

PHYH siRNA (m): sc-76128

BACKGROUND

PHYH (phytanoyl-CoA 2-hydroxylase), also known as RD, LN1, PAHX or LNP1, is a 338 amino acid protein that localizes to the peroxisome and plays an important role in fatty acid metabolism. Expressed in kidney, liver and T cells, PHYH uses iron and ascorbate as cofactors to catalyze the conversion of phytanoyl-CoA to 2-hydroxyphytanoyl-CoA, a reaction that is involved in the α -oxidation of 3-methyl branched fatty acids. Defects in the gene encoding PHYH are associated with Refsum disease (RD), an autosomal recessive disorder that is characterized by retinitis pigmentosa, peripheral neuropathy, cerebellar ataxia, nerve deafness, anosmia, skeletal abnormalities, ichthyosis, cataracts and cardiac impairment, all of which usually develop during the second or third decade of life.

REFERENCES

1. Jansen, G.A., et al. 1997. Phytanoyl-coenzyme A hydroxylase deficiency—the enzyme defect in Refsum's disease. *N. Engl. J. Med.* 337: 133-134.
2. Mihalik, S.J., et al. 1997. Identification of PAHX, a Refsum disease gene. *Nat. Genet.* 17: 185-189.
3. Jansen, G.A., et al. 1999. Phytanoyl-CoA hydroxylase deficiency. Enzymological and molecular basis of classical Refsum disease. *Adv. Exp. Med. Biol.* 466: 371-376.
4. Mukherji, M., et al. 2001. Structure-function analysis of phytanoyl-CoA 2-hydroxylase mutations causing Refsum's disease. *Hum. Mol. Genet.* 10: 1971-1982.
5. Kee, H.J., et al. 2003. A novel murine long-chain acyl-CoA synthetase expressed in brain participates in neuronal cell proliferation. *Biochem. Biophys. Res. Commun.* 305: 925-933.
6. Foulon, V., et al. 2003. Further studies on the substrate spectrum of phytanoyl-CoA hydroxylase: implications for Refsum disease? *J. Lipid Res.* 44: 2349-2355.

CHROMOSOMAL LOCATION

Genetic locus: Phyh (mouse) mapping to 2 A1.

PRODUCT

PHYH 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 PHYH shRNA Plasmid (m): sc-76128-SH and PHYH shRNA (m) Lentiviral Particles: sc-76128-V as alternate gene silencing products.

For independent verification of PHYH (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-76128A, sc-76128B and sc-76128C.

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 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

PHYH siRNA (m) is recommended for the inhibition of PHYH 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

PHYH (E-8): sc-376727 is recommended as a control antibody for monitoring of PHYH 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 PHYH gene expression knockdown using RT-PCR Primer: PHYH (m)-PR: sc-76128-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.