



## Soluble Epoxide Hydrolase Inhibitor Screening Assay Kit

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Item No. 10011671

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## GENERAL INFORMATION

### Materials Supplied

Kit will arrive packaged as a -80°C kit. After opening the kit, store individual components as stated below.

Item Number	Item	Quantity/Size	Storage
600036	sEH Assay Buffer (10X)	1 vial/3 ml	-20°C
401038	sEH Enzyme (human, recombinant)	2 vials/30 µl	-80°C
401039	sEH Substrate	1 vial/60 µl	-80°C
401048	sEH Inhibitor	1 vial/120 µl	-20°C
401049	sEH Assay Calibrator	1 vial/60 µl	-20°C
400017	96-Well Solid Plate (black)	1 plate	RT
400023	Foil Plate Cover	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.



**WARNING:** THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

## Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

## Precautions

**Please read these instructions carefully before beginning this assay.**

This kit may not perform as described if any reagent or procedure is replaced or modified.

## If You Have Problems

### **Technical Service Contact Information**

**Phone:** 888-526-5351 (USA and Canada only) or 734-975-3888

**Email:** techserv@caymanchem.com

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

## Storage and Stability

This kit will perform as specified if stored as directed in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

## Materials Needed But Not Supplied

1. A plate reader or fluorometer capable of measuring fluorescence with excitation and emission wavelengths of 330 nm (315-335 nm) and 465 nm (450-470 nm), respectively.
2. Adjustable pipettes; multichannel or repeating pipettor recommended
3. A source of pure water; glass-distilled is acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
4. DMSO
5. Materials used for **Sample Preparation** (see page 9)

### Background

Soluble epoxide hydrolase (sEH) is a member of the  $\alpha/\beta$ -hydrolase fold enzyme family that catalyzes the hydrolysis of bioactive fatty acid epoxides to inactive vicinal diols.<sup>1</sup> sEH is localized to the cytoplasm or to peroxisomes in a tissue-specific manner and is found in various tissues, including skin, lung, uterus, kidney, brain, and myocardium.<sup>2,3</sup> sEH is also expressed in the vasculature where inhibition attenuates pathogenic vascular remodeling and hypertension *via* preservation of cardioprotective epoxyeicosatrienoic acids (EpETrEs, also known as EETs) in rat models of atherosclerosis and hypertension, respectively.<sup>4</sup> Inhibition of sEH also has a protective role in various diseases, including inflammatory bowel disease, osteoarthritis, seizure, stroke, and Alzheimer's disease, as well as in various chronic pain states.<sup>5,6</sup> The development of small molecule inhibitors targeting sEH is a viable therapeutic strategy in many disease settings.

### About This Assay

Cayman's fluorescence-based sEH Inhibitor Screening Assay Kit provides a convenient method for screening human sEH inhibitors. The assay utilizes (3-phenyl-oxiranyl)-acetic acid cyano-(6-methoxy-naphthalen-2-yl)-methyl ester (PHOME) as a substrate. When the epoxide moiety of PHOME is hydrolyzed by sEH, an intramolecular cyclization occurs, which results in the release of a cyanohydrin under basic conditions. The cyanohydrin quickly decomposes into cyanide ion and the highly fluorescent 6-methoxy-2-naphthaldehyde,<sup>7</sup> which can be analyzed using a fluorescence plate reader at excitation and emission wavelengths of 330 and 465 nm, respectively.

### Reagent Preparation

#### 1. sEH Assay Buffer (10X) - (Item No. 600036)

This vial contains 3 ml of sEH Assay Buffer (10X). Thaw at room temperature and dilute the entire contents with 27 ml of pure water to make 30 ml of sEH Assay Buffer (1X). The sEH Assay Buffer (1X) will be stable for 3 months when stored at 4°C.

