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PPOX siRNA (m): sc-44784

BACKGROUND

Protoporphyrinogen oxidase, the penultimate enzyme in the heme biosynthetic pathway, catalyzes the 6-electron oxidation of protoporphyrinogen IX to form protoporphyrin IX. The PPOX protein localizes to the inner membrane of mitochondria from various tissues, including heart, brain, placenta, lung, liver, skeletal muscle, kidney and pancreas. Genetic deficiency of PPOX results in variegate porphyria, a low penetrance, autosomal dominant disorder characterized by cutaneous photosensitivity and/or various neurological manifestations. The rare homozygous variant of VP is characterized by severe PPOX deficiency, and results in the onset of photosensitization by porphyrins in early childhood, skeletal abnormalities of the hand, and, less constantly, short stature, mental retardation and convulsions.

REFERENCES

1. Taketani, S., et al. 1995. The human protoporphyrinogen oxidase gene (PPOX): organization and location to chromosome 1. *Genomics* 29: 698-703.
2. Nishimura, K., et al. 1995. Cloning of a human cDNA for protoporphyrinogen oxidase by complementation *in vivo* of a hemG mutant of *Escherichia coli*. *J. Biol. Chem.* 270: 8076-8080.
3. Puy, H., et al. 1996. Protoporphyrinogen oxidase: complete genomic sequence and polymorphisms in the human gene. *Biochem. Biophys. Res.* 226: 226-230.
4. Maneli, M.H., et al. 2003. Kinetic and physical characterisation of recombinant wild-type and mutant human protoporphyrinogen oxidases. *Biochim. Biophys. Acta* 1650: 10-21.
5. Wiman, A., et al. 2003. Nine novel mutations in the protoporphyrinogen oxidase gene in Swedish families with variegate porphyria. *Clin. Genet.* 64: 122-130.
6. Morgan, R.R., et al. 2004. Identification of sequences required for the import of human protoporphyrinogen oxidase to mitochondria. *Biochem. J.* 377: 281-287.

CHROMOSOMAL LOCATION

Genetic locus: Ppox (mouse) mapping to 1 H3.

PRODUCT

PPOX 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 PPOX shRNA Plasmid (m): sc-44784-SH and PPOX shRNA (m) Lentiviral Particles: sc-44784-V as alternate gene silencing products.

For independent verification of PPOX (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-44784A, sc-44784B and sc-44784C.

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

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

PPOX (C-12): sc-271768 is recommended as a control antibody for monitoring of PPOX 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 PPOX gene expression knockdown using RT-PCR Primer: PPOX (m)-PR: sc-44784-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.