SANTA CRUZ BIOTECHNOLOGY, INC.

CYP20A1 siRNA (h): sc-94544

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 arachidonic acid to hydroxyeicosatetraenoic acid (a regulator of blood pressure) and epoxyeicosatrienoic acid (a molecule involved in signaling events). CYP20A1 (cytochrome P450, family 20, subfamily A, polypeptide 1), also known as CYP-M, is a 462 amino acid single-pass membrane protein that belongs to the cytochrome P450 family. CYP20A1 is thought to carry its own oxygen as it lacks a conserved I-helix motif and one amino acid of its conserved heme binding site.

REFERENCES

1. Ito, O., Omata, K., Ito, S., Hoagland, K.M. and Roman, R.J. 2001. Effects of converting enzyme inhibitors on renal P-450 metabolism of arachidonic acid. Am. J. Physiol. Regul. Integr. Comp. Physiol. 280: R822-R830.
2. Jiang, J.H., Jia, W.H., Qin, H.D., Liang, H., Pan, Z.G. and Zeng, Y.X. 2004. Expression of cytochrome P450 enzymes in human nasopharyngeal carcinoma and non-cancerous nasopharynx tissue. Ai Zheng 23: 672-677.
3. Barbosa-Sicard, E., Markovic, M., Honeck, H., Christ, B., Muller, D.N. and Schunck, W.H. 2005. Eicosapentaenoic acid metabolism by cytochrome P450 enzymes of the CYP2C subfamily. Biochem. Biophys. Res. Commun. 329: 1275-1281.
4. Sue Masters, B. and Marohnic, C.C. 2006. Cytochromes P450—a family of proteins and scientists-understanding their relationships. Drug Metab. Rev. 38: 209-225.
5. Inui, H., Maeda, A. and Ohkawa, H. 2007. Molecular characterization of specifically active recombinant fused enzymes consisting of CYP3A4, NADPH-cytochrome P450 oxidoreductase, and cytochrome b5. Biochemistry 46: 10213-10221.
6. Munro, A.W., Girvan, H.M. and McLean, K.J. 2007. Cytochrome P450—redox partner fusion enzymes. Biochim. Biophys. Acta 1770: 345-359.
7. Schmidt, K., Hughes, C., Chudek, J.A., Goodyear, S.R., Aspden, R.M., Talbot, R., Gundersen, T.E., Blomhoff, R., Henderson, C., Wolf, C.R. and Tickle, C. 2009. Cholesterol metabolism: the main pathway acting downstream of cytochrome P450 oxidoreductase in skeletal development of the limb. Mol. Cell. Biol. 29: 2716-2729.

CHROMOSOMAL LOCATION

Genetic locus: CYP20A1 (human) mapping to 2q33.2.

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.

PRODUCT

CYP20A1 siRNA (h) is a pool of 2 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 CYP20A1 shRNA Plasmid (h): sc-94544-SH and CYP20A1 shRNA (h) Lentiviral Particles: sc-94544-V as alternate gene silencing products.

For independent verification of CYP20A1 (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-94544A and sc-94544B.

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

CYP20A1 siRNA (h) is recommended for the inhibition of CYP20A1 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CYP20A1 gene expression knockdown using RT-PCR Primer: CYP20A1 (h)-PR: sc-94544-PR (20 µl, 594 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.