#### 2. sEH Enzyme (human, recombinant) - (Item No. 401038)

Each vial contains 30 µl of recombinant human sEH. Prior to use in the assay, thaw the enzyme on ice and mix gently. Do not vortex. Dilute the enzyme 200-fold by mixing 15 µl with 3 ml of sEH Assay Buffer (1X). This is a sufficient volume to assay 50 wells. Scale as needed. The diluted sEH enzyme will be stable for one hour when stored on ice. Any undiluted enzyme can be aliquoted and stored at -80°C. Limit freeze-thaw cycles to three.

#### 3. sEH Substrate - (Item No. 401039)

This vial contains 60 µl of PHOME in DMSO. Dilute the substrate 200-fold by mixing 15 µl with 3 ml of sEH Assay Buffer (1X). This is a sufficient volume to assay 50 wells. Scale as needed. The diluted sEH substrate will be stable for one hour when stored on ice. Let the diluted sEH substrate warm up to room temperature for 30 minutes prior to use in the assay. Any undiluted substrate can be aliquoted and stored at -80°C. Limit freeze-thaw cycles to five.

#### 4. sEH Inhibitor (1 mM) - (Item No. 401048)

This vial contains 120 µl of 1 mM AUDA in DMSO, which can be used as an inhibitor control. Dilute the sEH Inhibitor to 12 µM by mixing 12 µl with 988 µl of DMSO for a final concentration of 300 nM in the reaction. The diluted inhibitor will be stable for two hours at room temperature. Any remaining sEH Inhibitor can be aliquoted and stored at -20°C. Limit freeze-thaw cycles to two.

#### 5. sEH Assay Calibrator - (Item No. 401049)

This vial contains 60 µl of sEH Assay Calibrator, which can be used to optimize instrument gain when running the assay kinetically. Calibrator wells are not required when running the assay in endpoint mode. To prepare the calibrator solution, dilute the sEH Assay Calibrator 20-fold by mixing 15 µl with 285 µl of sEH Assay Buffer (1X). Any undiluted calibrator can be aliquoted and stored at -20°C. Limit freeze-thaw cycles to four.

### Sample Preparation

#### Test Compounds

Test compounds can be dissolved in DMSO or ethanol. DMF and methanol are not compatible with this assay. Additional solvents have not been tested. The concentrated test compound stock solutions must be further diluted in sEH Assay Buffer (1X) to a concentration 40X the final assay (*i.e.* in-well) concentration. To minimize potential interference by solvents in the assay, refer to the **Effects of Solvents** section (on page 19) to determine the maximum solvent concentration tolerated by the assay. Appropriate vehicle control wells containing the same concentration of solvent used for the test compounds must be included in each assay.

## Plate Set Up

There is no specific pattern for using the wells on the plate. However, it is necessary to have two wells designated as vehicle control and two wells designated as background. It is suggested that each test compound and its vehicle control be assayed in duplicate. A typical layout of samples to be measured in duplicate is shown in Figure 1. It is suggested that the contents of each well are recorded on the template sheet provided (see page 25).

	1	2	3	4	5	6	7	8	9	10	11	12
A	BW	BW	6	6	14	14	22	22	30	30	38	38
B	VC	VC	7	7	15	15	23	23	31	31	39	39
C	IC	IC	8	8	16	16	24	24	32	32	40	40
D	1	1	9	9	17	17	25	25	33	33	41	41
E	2	2	10	10	18	18	26	26	34	34	42	42
F	3	3	11	11	19	19	27	27	35	35	43	43
G	4	4	12	12	20	20	28	28	36	36	44	44
H	5	5	13	13	21	21	29	29	37	37	C	C

BW = Background Wells  
 VC = Vehicle Control Wells  
 IC = Inhibitor Control Wells  
 1-44 = Test Compound Wells  
 C = Calibrator Wells (Kinetic Mode)

Figure 1. Sample plate format

### Pipetting Hints

- It is recommended that a multichannel pipette be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (*i.e.*, slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

### General Information

- The final volume in the assay is 200  $\mu\text{l}$  in all of the wells.
- All reagents should be prepared as described above. The diluted sEH Enzyme (human, recombinant) should be kept on ice and all other reagents should be at room temperature before beginning the assay.
- It is not necessary to use all the wells on the plate at one time.
- It is recommended that the samples be assayed at least in duplicate.
- The assay is performed at room temperature.
- Monitor the fluorescence with excitation and emission wavelengths of 330 and 465 nm, respectively.

