



CYPOR siRNA (m): sc-35148

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. CYPOR, also known as cytochrome P450 reductase and 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. CYPOR is localized to the endoplasmic reticulum, where it is also able to transfer electrons to heme oxygenase and cytochrome b5. CYPOR is structurally related to two separate flavoprotein families, ferredoxin nucleotide reductase (FNR) and flavodoxin. Electron transfer of CYPOR requires the binding of two flavin cofactors, FAD and FMN, to the FNR and flavodoxin domains, respectively.

REFERENCES

1. Vermilion, J.L. and Coon, M.J. 1978. Purified liver microsomal NADPH cytochrome P450 reductase. Spectral characterization of oxidation-reduction states. *J. Biol. Chem.* 253: 2694-2704.
2. Haniu, M., et al. 1989. Structural and functional analysis of NADPH cytochrome P450 reductase from human liver: complete sequence of human enzyme and NADPH-binding sites. *Biochemistry* 28: 8639-8645.
3. Shen, A.L., et al. 1989. Structural analysis of the FMN binding domain of NADPH cytochrome P450 oxidoreductase by site-directed mutagenesis. *J. Biol. Chem.* 264: 7584-7589.
4. Ohgiya, S., et al. 1994. Mouse NADPH cytochrome P450 oxidoreductase: molecular cloning and functional expression in yeast. *Biochem. Biophys. Acta* 1186: 137-141.

CHROMOSOMAL LOCATION

Genetic locus: Por (mouse) mapping to 5 G2.

PRODUCT

CYPOR 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CYPOR shRNA Plasmid (m): sc-35148-SH and CYPOR shRNA (m) Lentiviral Particles: sc-35148-V as alternate gene silencing products.

For independent verification of CYPOR (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-35148A, sc-35148B and sc-35148C.

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

CYPOR shRNA (m) Lentiviral Particles is recommended for the inhibition of CYPOR 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 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

CYPOR (F-10): sc-25270 is recommended as a control antibody for monitoring of CYPOR 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 CYPOR gene expression knockdown using RT-PCR Primer: CYPOR (m)-PR: sc-35148-PR (20 μ l, 575 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Aguilar, A., et al. 2009. P450 oxidoreductase expressed in rat chondrocytes modulates chondrogenesis via cholesterol- and Indian hedgehog-dependent mechanisms. *Endocrinology* 150: 2732-2739.
2. Ilangoan, G., et al. 2021. Defining the reducing system of the NO dioxygenase cytoglobin in vascular smooth muscle cells and its critical role in regulating cellular NO decay. *J. Biol. Chem.* 296: 100196.

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.