Soluble Epoxide Hydrolase Cell-Based Assay Kit

Item No. 600090

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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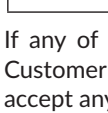
GENERAL INFORMATION

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 (480 well)	Storage
600091	Cell-Based sEH Assay Buffer (10X)	1 vial/60 ml	RT
600092	CBA Digitonin Solution	1 vial/250 µl	-20°C
600093	CBA sEH Positive Control	1 vial/10 µl	-80°C
600094	CBA 6-methoxy-2-Naphthaldehyde Standard	1 vial/100 µl	-20°C
600095	CBA sEH Substrate	1 vial/100 µl	-20°C
600096	CBA sEH Inhibitor (10 mM)	1 vial/50 µl	-20°C
10011297	96-Well Solid Plate (black) with lid	5 plates	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.

If You Have Problems

Technical Service Contact Information

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

Fax: 734-971-3641

Email: 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 and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. Primary liver cells such as Huh-7 human liver primary cells. Other cells such as human liver cell line HepG2 cells (can be obtained from ATCC) can also be used
2. A fluorometer with the capacity to measure fluorescence using an excitation wavelength of 340 nm and an emission wavelength of 465 nm
3. Adjustable pipettes and a repeating pipettor
4. A source of pure water; glass distilled water or HPLC-grade water is acceptable

INTRODUCTION

Background

Mammalian soluble epoxide hydrolase (sEH) is a member of the α/β-hydrolase fold family of enzymes that catalyze 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 vasodilatory 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 sEH Cell-Based Assay Kit provides a convenient 96-well plate, fluorescence-based method for detecting epoxide hydrolase activity in whole cells. The assay utilizes Epoxy Fluor 7, a sensitive fluorescent substrate for sEH that can be used to monitor the activity of both human and murine enzymes.[4] Hydrolysis of the substrate epoxide yields a highly fluorescent product, 6-methoxy-2-Naphthaldehyde, that can be monitored at excitation and emission wavelengths of 330 and 465 nm, respectively. 6-Methoxy-2-Naphthaldehyde is included to quantify enzyme activity and a recombinant sEH is included as a positive control. An sEH inhibitor AUDA is also included for checking specificity of the reaction. This assay parallels Cayman's Soluble Epoxide Hydrolase Inhibitor Screening Assay Kit (Item No. 10011671) which uses recombinant protein rather than whole cells for the assay. Together, both assays will help to identify whether or not an inhibitor/activator has a direct or indirect effect on the enzyme.

PRE-ASSAY PREPARATION

Cell Culture Preparation

1. Seed cells in a 96-well plate at a density of (2×10^4) - (5×10^4) cells/well in 100 µl of culture medium with or without compounds to be tested. We recommend that each treatment be performed in triplicate.
2. Culture the cells in a CO_2 incubator at 37°C for 24-48 hours, or for a period of time according to your typical experimental protocol.

Reagent Preparation

1. **Assay Buffer**

   To prepare an Assay Buffer, add 10 ml of the stock Cell-Based sEH Assay Buffer (10X) (Item No. 600091) to 90 ml of distilled water.

2. **Lysis Buffer**

   To prepare a Lysis Buffer, add 50 µl of the CBA Digitonin Solution (Item No. 600092) to 10 ml of diluted Assay Buffer. Keep the Lysis Buffer on ice. It is recommended that you prepare fresh Lysis Buffer for each assay.

3. **6-methoxy-2-Naphthaldehyde Standard**

   The CBA 6-methoxy-2-Naphthaldehyde Standard vial (Item No. 600094) contains 100 µl of 100 µM 6-methoxy-2-Naphthaldehyde in dimethylsulfoxide (DMSO).

   To run a 6-methoxy-2-Naphthaldehyde standard curve, obtain eight clean test tubes and label them #1 to #8. Add 980 µl of diluted Assay Buffer into tube #1 and 500 µl into tubes #2-8. Transfer 20 µl of the CBA 6-methoxy-2-Naphthaldehyde Standard (Item No. 600094) to tube #1 and mix thoroughly. The concentration of this standard, the first point on the standared curve, is 2 µM. Serially dilute the standard by removing 500 µl from tube #1 and placing it into tube #2; mix thoroughly. Next remove 500 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat the procedure for tubes #4-7. Do not add any standard to tube #8. This tube will be your blank.

4. **Soluble Epoxide Hydrolase Positive Control**

   The CBA sEH Positive Control vial (Item No. 600093) contains 10 µl of 1 mg/ml recombinant human sEH. Thaw and store the enzyme on ice while preparing the reagents for the assay. Prior to assaying, dilute 1 µl of sEH Positive Control with 1 ml of diluted Assay Buffer and mix thoroughly. This is the stock sEH Positive Control and is stable for two hours on ice.

