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PPOX shRNA (m) Lentiviral Particles: sc-44784-V

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.

CHROMOSOMAL LOCATION

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

PRODUCT

PPOX shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see PPOX siRNA (m): sc-44784 and PPOX shRNA Plasmid (m): sc-44784-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

PPOX shRNA (m) Lentiviral Particles is recommended for the inhibition of PPOX expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

PPOX (H-180): sc-67040 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.