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DD4 shRNA (h) Lentiviral Particles: sc-44463-V

BACKGROUND

Human liver contains isoforms of dihydrodiol dehydrogenase (DD1, DD2, DD3 and DD4), which belong to the aldo-oxo reductase/aldo-keto reductase (AKR) superfamily, have 20 α - or 3 α -hydroxysteroid dehydrogenase (HSD) activity. DD1 is also designated AKR1C1, DDH or DDH1 while DD2 also can be designated AKR1C2, dDD, BABP or DDH2. AKR1C3 and 3 α -HSD are alternate designations for DD3, while DD4 also can be called AKR1C4, CD or CHDR. DD1 and DD2 are 20 α -HSDs, whereas DD3 and DD4 are the 3 α -HSDs. The multiple human cytosolic dihydrodiol dehydrogenases are involved in the metabolism of xenobiotics, such as polycyclic aromatic hydrocarbons, pesticides and steroid hormones, and are responsible for the reduction of ketone-containing drugs by using NADH or NADPH as a cofactor. The 20 α -HSD catalyzes the reaction of progesterone to the inactive form 20 α -hydroxyprogesterone. The 3 α -HSD is a cytosolic, monomeric, NADPH-dependent oxidoreductase that reduces 3-keto-5-dihydrosteroids to their tetrahydro products. DD1 and DD2 are ubiquitously expressed, whereas DD4 mRNA is restricted to the liver. DD3 is a unique enzyme that can specifically catalyze the dehydrogenation of *trans*-benzenedihydrodiol and *trans*-naphthalenedihydrodiol.

REFERENCES

1. Binstock, J.M., et al. 1992. Human hepatic 3 α -hydroxysteroid dehydrogenase: possible identity with human hepatic chlordecone reductase. *Biochem. Biophys. Res. Commun.* 187: 760-766.
2. Mizoguchi, T., et al. 1992. A novel dihydrodiol dehydrogenase in bovine liver cytosol: purification and characterization of multiple forms of dihydrodiol dehydrogenase. *J. Biochem.* 12: 523-529.
3. Nanjo, H., et al. 1995. Enzymatic characterization of a novel bovine liver dihydrodiol dehydrogenase—reaction mechanism and bile acid dehydrogenase activity. *Biochim. Biophys. Acta* 1244: 53-61.
4. Khanna, M., et al. 1995. Localization of multiple human dihydrodiol dehydrogenase (DDH1 and DDH2) and chlordecone reductase (CHDR) genes in chromosome 10 by the polymerase chain reaction and fluorescence *in situ* hybridization. *Genomics* 25: 588-590.

CHROMOSOMAL LOCATION

Genetic locus: AKR1C4 (human) mapping to 10p15.1.

PRODUCT

DD4 shRNA (h) 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 μ l frozen stock containing 1.0×10^6 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 DD4 siRNA (h): sc-44463 and DD4 shRNA Plasmid (h): sc-44463-SH as alternate gene silencing products.

STORAGE

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

APPLICATIONS

DD4 shRNA (h) Lentiviral Particles is recommended for the inhibition of DD4 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

DD4 (F-16): sc-27920 is recommended as a control antibody for monitoring of DD4 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor DD4 gene expression knockdown using RT-PCR Primer: DD4 (h)-PR: sc-44463-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ 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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