### Performing the Assay

*NOTE: The assay may be read kinetically or as an endpoint. See steps 1 and 5 for details.*

1. **Calibrator Well (Kinetic Mode)** - Add 200  $\mu\text{l}$  of calibrator solution to the designated wells.  
Read the plate to determine the instrument's optimal *gain* setting at an excitation wavelength of 330 nm and an emission wavelength of 465 nm. The optimal *gain* setting will be used when assaying with kinetic measurements (see step 5).
2. Add the appropriate amount of reagents to the designated wells according to Table 1, below. Add the enzyme last. Thoroughly mix by pipetting up and down, ensuring no bubbles are formed.

	Background Wells	Vehicle Control Wells	Test Compound Wells	Inhibitor Control Wells
sEH Assay Buffer (1X)	145 $\mu\text{l}$	95 $\mu\text{l}$	95 $\mu\text{l}$	95 $\mu\text{l}$
Solvent	5 $\mu\text{l}$	5 $\mu\text{l}$	--	--
Test Compound	--	--	5 $\mu\text{l}$	--
Diluted sEH Inhibitor	--	--	--	5 $\mu\text{l}$
Diluted sEH Enzyme (human, recombinant)	--	50 $\mu\text{l}$	50 $\mu\text{l}$	50 $\mu\text{l}$

Table 1. Pipetting summary

3. Cover the plate with the Foil Plate Cover (Item No. 400023) and incubate for 15 minutes at room temperature.
4. Initiate the reactions by adding 50  $\mu$ l of diluted sEH Substrate to all wells being used. Thoroughly mix by pipetting up and down, ensuring no bubbles are formed.
5. Fluorescence Measurement
  - a. **Kinetic** - Read fluorescence every minute for 30 minutes at excitation and emission wavelengths of 330 and 465 nm, respectively.
  - b. **Endpoint** - Cover the plate with the Foil Plate Cover and incubate for 30 minutes at room temperature. Remove the plate cover and read the fluorescence using excitation and emission wavelengths of 330 and 465 nm, respectively.

## ANALYSIS

### Calculations

1. If read kinetically, determine the initial rate (RFU/min) based on the linear portion of the kinetic curve, which typically falls between 10-30 minutes.
2. Determine the average endpoint fluorescence or the average initial rate for each replicate.
3. Subtract the average fluorescence or initial rate of the background from the average fluorescence or initial rate of the vehicle control and test compounds, respectively. These are the corrected values.
4. Using the corrected values, determine the percent inhibition or percent activity for each test compound using one of the following equations:

$$\% \text{ inhibition} = \left[ 1 - \frac{\text{corrected value of test compound}}{\text{corrected value of vehicle}} \right] \times 100$$

$$\% \text{ activity} = \frac{\text{corrected value of test compound}}{\text{corrected value of vehicle}} \times 100$$

5. Graph the percent inhibition or percent activity as a function of test compound concentration to determine the IC<sub>50</sub> value (the concentration at which there is 50% inhibition) of the inhibitor. Inhibition of recombinant human sEH by the inhibitor control is shown in Figure 3 (see page 18).

