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DD3 siRNA (h): sc-44464

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, δ DD, 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.
5. Hara, A., et al. 1996. Relationship of human liver dihydrodiol dehydrogenase to hepatic bile-acid-binding protein and an oxidoreductase of human colon cells. *Biochem. J.* 313: 373-376.

CHROMOSOMAL LOCATION

Genetic locus: AKR1C3 (human) mapping to 10p15.1.

PRODUCT

DD3 siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see DD3 shRNA Plasmid (h): sc-44464-SH and DD3 shRNA (h) Lentiviral Particles: sc-44464-V as alternate gene silencing products.

For independent verification of DD3 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44464A, sc-44464B and sc-44464C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

DD3 siRNA (h) is recommended for the inhibition of DD3 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 μ M in 66 μ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

DD3 (D-9): sc-398596 is recommended as a control antibody for monitoring of DD3 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor DD3 gene expression knockdown using RT-PCR Primer: DD3 (h)-PR: sc-44464-PR (20 μ l, 576 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.