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SANTA CRUZ BIOTECHNOLOGY, INC.

CYP3A4 siRNA (h): sc-43711

BACKGROUND

Cytochrome P450 3A (CYP3A) genes encode monooxygenases—enzymes which catalyze drug metabolism and the synthesis of cholesterol, steroids and other lipids. CYP3A, the most abundant p450 enzyme in human liver, is responsible for the metabolism of more than 50% of all clinical drugs. CYP3A family members localize in organs that associate with drug disposition, including the liver, gastrointestinal tract and kidney. The CYP3A cluster maps to gene locus 7q22.1 and consists of four genes (CYP3A4, CYP3A5, CYP3A7 andCYP3A43) and two pseudogenes (CYP3A5P1 and CYP3A5P2). CYP3A4 is abundant in the endoplasmic reticulum of liver cells and upper intestinal enterocytes. CYP3A4 expression is inducible by glucocorticoids pharmacological agents.

REFERENCES

1. Murray, G.I., et al. 1988. The immunocytochemical localization and distribution of cytochrome P450 in normal human hepatic and extrahepatic tissues with a monoclonal antibody to human cytochrome P450. Br. J. Clin. Pharmacol. 25: 465-475.
2. Wienkers, L.C. 2001. Problems associated with *in vitro* assessment of drug inhibition of CYP3A4 and other P450 enzymes and its impact on drug discovery. J. Pharmacol. Toxicol. Methods 45: 79-84.
3. Patel, J., et al. 2001. Strategies to overcome simultaneous P-glycoprotein-mediated efflux and CYP3A4-mediated metabolism of drugs. Pharmacogenomics 2: 401-415.
4. Kapucuoglu, N., et al. 2003. Expression of CYP3A4 in human breast tumor and non-tumor tissues. Cancer Lett. 202: 17-23.
5. Williams, P.A., et al. 2004. Crystal structures of human cytochrome P450 3A4 bound to metyrapone and progesterone. Science 305: 683-686.
6. Stedman, C., et al. 2004. Feed-forward regulation of bile acid detoxification by CYP3A4: studies in humanized transgenic mice. J. Biol. Chem. 279: 11336-11343.

CHROMOSOMAL LOCATION

Genetic locus: CYP3A4 (human) mapping to 7q22.1.

PRODUCT

CYP3A4 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 CYP3A4 shRNA Plasmid (h): sc-43711-SH and CYP3A4 shRNA (h) Lentiviral Particles: sc-43711-V as alternate gene silencing products.

For independent verification of CYP3A4 (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-43711A, sc-43711B and sc-43711C.

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

CYP3A4 siRNA (h) is recommended for the inhibition of CYP3A4 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

CYP3A4 (HL3): sc-53850 is recommended as a control antibody for monitoring of CYP3A4 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 CYP3A4 gene expression knockdown using RT-PCR Primer: CYP3A4 (h)-PR: sc-43711-PR (20 µl, 575 bp). 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.