## Performance Characteristics

### Z' Factor

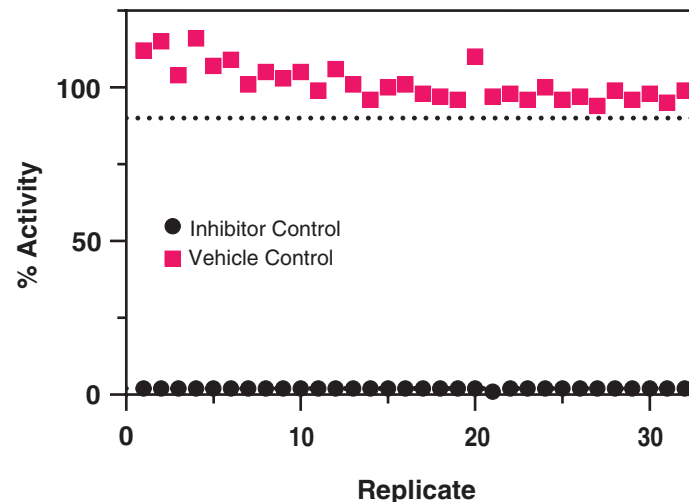
Z' factor is a term used to describe the robustness of an assay, which is calculated using the equation below.<sup>8</sup>

$$Z' = 1 - \frac{3\sigma_{c+} + 3\sigma_{c-}}{|\mu_{c+} - \mu_{c-}|}$$

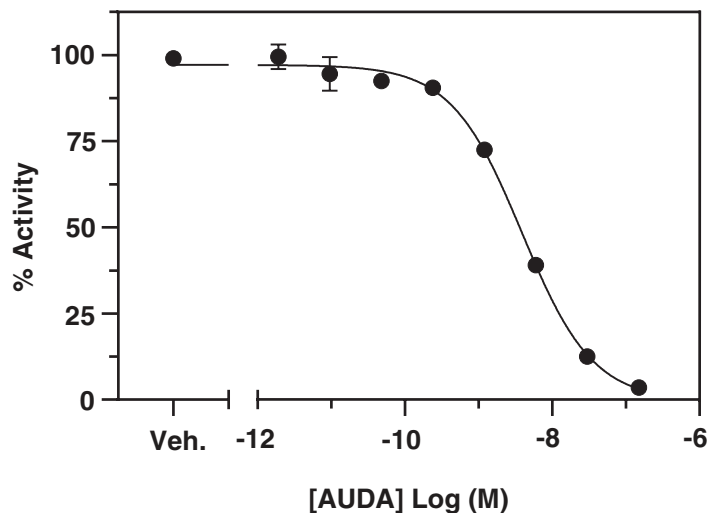
Where  $\sigma$ : Standard deviation  
 $\mu$ : Mean  
c+: Positive control  
c-: Negative control

The theoretical upper limit for the Z' factor is 1.0. A robust assay has a Z' factor >0.5. The Z' factor for Cayman's sEH Inhibitor Screening Assay Kit was determined to be 0.88.

### Sample Data:



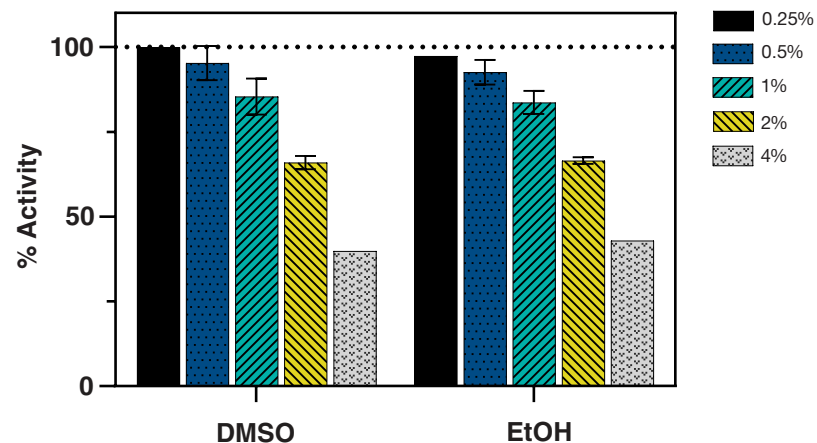
**Figure 2. Typical performance data for the sEH Inhibitor Screening Assay Kit.** The percent activities were calculated using initial rates. Data shown are from 32 replicates each for vehicle control and 300 nM AUDA prepared as described in the kit booklet. The calculated Z' factor for this experiment was 0.88. The dotted lines correspond to three standard deviations from the mean for each control value.



