Soluble Epoxide Hydrolase Inhibitor Screening Assay Kit

Item No. 10011671

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GENERAL INFORMATION

Materials Supplied

Kit will arrive packaged as a -80°C kit. For best results, remove components and store as stated below.

Item Number	Item	Quantity/Size	Storage
600036	sEH Assay Buffer (10X)	1 vial/3 ml	-20°C
600037	sEH (human recombinant)	2 vials/12 µl	-80°C
600038	sEH Substrate	1 vial/600 µl	-80°C
400017	96-Well Solid Plate (black)	1 plate	RT
400012	96-Well Cover Sheet	1 cover	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.

The reagents in this kit have been tested and formulated to work exclusively with Cayman's Soluble Epoxide Hydrolase Inhibitor Screening Assay Kit. 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

Fax: 734-971-3641

E-Mail: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

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, on page 3, and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A fluorometer capable of measuring fluorescence using excitation and emission wavelengths of 330 and 465 nm, respectively
2. Adjustable pipettes and a repeating pipettor
3. A source of UltraPure water (Milli-Q or HPLC-grade water)

INTRODUCTION

Background

Mammalian soluble epoxide hydrolase (sEH) is a member of the α/β-hydrolase fold family of enzymes that catalyzes the hydrolysis of exogenous and endogenous epoxides to vicinal diols. sEH is a homodimer consisting of two domains.[1] The C-terminal domain is responsible for the epoxide hydrolase activity while the N-terminal domain has a catalytic center with phosphatase activity. Endogenous substrates for sEH include epoxyeicosatrienoic acids (EETs) which exhibit vasodialatory and anti-inflammatory activity.[2] Inhibition of sEH in animal models was shown to effectively treat hypertension and vascular inflammation as well as related syndromes.[3] These studies demonstrate the value for targeting sEH for development of small molecule inhibitors as therapeutics.

About This Assay

Cayman's fluorescence-based sEH Inhibitor Screening Assay Kit provides a convenient method for screening human epoxide hydrolase inhibitors. The assay utilizes (3-phenyl-oxiranyl)-acetic acid cyano-(6-methoxy-naphthalen-2-yl)-methyl ester (PHOME) as substrate. When the epoxide moiety of PHOME is hydrolyzed by epoxide hydrolase, 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[4] which can be analyzed using an excitation wavelength of 330 nm and an emission wavelength of 465 nm.

PRE-ASSAY PREPARATION

NOTE: Water used to prepare all reagents and buffers must be deionized. Glass distilled water, HPLC-grade water, and sterile water (for injections) are adequate for this kit. UltraPure water may be purchased from Cayman (Item No. 400000).

Reagent Preparation

1. **sEH Assay Buffer (10X)**

   Dilute the 3 ml of sEH Assay Buffer (10X) concentrate (Item No. 600036) with 27 ml of HPLC-grade water. This final Assay Buffer, 25 mM *bis*-Tris, pH 7.0, should be used in the assay and for the dilution of epoxide hydrolase. When stored at -20°C, this diluted Assay Buffer is stable for at least six months.

2. **sEH (human recombinant)**

   Each vial (Item No. 600037) contains 12 µl of human recombinant sEH. Thaw the enzyme on ice. Prior to assaying, add 588 µl of cold Assay Buffer directly to one enzyme vial. This is sufficient enzyme for the full 96-well plate. If not utilizing the entire plate, adjust the amount of diluted enzyme accordingly by diluting the enzyme 1:50 with cold Assay Buffer before use. The diluted enzyme is stable for four hours on ice. Prepare aliquots of the remaining undiluted enzyme and store at -80°C.

3. **sEH Substrate**

   The vial (Item No. 600038) contains 600 µl of a 10 µM solution of PHOME in dimethyl sulfoxide (DMSO). The Substrate is ready to use as supplied. The final concentration of Substrate is 0.25 µM which is below the estimated K_m value of 1.5 µM. For competitive inhibitors, the IC_{50} is dependent upon the Substrate concentration and should be reported when publishing the experimental results.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, it is necessary to have three wells designated as background wells and three wells designated as 100% Initial Activity wells. We suggest that each Inhibitor sample be assayed in triplicate and the contents of each well recorded on the template sheet provided on page 14. A typical layout of samples and inhibitors to be measured in triplicate is given below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	BW	BW	BW	7	7	7	15	15	15	23	23	23
B	A	A	A	8	8	8	16	16	16	24	24	24
C	1	1	1	9	9	9	17	17	17	25	25	25
D	2	2	2	10	10	10	18	18	18	26	26	26
E	3	3	3	11	11	11	19	19	19	27	27	27
F	4	4	4	12	12	12	20	20	20	28	28	28
G	5	5	5	13	13	13	21	21	21	29	29	29
H	6	6	6	14	14	14	22	22	22	30	30	30

BW - Background Wells
A - 100% Initial Activity Wells
1-30 - Inhibitor Wells

Figure 1. Inhibitor screening plate format

Pipetting Hints

- Use different tips to pipette the buffer, substrate, inhibitors, and protein.
- 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 of the assay is 200 µl in all the wells.
- It is not necessary to use all the wells on the plate at one time.
- The assay temperature is 25°C. Pre-warm the Assay Buffer and Substrate to room temperature before assaying.
- It may be necessary to assay inhibitors at several concentrations to determine an effective concentration.
- If using DMSO as a solvent, it is recommended that all dilutions be performed using glass vials. Use of plastic vials may cause leaching of bioactive contaminants that may interfere with the assay.
- We recommend assaying samples in triplicate, but it is the user's discretion to do so.
- Thirty inhibitor samples can be assayed in triplicate or 45 in duplicate.