   Immediately before assaying, dilute the stock sEH Positive Control 1:10-1:100 in the diluted Assay Buffer. The concentration of this Positive Control is 10 ng/ml.

5. **Soluble Epoxide Hydrolase Substrate**

   The CBA sEH Substrate vial (Item No. 600095) contains 100 µl of Epoxy Fluor 7 in DMSO. Prior to assaying, dilute 10 µl of CBA sEH Substrate with 10 ml of diluted Assay Buffer.

6. **Soluble Epoxide Hydrolase Inhibitor AUDA**

   The CBA sEH Inhibitor (10 mM) vial (Item No. 600096) contains 50 µl of 10 mM AUDA in DMSO. Prior to assaying, dilute 10 µl with 500 µl of diluted Assay Buffer. The diluted solution is stable for two hours at room temperature.

ASSAY PROTOCOL

Plate Set Up

There is no specific pattern for using the wells on the plate. However, a positive control in duplicate has to be assayed with the sample. We suggest that each sample be assayed at least in duplicate and to have two wells designated as background wells. We also recommend assaying each sample in the presence and absence of the sEH inhibitor to allow for the correction of sEH-independent fluorescence. Record the contents of each well on the template sheet provided on page 18.

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- 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.

1. Centrifuge the plate in a plate centrifuge at 800 x g for five minutes.
2. Aspirate the culture medium.
3. Add 200 µl of diluted Assay Buffer to each well and centrifuge the plate at 800 x g for five minutes.
4. Aspirate the supernatant.
5. Add 100 µl of Lysis Buffer prepared above to each well.
6. Incubate with gentle shaking on an orbital shaker for 30 minutes at room temperature.
7. Centrifuge the plate at 3,000 x g for 20 minutes at 4°C.
8. Transfer 90 µl of the supernatants to the 96-Well Solid Plate (black) with lid (Item No. 10011297). Add 10 µl of Assay Buffer (sample activity measurement) or 10 µl of the AUDA solution (to test assay specificity) to appropriate wells. For positive control wells, add 100 µl of the 10 ng/ml sEH Positive Control prepared above to two wells.
9. Add 200 µl of 6-methoxy-2-Naphthaldehyde Standards prepared above to corresponding wells of the black plate.
10. Add 100 µl of the Substrate Solution to each well, except the standards, and incubate the plate at 37°C for 30 minutes.
11. Read the fluorescent intensity of each well (excitation = 330 nm; emission = 465 nm).

Well	Standard	Sample Supernatant	Assay Buffer	AUDA	sEH Positive Control	Substrate Solution
Standard	200 µl	-	-	-	-	-
Sample	-	90 µl	10 µl	-	-	100 µl
Inhibitor Control	-	90 µl	-	10 µl	-	100 µl
Positive Control	-	-	-	-	100 µl	100 µl

Table 1. Pipetting summary

ANALYSIS

Calculations

Plot the Standard Curve

1. Determine the average fluorescence of the standards. Subtract the fluorescence value of the blank (standard tube #8) from itself and all other standards. This is the corrected fluorescence.
2. Plot the corrected fluorescence values (from step 1 above) of each standard as a function of the final concentration of 6-methoxy-2-Naphthaldehyde. See Figure 1, on page 14, for a typical standard curve.

Determination of sEH Activity

1. Determine the average fluorescence of each sample and sample plus inhibitor AUDA.
2. Subtract the AUDA sample fluorescence from the non-AUDA sample fluorescence to yield the corrected sample fluorescence.
3. Calculate the sEH activity (represent by the fluorencence product 6-methoxy-2-Naphthaldehyde) of the samples using the equation obtained from the linear regression of the standard curve substituting corrected fluorescence values for each sample.

$$\text{sEH Activity (pmol/min/ml)} = \frac{[(\text{Corrected Sample Fluorescence} - (\text{y-intercept}))/\text{slope}] \times \text{dilution}}{30 \text{ minutes}}$$

If you anticipate a high production of sEH in the samples, serial dilution may be required to obtain values that fall on the standard curve.