**Figure 3. Inhibition of recombinant human sEH by sEH Inhibitor.** Data are plotted as the mean of duplicate kinetic measurements  $\pm$  the standard deviation. The vehicle control (Veh.) represents 100% activity. The  $IC_{50}$  value of sEH Inhibitor is 3.9 nM.

#### Effects of Solvents:

Compounds may be prepared in organic solvents such as DMSO and ethanol. A titration of organic solvents showed that activity decreases with increasing solvent concentration, so the proper vehicle control for each solvent and corresponding concentrations should be included in the assay.



**Figure 4. The effect of solvent on the readout of sEH activity.** The data are shown as the mean  $\pm$  standard deviation for triplicate reactions containing the indicated concentration of solvents. The percent activities were calculated using initial rates. The dotted line represents the no-solvent control, which is set as 100% activity.

## Precision:

Intra-assay precision was determined by analyzing 23 measurements of the background and 32 measurements of the vehicle and inhibitor control on the same day. The intra-assay coefficients of variation were 15, 6, and 11%, respectively. The intra-assay coefficient of variation for the IC<sub>50</sub> value of 11 inhibition curves performed on the same day was 7%.

Inter-assay precision was determined by analyzing inhibition with the sEH inhibitor in 7 separate assays on different days. The inter-assay coefficient of variation for the IC<sub>50</sub> value was 8%.

## RESOURCES

### Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of replicates	A. Poor pipetting/technique B. Bubble in the well(s)	A. Be careful not to splash the contents of the wells B. Carefully tap the side of the plate with your finger to remove bubbles
No enzymatic activity observed in vehicle control wells	A. Enzyme or substrate was not added to the well(s) B. Solvent concentration is too high	A. Make sure to add all components to the wells; use fresh enzyme/substrate and ensure proper storage and handling B. Check solvent tolerance limits
Inhibitor control shows no inhibition	Inhibitor is used at incorrect concentration or has degraded	Prepare fresh inhibitor stock; verify concentration, storage, and handling; confirm correct dilution and addition step

## Assay Summary

Item No.	Reagent	Procedure
600036	sEH Assay Buffer (10X)	Dilute to 1X by adding 27 ml of pure water to the vial
401038	sEH Enzyme (human, recombinant)	Dilute 1:200 with sEH Assay Buffer (1X)
401039	sEH Substrate	Dilute 1:200 with sEH Assay Buffer (1X)
401048	sEH Inhibitor (1 mM)	Dilute to 12 $\mu$ M with DMSO
401049	sEH Assay Calibrator	Dilute 1:20 with sEH Assay Buffer (1X)

Table 2. Preparation summary

Procedure	Background Wells	Vehicle Control Wells	Test Compound Wells	Inhibitor Control Wells	Calibrator Wells
Add Calibrator Solution	--	--	--	--	200 $\mu$ l
Add sEH Assay Buffer (1X)	145 $\mu$ l	95 $\mu$ l	95 $\mu$ l	95 $\mu$ l	--
Add Solvent	5 $\mu$ l	5 $\mu$ l	--	--	--
Add Test Compound	--	--	5 $\mu$ l	--	--
Add diluted sEH Inhibitor	--	--	--	5 $\mu$ l	--
Add diluted sEH Enzyme (human, recombinant)	--	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l	--
Mix thoroughly, cover the plate, and incubate for 15 minutes at room temperature					Kinetic: Use the calibrator wells to determine optimal gain setting
Add diluted sEH Substrate	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l	50 $\mu$ l	
Mix thoroughly Endpoint: Incubate at room temperature for 30 minutes, read Ex <sub>330</sub> /Em <sub>465</sub> Kinetic: read Ex <sub>330</sub> /Em <sub>465</sub> every minute for 30 minutes					

Table 3. Assay summary

## References

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