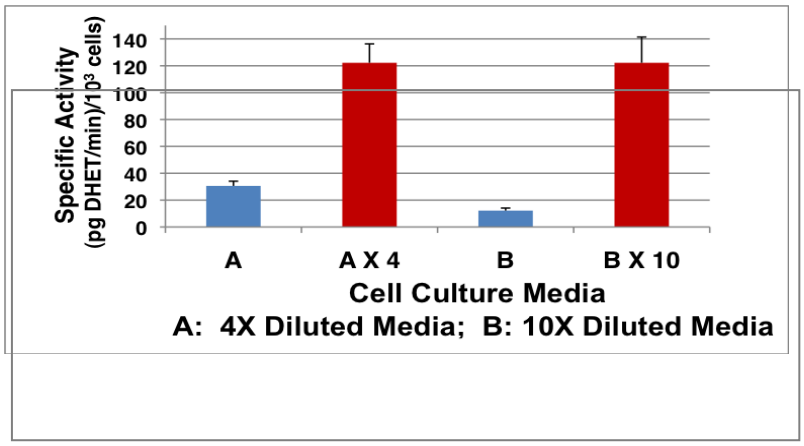
Metro Center for High Technology Bldg.
2727 Second Ave. Suite 4113
Detroit, MI 48201
Phone: (313) 961-1606; Fax: (313)963-7130
Email: info@DetroitRandD.com
Web: www.DetroitRandD.com

Activity Assay and Toxicant and Drug Candidate Screening Kit
Cell-Based Soluble Epoxide Hydrolase (sEH)

Cat # SH 1: ELISA kit for measuring sEH activity in biological samples

The 14,15-DHET is a metabolite of soluble epoxide hydrolase (sEH)-mediated metabolism of 14,15-EET which is generated by arachidonic acid epoxygenase activity of cytochromes P450 (CYPs) 2C and 2J. This competitive ELISA kit with an HRP system can be used to determine 14,15-DHET levels in biological samples (tissue, plasma, and urine) and cell culture media as a measure of sEH activity. In addition, this assay can also be used to screen for inhibitors of soluble epoxide hydrolase activity. The kit is similar to our 14,15-DHET/EET ELISA kit (DH 2) but also contains the EET substrate. Each kit for a 96-well plate is good for triplicate analyses of up to 24 samples.



HepG2 Cell Culture Media Experiment

ELISA kit measurement of 14,15-DHET-formation in either 4X- or 10X-diluted cell culture media following incubation of 10^3 HepG2 cells with 14,15-EET. see Page 2 **Protocols, sEH Activity Protocol for Adherent Cells.**

References for Assay Utility: 14,15-DHET ELISA can be used for (a) quantification of 14,15-EET and DHET and glucuronidated 14,15-DHET In biological samples and cell culture, (b) measurement of enzymatic activities and (c) development of hypertension, stroke and diabetes drugs. H, human; R, rat; M, mouse; and B, bovine.

For a list of publications on sEH-induced disease studies using Detroit R&D ***Bio*Target®** Hypertension ELISA go to**:** **https://www.detroitrandd.com/news/detroit-rd-biotarget-hypertension-elisa**

Related Products:

Other Hypertension & Oxidative Stress ELISAs
14,15-DHET/EET ELISA (**Cat# DH 2**)
11,12-DHET/EET ELISA **Cat.# DH 5**)
20-HETE ELISA (**Cat.# 20H1**)
8-Isoprostane Oxidative Stress ELISA (**Cat.# 8iso1**)

Antibodies
Polyclonal Rabbit anti-P450 1B1 *Human* (**Cat.# PH1B1)**
Polyclonal Rabbit anti-P450 2C9/2C10 *Human* (**Cat.# P2C**)
Polyclonal Rabbit anti-P450 2C23 *Rat* (**Cat.# P2C23DR**)
Polyclonal Rabbit anti-P450 4A (*Rat, Human*) (**Cat.# P4AJC**)

Metro Center for High Technology Bldg.
2727 Second Ave. Suite 4113
Detroit, MI 48201
Phone: (313) 961-1606; Fax: (313)963-7130
Email: info@DetroitRandD.com
Web: www.DetroitRandD.com

Protocols

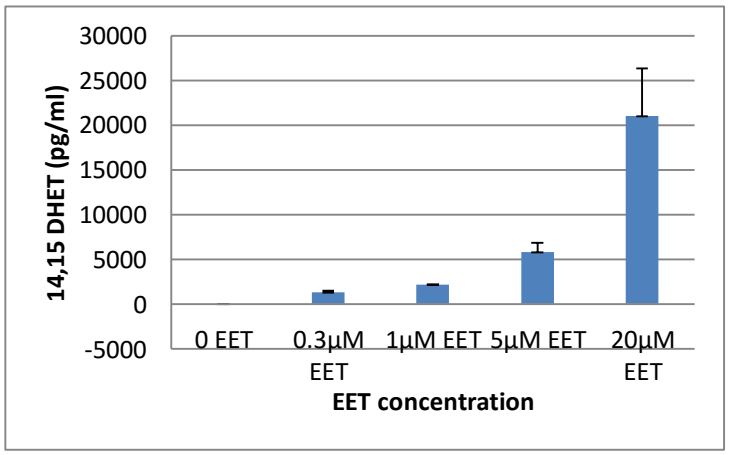
Recommended sEH Activity & Inhibitor screening Protocol for Adherent Cells:

1) Cells are detached from the growing surface by trypsinization.
2) Cells are washed once with PBS and resuspended in 10 mL media . Optimal cell density is approximately 180,000 cells/mL.
3) Cells are transferred to a 96-well clear bottom assay plate (900 cells in 50ul/well) and are allowed to attach to the plate for 12-18 hours in an humidified incubator under 37C.
4) Wells are washed with PBS and 88ul of media (serum free) are added to each well. If screening for an inhibitor of soluble epoxide hydrolase, dilute the test compound to the appropriate concentration(s) and add to the well (recommended final volume of 11ul) and incubate for 30 minutes. Better results may be observed with 2 hours of incubation.
5) Prepare the 14,15-EET solution by diluting the 100 μM stock EET solution (supplied with the kit) with media to a concentration of 10 μM. Add 11ul of diluted EET to each well (final concentration of 1μM EET).
6) Incubate cell mixture for 1 hour at room temperature.
7) Transfer 100ul of media to the 14,15 DHET ELISA plate. (We found that no dilution works best).

sEH Activity Protocol for Cytosol preparations (recommended protocol based on liver cytosol; adjust as necessary if using cytosol prepared from other tissue)

1. Mix 400μl of 1X sample dilution buffer and 50 μl cytosol (final protein concentration should be between 4 and 200ug/mL) in a microtube
2. If screening for inhibitors of soluble epoxide hydrolase activity, dilute the test compound(s) to the appropriate concentration (100X inhibitor stock solutions).

   Add 5 μL of inhibitor to to 395 μL of 1X sample dilution buffer and then add 50 μL cytosol. Incubate for 30 minutes with shaking at room temperature. Better results may be observed with 2 hours of incubation.
3. For all reactions, add 50 μl of 10 μM EET (final concentration 1 μM).
4. Incubate at 37°C for 30 minutes
5. Add 100 μl to each well of the 14,15 DHET Elisa plate and follow direction for how to use the ELISA kit



Cytosolic Soluble epoxide hydrolase mediated 14,15-DHET formation with different concentrations of 14,15-DHET