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CYP24 siRNA (h): sc-44652

BACKGROUND

P450 enzymes constitute a family of monooxygenase enzymes that are involved in the metabolism of a wide array of endogenous and xenobiotic compounds. P450 enzymes can be classified, based on their sequence similarities, into distinct subfamilies, which include CYP1A and CYP2A. The P450 family member, CYP19, catalyzes the conversion of C19 steroids to estrogens in various tissues, including placenta, gonads, adipose tissue, skin and brain. 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 D 25-hydroxylation and is localized to the mitochondria in kidney and liver. Overexpression of CYP24 (encoding vitamin D 24 hydroxylase) is likely to lead to abrogation of growth control mediated by vitamin D.

REFERENCES

1. Nelson, D.R., et al. 1996. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics* 6: 1-42.
2. Peterson, J.A., et al. 1997. P450BM-3; a tale of two domains—or is it three? *Steroids* 62: 117-123.
3. Bulun, S.E., et al. 1997. Endocrine disorders associated with inappropriately high aromatase expression. *J. Steroid Biochem. Mol. Biol.* 61: 133-139.
4. Braunstein, G.D. 1999. Aromatase and gynecomastia. *Endocr. Relat. Cancer* 6: 315-324.
5. 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: CYP24A1 (human) mapping to 20q13.2.

PRODUCT

CYP24 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 CYP24 shRNA Plasmid (h): sc-44652-SH and CYP24 shRNA (h) Lentiviral Particles: sc-44652-V as alternate gene silencing products.

For independent verification of CYP24 (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-44652A, sc-44652B and sc-44652C.

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

CYP24 siRNA (h) is recommended for the inhibition of CYP24 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

CYP24 (E-7): sc-365700 is recommended as a control antibody for monitoring of CYP24 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 CYP24 gene expression knockdown using RT-PCR Primer: CYP24 (h)-PR: sc-44652-PR (20 μ l, 540 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Osanai, M. and Lee, G.H. 2016. CYP24A1-induced vitamin D insufficiency promotes breast cancer growth. *Oncol. Rep.* 36: 2755-2762.
2. Hu, N. and Zhang, H. 2018. CYP24A1 depletion facilitates the antitumor effect of vitamin D₃ on thyroid cancer cells. *Exp. Ther. Med.* 16: 2821-2830.

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.