SANTA CRUZ BIOTECHNOLOGY, INC.

CYP1A1 siRNA (m): sc-41484

BACKGROUND

P450 enzymes constitute a family of monooxygenase enzymes that are involved in the metabolism of a wide array of endogenous and xenobiotic compounds. Several P450 enzymes have been classified by sequence similarities as members of the CYP1A and CYP2A subfamilies. NADPH cytochrome P450 reductase is a microsomal enzyme responsible for the transfer of electrons from NADPH to cytochrome P450 enzymes during the P450 catalytic cycle. NADPH cytochrome P450 reductase is localized to the endoplasmic reticulum where it is also able to transfer electrons to heme oxygenase and cytochrome b5. NADPH cytochrome P450 reductase is structurally related to two separate flavoprotein families, ferredoxin nucleotide reductase (FNR) and flavodoxin. Electron transfer of NADPH cytochrome P450 reductase requires the binding of two flavin cofactors, FAD and FMN, to the FNR and flavodoxin domains, respectively.

REFERENCES

1. Vermilion, J.L., et al. 1978. Purified liver microsomal NADPH-cytochrome P-450 reductase. Spectral characterization of oxidation-reduction states. J. Biol. Chem. 253: 2694-2704.
2. Shen, A.L., et al. 1989. Structural analysis of the FMN binding domain of NADPH-cytochrome P-450 oxidoreductase by site-directed mutagenesis. J. Biol. Chem. 264: 7584-7589.
3. Haniu, M., et al. 1989. Structural and functional analysis of NADPH-cytochrome P-450 reductase from human liver: complete sequence of human enzyme and NADPH-binding sites. Biochemistry 28: 8639-8645.
4. Ohgiya, S., et al. 1994. Mouse NADPH-cytochrome P-450 oxidoreductase: molecular cloning and functional expression in yeast. Biochim. Biophys. Acta 1186: 137-141.
5. Sevrioukova, I.F., et al. 1995. NADPH-P-450 reductase: structural and functional comparisons of the eukaryotic and prokaryotic isoforms. Biochimie 77: 562-572.
6. Nelson, D.R., et al. 1996. P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics 6: 41-42.

CHROMOSOMAL LOCATION

Genetic locus: Cyp1a1 (mouse) mapping to 9 B.

PRODUCT

CYP1A1 siRNA (m) 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 CYP1A1 shRNA Plasmid (m): sc-41484-SH and CYP1A1 shRNA (m) Lentiviral Particles: sc-41484-V as alternate gene silencing products.

For independent verification of CYP1A1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-41484A, sc-41484B and sc-41484C.

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

CYP1A1 siRNA (m) is recommended for the inhibition of CYP1A1 expression in mouse 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

CYP1A1 (B-4): sc-25304 is recommended as a control antibody for monitoring of CYP1A1 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 CYP1A1 gene expression knockdown using RT-PCR Primer: CYP1A1 (m)-PR: sc-41484-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.