Performing the Assay

NOTE: See Table 1; Pipetting summary below for additional clarity.

1. **Background Wells -** add 190 µl of Assay Buffer and 5 µl of solvent (which ever solvent you dissolved your inhibitor in) to three wells.
2. **100% Initial Activity Wells -** add 185 µl of Assay Buffer, 5 µl of sEH, and 5 µl of solvent (which ever solvent you dissolved your inhibitor in) to three wells.
3. **Inhibitor Wells -** add 185 µl of Assay Buffer, 5 µl of inhibitor*, and 5 µl of sEH to three wells.
4. Initiate the reactions by adding 5 µl of Substrate to all wells being used. Carefully shake the microwell plate for 10 seconds to mix and cover with the plate cover. Incubate for 15 minutes at 25°C. (This can also be read in the kinetic mode if desired.)
5. Remove the plate cover and read the fluorescence using an excitation wavelength of 330 nm and an emission wavelength of 465 nm.

*Inhibitors can be dissolved in methanol, DMSO, or ethanol and should be added to the assay in a final volume of 5 µl.

Well	Assay Buffer	Solvent	Inhibitor	sEH	Substrate
Background	190 µl	5 µl	-	-	5 µl
100% Initial Activity	185 µl	5 µl	-	5 µl	5 µl
Inhibitor	185 µl	-	5 µl	5 µl	5 µl

Table 1. Pipetting summary

ANALYSIS

Calculations

1. Determine the average fluorescence (AF) of the background wells, 100% Initial Activity wells, and each of the inhibitors.
2. Subtract the background AF from the 100% Initial Activity and Inhibitor AFs.
3. Use the following equation to calculate the percent activity remaining:

$$\%\ \text{Initial Activity} = \left[\frac{\text{Inhibited AF}}{\text{100\% Initial Activity}}\right] \times 100\%$$

4. If multiple concentrations of initial inhibition are tested, graph the percent activity as a function of the Inhibitor concentration to determine the IC_{50} value (concentration at which there is 50% inhibition). An example of human recombinant epoxide hydrolase inhibition by a specific inhibitor, AUDA, is shown in Figure 2 on page 11.

Performance Characteristics

Precision:

Intra-assay coefficient of variation = 7.0% (n = 12). Inter-assay coefficient of variation = 7% (n = 5).

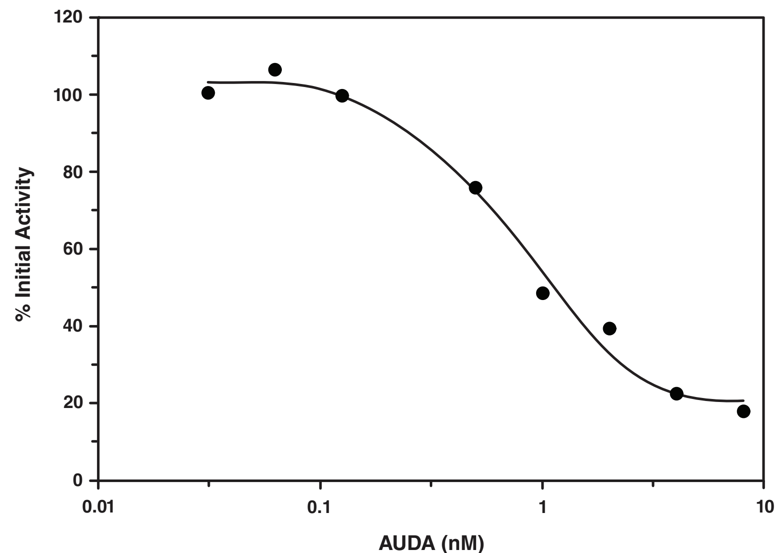

Figure 2. Inhibition of human recombinant sEH by AUDA (IC_{50} = 1 nM)

RESOURCES

Interferences

The following reagents were tested in the assay for interference:

	Reagent	Will Interfere (Yes or No)
Buffers	Borate	No
	Phosphate	No
	Tris	No
Detergents/Chelators	EDTA (≤2.5 mM)	No
	EGTA (≤2.5 mM)	No
	Triton 20 (≤1%)	No
	Triton X-100 (≤1%)	No
Solvents	Dimethylsulfoxide (10%)	No
	Ethanol (10%)	No
	Methanol (10%)	No
Others	BSA (≤0.1%)	No
	Glycerol (≤5%)	No

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates/triplicates	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 fluorescence above background is seen in the Inhibitor wells	A. Enzyme or substrate was not added to the well(s) B. Inhibitor concentration is too high resulting in complete loss of enzyme activity	A. Make sure to add all components to the wells B. Reduce the concentration of the inhibitor and re-assay
No inhibition seen with inhibitor	The inhibitor concentration is not high enough or the compound is not an inhibitor of the enzyme	Increase the inhibitor concentration and re-assay

References

1. Chiamvimonvat, N., Ho, C.-M., Tsai, H.-J., *et al.* The soluble epoxide hydrolase as a pharmaceutical target for hypertension. *J. Cardiovasc. Pharmacol.* **50(3)**, 225-237 (2007).
2. Yu, Z., Xu, F., Huse, L.M., *et al.* Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. *Circ. Res.* **87**, 992-998 (2000).
3. Imig, J.D., Zhao, X., Zaharis, C.Z., *et al.* An orally active epoxide hydrolase inhibitor lowers blood pressure and provides renal protection in salt-sensitive hypertension. *Hypertension* **46(2)**, 975-981 (2005).
4. Wolf, N.M., Morisseau, C., Jones, P.D., *et al.* Development of a high-throughput screen for soluble epoxide hydrolase inhibition. *Anal. Biochem.* **355**, 71-80 (2006).

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.

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