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sEH shRNA (m) Lentiviral Particles: sc-44392-V

BACKGROUND

Epoxide hydrolase (EHs) are biotransformation enzymes that catalyze the hydrolysis of arene and aliphatic epoxides to less reactive and more water soluble dihydrodiols by the *trans* addition of water. The enzymatic hydration is essentially irreversible and produces mainly metabolites of lower reactivity that can be conjugated and excreted, and, therefore, are generally regarded as detoxifying. Soluble EH (sEH) is a ubiquitous mammalian enzyme for which liver and kidney are reported to have the highest activity. Microsomal EH (mEH) exhibits a broad substrate specificity, while sEH is an enzyme with a "complementary" substrate specificity to mEH. sEH is expressed in 3T3 and HeLa cells. sEH is encoded by the EPHX2 gene, which maps to chromosome 8p21.2.

REFERENCES

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2. Lancaster, J.M., et al. 1996. Microsomal epoxide hydrolase polymorphism as a risk factor for ovarian cancer. *Mol. Carcinog.* 17: 160-162.
3. Seidegard, J., et al. 1997. The role of human glutathione transferases and epoxide hydrolases in the metabolism of xenobiotics. *Environ. Health Perspect.* 105 Suppl. 4: 791-799.
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CHROMOSOMAL LOCATION

Genetic locus: Ephx2 (mouse) mapping to 14 D1.

PRODUCT

sEH shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see sEH siRNA (m): sc-44392 and sEH shRNA Plasmid (m): sc-44392-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

sEH shRNA (m) Lentiviral Particles is recommended for the inhibition of sEH expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

sEH (A-5): sc-166961 is recommended as a control antibody for monitoring of sEH gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor sEH gene expression knockdown using RT-PCR Primer: sEH (m)-PR: sc-44392-PR (20 µl, 565 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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