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SANTA CRUZ BIOTECHNOLOGY, INC.

CYP8B1 siRNA (m): sc-41495

BACKGROUND

CYP8B1 (sterol 12-α-hydroxylase) is a member of the cytochrome P450 superfamily of monooxygenase enzymes that are involved in the metabolism of a wide array of endogenous and xenobiotic compounds. CYP8B1 is highly expressed in liver and is an important enzyme for bile acid synthesis. Specifically, CYP8B1 moderates the ratio of cholic acid over chenodeoxycholic acid to control the solubility of cholesterol. The gene encoding human CYP8B1 maps to chromosome 3p22.1. The CYP8B1 gene encodes a 501-amino acid protein and does not contain any introns. The CYP8B1 gene promoter is trans-activated by hepatocyte nuclear factor 4α. In mice, disruption of the CYP8B1 gene prevents the synthesis of cholate, which is a primary bile acid.

REFERENCES

1. Eggertsen, G., Olin, M., Andersson, U., Ishida, H., Kubota, S., Hellman, U., Okuda, K.I. and Bjorkhem, I. 1996. Molecular cloning and expression of rabbit sterol 12α-hydroxylase. J. Biol. Chem. 271: 32269-32275.
2. Peterson, J.A., Sevrioukova, I., Truan, G. and Graham-Lorence, S.E. 1997. P450BM-3; a tale of two domains—or is it three? Steroids 62: 117-123.
3. Gafvels, M., Olin, M., Chowdhary, B.P., Raudsepp, T., Andersson, U., Persson, B., Jansson, M., Bjorkhem, I. and Eggertsen, G. 1999. Structure and chromosomal assignment of the sterol 12α-hydroxylase gene (CYP8B1) in human and mouse: eukaryotic cytochrome P450 gene devoid of introns. Genomics 56: 184-196.
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5. Li-Hawkins, J., Gafvels, M., Olin, M., Lund, E.G., Andersson, U., Schuster, G., Bjorkhem, I., Russell, D.W. and Eggertsen, G. 2002. Cholic acid mediates negative feedback regulation of bile acid synthesis in mice. J. Clin. Invest. 110: 1191-1200.

CHROMOSOMAL LOCATION

Genetic locus: Cyp8b1 (mouse) mapping to 9 F4.

PRODUCT

CYP8B1 siRNA (m) 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 CYP8B1 shRNA Plasmid (m): sc-41495-SH and CYP8B1 shRNA (m) Lentiviral Particles: sc-41495-V as alternate gene silencing products.

For independent verification of CYP8B1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41495A, sc-41495B and sc-41495C.

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

CYP8B1 siRNA (m) is recommended for the inhibition of CYP8B1 expression in mouse 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

CYP8B1 (M15-P3B7): sc-101387 is recommended as a control antibody for monitoring of CYP8B1 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 CYP8B1 gene expression knockdown using RT-PCR Primer: CYP8B1 (m)-PR: sc-41495-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.