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CYP27A1 siRNA (m): sc-41501

BACKGROUND

P450 enzymes constitute a family of monooxygenase enzymes that are involved in the metabolism of a wide array of endo-genous and xenobiotic compounds. P450 enzymes can be classified, based on their sequence similarities, into distinct subfamilies, which include CYP1A and CYP2A. Other P450 family members include CYP19, also designated aromatase (P450arom), which catalyzes the conversion of C19 steroids to estrogens in various tissues, including placenta, gonads, adipose tissue, skin and brain. CYP19 expression is controlled by hormonally regulated promoters in different tissues and increased aromatase activity is associated with familial gynecomastia. Also, a polymorphic allele of CYP19 (repeat (TTTA)₁₂) is present in a majority of breast cancer patients. P450 cholesterol 7 α -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 D₃ 25-hydroxylation and is localized to the mitochondria in kidney and liver.

REFERENCES

- Nelson, D.R., et al. 1996. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* 6: 1-42.
- Peterson, J.A., et al. 1997. P450BM-3; a tale of two domains—or is it three? *Steroids* 62: 117-123.
- Bulun, S.E., et al. 1997. Endocrine disorders associated with inappropriately high aromatase expression. *J. Steroid Biochem. Mol. Biol.* 61: 133-139.
- Braunstein, G.D. 1999. Aromatase and gynecomastia. *Endocr. Relat. Cancer* 6: 315-324.
- Kristensen, V.N., et al. 2000. Genetic variants of CYP19 (aromatase) and breast cancer risk. *Oncogene* 19: 1329-1333.
- Repa, J.J., et al. 2000. Regulation of absorption and ABC1-mediated efflux of cholesterol by RXR heterodimers. *Science* 289: 1524-1529.

CHROMOSOMAL LOCATION

Genetic locus: Cyp27a1 (mouse) mapping to 1 C3.

PRODUCT

CYP27A1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CYP27A1 shRNA Plasmid (m): sc-41501-SH and CYP27A1 shRNA (m) Lentiviral Particles: sc-41501-V as alternate gene silencing products.

For independent verification of CYP27A1 (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-41501A, sc-41501B and sc-41501C.

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 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

CYP27A1 siRNA (m) is recommended for the inhibition of CYP27A1 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 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

CYP27A1 (G-2): sc-390974 is recommended as a control antibody for monitoring of CYP27A1 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 CYP27A1 gene expression knockdown using RT-PCR Primer: CYP27A1 (m)-PR: sc-41501-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.