein concentration should range between 0.2 and 2 µg/µl*). Dilute the lysate if needed using sEH Assay Buffer. Carry out ammonium sulfate precipitation of the lysate using 80% saturated $(NH_4)_2SO_4$ *(BV# 7096 or similar)* on ice for 30 minutes. Centrifuge at 10,000 x g for 5 minutes at 4ºC. Discard the supernatant and wash the pellet with 80% saturated $(NH_4)_2SO_4$ followed again by centrifugation at 10,000 x g for 5 minutes 4ºC. Discard supernatant and re-suspend the pellet in the same volume of sEH assay buffer as was used to carry out lysis. *We recommend using the samples for activity analysis immediately, if that is not possible, they may be stored at -80 ºC for 3-4 days.* Prepare three wells for each sample labeled "Sample Background Control" (BC), "Sample" (S) and "Sample + Inhibitor" (SI). Add 5 -10 µl sample (1 – 5 µg protein) into each of these wells. For SI well add 10 µl 1X sEH Inhibitor in addition to sample. S*everal dilutions of the sample may be tested.* For Positive Control, add 5-10 µl of the provided sEH Positive Control into the desired well. Adjust volume in each well to 50 µl with sEH Assay Buffer. For Assay Background Control (i.e., substrate background), add 50 µl of sEH Assay Buffer to a well. Incubate the plate at room temperature for 10 minutes.

   Notes:

a. For unknown samples, we suggest testing several concentrations to ensure the readings are within the Standard Curve range.

b. For samples having low activity, white plate may be used. *If white plate is used, prepare standard curve in a white plate as well.*

2. **Standard Curve Generation:** Dilute the Fluorescence Standard by adding 10 µl of the 5 mM stock to 990 µl sEH Assay Buffer to obtain a 50 µM Standard solution. Add 0, 2, 4, 6, 8 and10 µl of the 50 µM solution into a series of wells in a clear 96-well plate and adjust the volume of each well to 100 µl with sEH Assay Buffer, yielding 0, 100, 200, 300, 400 and 500 pmol/well Fluorescence Standard.

3. **Reaction Mix:** Mix enough reagents for the number of assays to be performed. Add BC Mix to "Sample Background Control" wells and Reaction Mix to Assay Background Control, Sample, Sample + Inhibitor and Positive Control wells. For each well, prepare 50 µl :

	BC Mix	Reaction Mix
sEH Assay Buffer	50 µl	48 µl
sEH Substrate	-	2 µl

Add the reaction mix to wells of a 96-well clear plate.

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

4. **Measurement:** Immediately start recording fluorescence at 30 second intervals for 20 - 30 minutes. Standard curve can be read in either kinetic or end point mode.

5. **Calculation:** Subtract the standard background from standard RFU values, and sample background control RFU values from the sample RFU values respectively. *If assay background control RFU values are higher than sample background control, subtract that value from sample RFU values instead*. Estimate amount of sEH metabolite in the reaction using the fluorescence standard curve. Calculate ΔM, which is the change in amount of sEH metabolite between time t_1 and t_2, such that t_1 and t_2 both fall in the linear portion of the reaction. sEH activity may be calculated using the following equation:

Detected activity = ΔM / (Δt x P) (pmol / (min x µg)) = µUnits / µg = mUnits / mg

Where: **ΔM =** linear change in sEH metabolite concentration during Δt (pmol)
Δt = t2 – t1 (min)
P = sample protein content added to well (µg)

Specific sEH activity in sample = detected activity in S – detected activity in SI

Unit Definition: One unit of sEH is the amount of enzyme that produces1 µmol of fluorescent sEH metabolite per minute at pH 7.4 at RT.

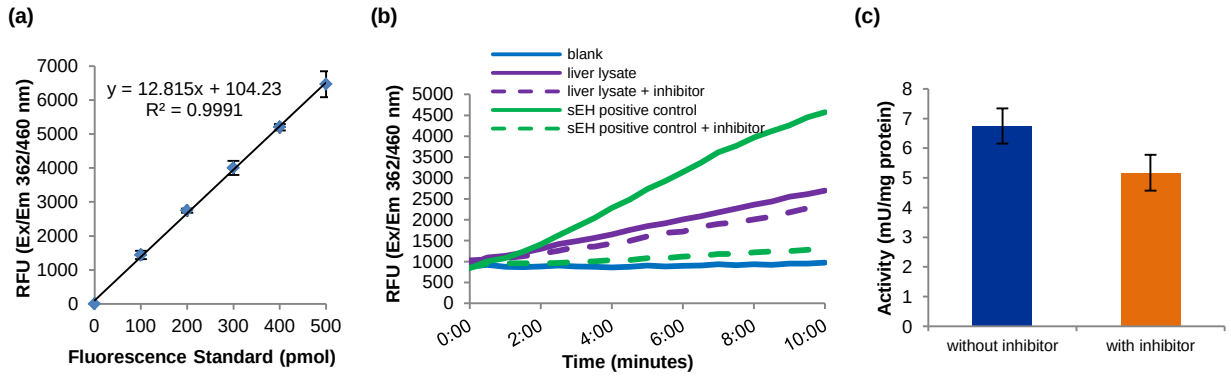


Figure 1: (**a**) Fluorescence standard curve for sEH metabolite (**b**) Enzyme kinetics in presence and absence of inhibitor for sEH positive control, and rat liver lysate (3.6 µg protein per well). **(c)** sEH activity in rat liver tissue lysate in absence and presence of inhibitor.

VIII. RELATED PRODUCTS:

Cytochrome P450 2C9 (CYP2C9) Activity Assay Kit (Fluorometric) (K895)
Cytochrome P450 2C19 (CYP2C19) Activity Assay Kit (Fluorometric) (K848)
Cytochrome P450 2C9 (CYP2C9) Inhibitor Screening Kit (Fluorometric) (K896)
Cyclooxygenase (COX) Activity Assay Kit (Fluorometric) (K549)
Cyclooxygenase-1 (COX-1) Inhibitor Screening Kit (Fluorometric) (K548)
COX-2 Inhibitor Screening Kit (Fluorometric) (K547)

FOR RESEARCH USE ONLY! Not to be used on humans.