SANTA CRUZ BIOTECHNOLOGY, INC.

CYP4A11 siRNA (h): sc-88588

BACKGROUND

Cytochrome P450 proteins are heme-thiolate monooxygenases that mediate NADPH-dependent electron transport and function to oxidize a variety of structurally unrelated compounds, including steroids, fatty acids and xenobiotics. Specifically, Cytochrome P450s are responsible for metabolizing ara-chidonic acid to hydroxyeicosatetraenoic acid (a regulator of blood pressure) and epoxyeicosatrienoic acid (a molecule involved in signaling events). Cytochrome P450 4A11 (CYP4A11), also known as CP4Y, CYP4A2, P450HL-ω or CYP4AII, is a 519 amino acid protein that is expressed in kidney and liver. Localized to the membrane of the endoplasmic reticulum, CYP4A11 uses heme as a cofactor to catalyze the oxygen-dependent hydroxylation of medium-chain fatty acids, such as myristate, laurate and palmitate, thereby playing an important role in fatty acid metabolism. CYP4A11 exists as two isoforms that are produced by alternative splicing events.

REFERENCES

1. Imaoka, S., et al. 1993. Complete cDNA sequence and cDNA-directed expression of CYP4A11, a fatty acid ω-hydroxylase expressed in human kidney. DNA Cell Biol. 12: 893-899.
2. Powell, P.K., et al. 1998. Metabolism of arachidonic acid to 20-hydroxy-5,8,11,14-eicosatetraenoic acid by P450 enzymes in human liver: involvement of CYP4F2 and CYP4A11. J. Pharmacol. Exp. Ther. 285: 1327-1336.
3. Chang, Y.T. and Loew, G.H. 1999. Homology modeling and substrate binding study of human CYP4A11 enzyme. Proteins 34: 403-415.
4. Lasker, J.M., et al. 2000. Formation of 20-hydroxyeicosatetraenoic acid, a vasoactive and natriuretic eicosanoid, in human kidney. Role of CYP4F2 and CYP4A11. J. Biol. Chem. 275: 4118-4126.
5. Hoch, U. and Ortiz De Montellano, P.R. 2001. Covalently linked heme in cytochrome P4504a fatty acid hydroxylases. J. Biol. Chem. 276: 11339-11346.
6. Gainer, J.V., et al. 2005. Functional variant of CYP4A11 20-hydroxyeicosatetraenoic acid synthase is associated with essential hypertension. Circulation 111: 63-69.
7. Fu, Z., et al. 2008. Haplotype-based case study of human CYP4A11 gene and cerebral infarction in Japanese subject. Endocrine 33: 215-222.

CHROMOSOMAL LOCATION

Genetic locus: CYP4A11 (human) mapping to 1p33.

PRODUCT

CYP4A11 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 CYP4A11 shRNA Plasmid (h): sc-88588-SH and CYP4A11 shRNA (h) Lentiviral Particles: sc-88588-V as alternate gene silencing products.

For independent verification of CYP4A11 (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-88588A, sc-88588B and sc-88588C.

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

CYP4A11 siRNA (h) is recommended for the inhibition of CYP4A11 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

CYP4A11 (M25-P2A10): sc-101385 is recommended as a control antibody for monitoring of CYP4A11 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 CYP4A11 gene expression knockdown using RT-PCR Primer: CYP4A11 (h)-PR: sc-88588-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.

PROTOCOLS

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