

AKR1D1 siRNA (h): sc-61964

BACKGROUND

AKR1D1 (aldo-keto reductase family 1 member D1), also known as δ^4 -3-oxosteroid 5- β -reductase (3o5bred) or steroid 5- β -reductase (SRD5B1), is responsible for catalyzing bile acid intermediates and steroid hormones possessing a δ^4 -3-one structure to 5 β reduced metabolites. The AKR family of proteins are soluble NADPH oxidoreductases. They play important roles in the metabolism of drugs, carcinogens and reactive aldehydes. AKR1D1 is highly expressed in liver, colon and testis. Substrates for AKR1D1 include Testosterone, androstenedione, Progesterone, 17- α -hydroxyprogesterone and the bile acid intermediates 7 α -hydroxy-4-cholesten-3-one and 7- α , 12- α -dihydroxy-4-cholesten-3-one. A deficiency in AKR1D1 may be involved in hepatic dysfunction.

REFERENCES

1. Kondo, K.H., et al. 1994. Cloning and expression of cDNA of human δ^4 -3-oxosteroid 5 β -reductase and substrate specificity of the expressed enzyme. *Eur. J. Biochem.* 219: 357-363.
2. Clayton, P.T., et al. 1996. δ^4 -3-oxosteroid 5 β -reductase deficiency: failure of ursodeoxycholic acid treatment and response to chenodeoxycholic acid plus cholic acid. *Gut* 38: 623-628.
3. Sumazaki, R., et al. 1997. Gene analysis in δ^4 -3-oxosteroid 5 β -reductase deficiency. *Lancet* 349: 329.
4. Charbonneau, A. and Luu-The, V. 1999. Assignment of steroid 5 β -reductase (SRD5B1) and its pseudogene (SRD5BP1) to human chromosome bands 7q32→q33 and 1q23→q25, respectively, by *in situ* hybridization. *Cytogenet. Cell Genet.* 84: 105-106.
5. Charbonneau, A. and The, V.L. 2001. Genomic organization of a human 5 β -reductase and its pseudogene and substrate selectivity of the expressed enzyme. *Biochim. Biophys. Acta* 1517: 228-235.
6. Lemonde, H.A., et al. 2003. Mutations in SRD5B1 (AKR1D1), the gene encoding δ^4 -3-oxosteroid 5 β -reductase, in hepatitis and liver failure in infancy. *Gut* 52: 1494-1499.
7. Gonzales, E., et al. 2004. SRD5B1 (AKR1D1) gene analysis in δ^4 -3-oxosteroid 5 β -reductase deficiency: evidence for primary genetic defect. *J. Hepatol.* 40: 716-718.

CHROMOSOMAL LOCATION

Genetic locus: AKR1D1 (human) mapping to 7q33.

PRODUCT

AKR1D1 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 AKR1D1 shRNA Plasmid (h): sc-61964-SH and AKR1D1 shRNA (h) Lentiviral Particles: sc-61964-V as alternate gene silencing products.

For independent verification of AKR1D1 (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-61964A, sc-61964B and sc-61964C.

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

AKR1D1 siRNA (h) is recommended for the inhibition of AKR1D1 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

AKR1D1 (C-2): sc-365932 is recommended as a control antibody for monitoring of AKR1D1 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 AKR1D1 gene expression knockdown using RT-PCR Primer: AKR1D1 (h)-PR: sc-61964-PR (20 μ l). 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.