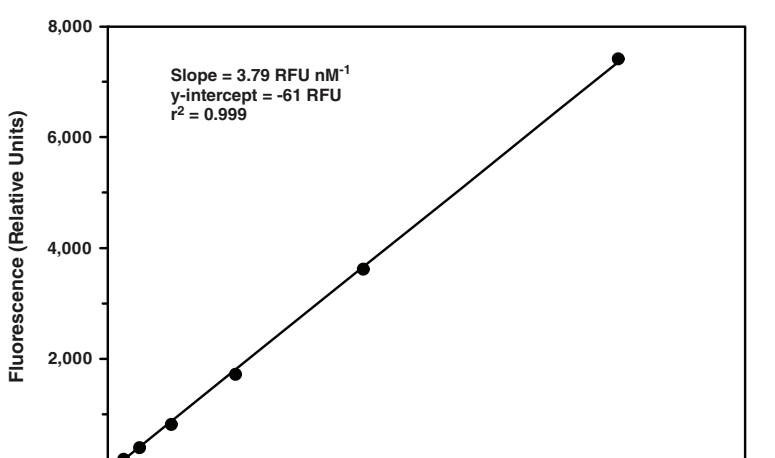


Figure 1. 6-methoxy-2-Naphthaldehyde standard curve

Performance Characteristics

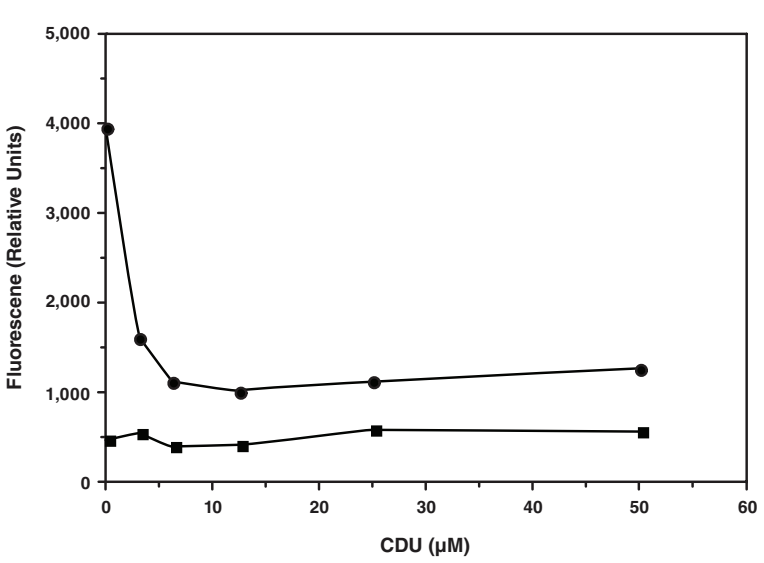


Figure 2. Effect of CDU on sEH activity in Huh-7 cells. HuH-7 cells were seeded at 2 x 10^4 cells/well in RPMI 1640 culture medium and incubated overnight at 37°C. The next day, cells were treated with different concentrations of CDU as indicated on the chart. On the third day, sEH activity in each group of cells were measured using the protocol described in this booklet.

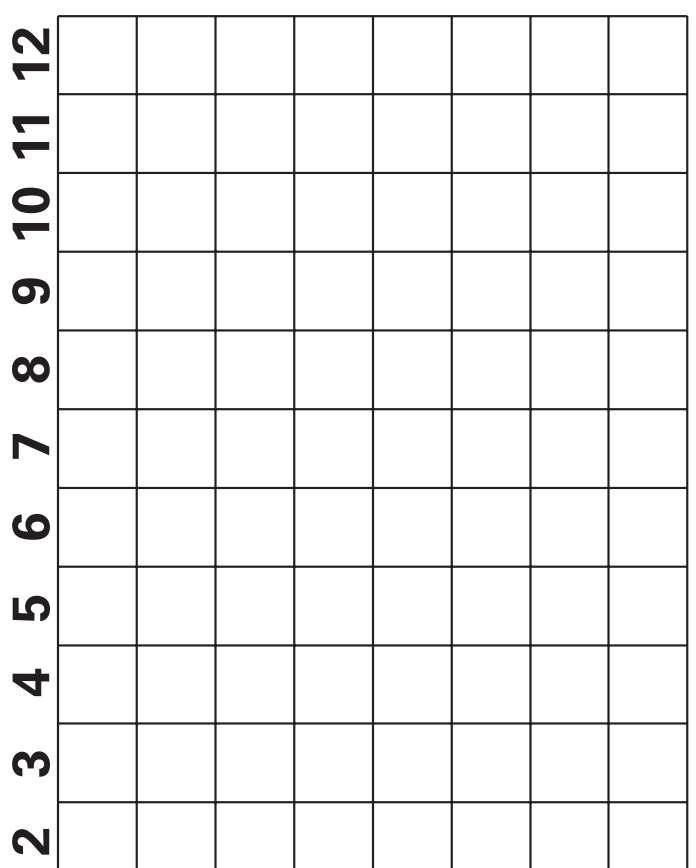
RESOURCES

Troubleshooting

Problem	Possible Causes	Recommended Solutions
Erratic values; dispersion of duplicates	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 the bubbles
Erratic response curve of compound treatments	Unequal number of cells in each well	Make sure each well contains the same number of cells
The fluorometer exhibited 'MAX' values for the wells	The GAIN setting is too high	Reduce the GAIN and re-read
High reading in all wells	Cell density is too high	Plate cells more sparsely

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. Jones, P.D., Wolf, N.M., Morisseau, C., *et al.* Fluorescent substrates for soluble epoxide hydrolase and application to inhibition studies. *Anal. Biochem.* **343**, 66-75 (2005).



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.