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SANTA CRUZ BIOTECHNOLOGY, INC.

CYP7B1 siRNA (h): sc-41492

BACKGROUND

P450 enzymes constitute a family of monooxygenase enzymes that are involved in the metabolism of a wide array of endogenous and xenobiotic compounds including cholesterol. CYP8B1 moderates the ratio of cholic acid over chenodeoxycholic acid to control the solubility of cholesterol. P450 cholesterol 7-hydroxylase, CYP7A1, is the rate limiting enzyme of bile acid synthesis in the liver, and its expression is mediated by the bile acid receptor FXR. CYP27A1 catalyzes vitamin D_3 25-hydroxylation and is localized to the mitochondria in kidney and liver. CYP7B1 (oxysterol 7-α-hydroxylase) functions as an enzyme in the alternate bile acid synthesis pathway. Specifically, CYP7B1 metabolizes 25- and 27-hydroxycholesterol. The gene encoding human CYP7B1 maps to chromosome 8q12.3. Mutations in the CYP7B1 gene may cause a metabolic defect in bile acid synthesis characterized by elevated urinary bile acid excretion, severe cholestasis, cirrhosis and liver synthetic failure.

REFERENCES

1. Eggertsen, G., et al. 1996. Molecular cloning and expression of rabbit sterol 12α-hydroxylase. J. Biol. Chem. 271: 32269-32275.
2. Peterson, J.A., et al. 1997. P450BM-3; a tale of two domains—or is it three? Steroids 62: 117-123.
3. Setchell, K.D., et al. 1998. Identification of a new inborn error in bile acid synthesis: mutation of the oxysterol 7α-hydroxylase gene causes severe neonatal liver disease. J. Clin. Invest. 102: 1690-1703.
4. Repa, J.J., et al. 2000. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. Science 289: 1524-1529.
5. Sawada, N., et al. 2000. Metabolism of vitamin D_3 by human CYP27A1. Biochem. Biophys. Res. Commun. 273: 977-984.
6. Li-Hawkins, J., et al. 2000. Disruption of the oxysterol 7-α-hydroxylase gene in mice. J. Biol. Chem. 275: 16536-16542.

CHROMOSOMAL LOCATION

Genetic locus: CYP7B1 (human) mapping to 8q12.3.

PRODUCT

CYP7B1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CYP7B1 shRNA Plasmid (h): sc-41492-SH and CYP7B1 shRNA (h) Lentiviral Particles: sc-41492-V as alternate gene silencing products.

For independent verification of CYP7B1 (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-41492A, sc-41492B and sc-41492C.

PROTOCOLS

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

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

CYP7B1 siRNA (h) is recommended for the inhibition of CYP7B1 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 µM in 66 µl. 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

CYP7B1 (WW-H9): sc-134309 is recommended as a control antibody for monitoring of CYP7B1 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 CYP7B1 gene expression knockdown using RT-PCR Primer: CYP7B1 (h)-PR: sc-41492-PR (20 µl). 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.