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CYP3A5 siRNA (h): sc-44703

BACKGROUND

CYP3A genes encode monooxygenases, enzymes which catalyze drug metabolism and the synthesis of cholesterol, steroids and other lipids. CYP3A (cytochrome P450, family 3, subfamily A), the most abundant p450 enzyme in human liver, is responsible for the metabolism of more than 50% of all clinical drugs. CYP3A members localize in organs that associate with drug disposition, including the liver, gastrointestinal tract and kidney. The CYP3A cluster consists of four genes: CYP3A43, CYP3A4, CYP3A7 and CYP3A5, and two pseudogenes: CYP3A5P1 and CYP3A5P2. The CYP3A cluster maps to gene locus 7q22.1.

REFERENCES

1. Paulussen, A., et al. 2000. Two linked mutations in transcriptional regulatory elements of the CYP3A5 gene constitute the major genetic determinant of polymorphic activity in humans. *Pharmacogenetics* 10: 415-424.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606534. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
3. 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.
4. Williams, P.A., et al. 2004. Crystal structures of human cytochrome P450 3A4 bound to metyrapone and progesterone. *Science* 305: 683-686.
5. LocusLink Report (LocusID: 1574). <http://www.ncbi.nlm.nih.gov/LocusLink/>

CHROMOSOMAL LOCATION

Genetic locus: CYP3A5 (human) mapping to 7q22.1.

PRODUCT

CYP3A5 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 CYP3A5 shRNA Plasmid (h): sc-44703-SH and CYP3A5 shRNA (h) Lentiviral Particles: sc-44703-V as alternate gene silencing products.

For independent verification of CYP3A5 (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-44703A, sc-44703B and sc-44703C.

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

CYP3A5 siRNA (h) is recommended for the inhibition of CYP3A5 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

CYP3A5 (F18 P3 B6): sc-53616 is recommended as a control antibody for monitoring of CYP3A5 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 CYP3A5 gene expression knockdown using RT-PCR Primer: CYP3A5 (h)-PR: sc-44703-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.

PROTOCOLS

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