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PTPS siRNA (m): sc-155952

BACKGROUND

The tetrahydrobiopterin (BH4) cofactor is essential for hepatic hydroxylase, which is involved in phenylalanine degradation and catecholamine and serotonin biosynthesis. BH4 is also an essential and limiting cofactor for all types of nitric oxide synthase. BH4 deficiency results in hyperphenylalaninemia and monoamine neurotransmitter depletion and is most commonly due to autosomal recessive mutation in 6-pyruvoyltetrahydropterin synthase (PTPS), the second enzyme for BH4 biosynthesis. The active site of PTPS consists of the pterin-anchoring Glu A107 neighbored by two catalytic motifs: a Zn(II) binding site and an intersubunit catalytic triad formed by Cys A42, Asp B88 and His B89. The active site of PTPS undergoes a Zn and Mg-dependent reaction that includes a triphosphate elimination, a stereospecific reduction and the oxidation of both side hydroxyl groups. The catalytic triad of PTPS is involved in the deprotonation of the side-chain carbons of substrates. In addition, Ser 19 of human PTPS may be a substrate for cGMP-dependent protein kinase type II phosphorylation *in vivo*, which is essential for normal activity of PTPS.

REFERENCES

1. Thöny, B., et al. 1992. Human 6-pyruvoyltetrahydropterin synthase: cDNA cloning and heterologous expression of the recombinant enzyme. *Biochem. Biophys. Res. Commun.* 189: 1437-1443.
2. Thöny, B., et al. 1994. Chromosomal location of two human genes encoding tetrahydrobiopterin-metabolizing enzymes: 6-pyruvoyl-tetrahydropterin synthase maps to 11q22.3-q23.3, and pterin-4 a-carbinolamine dehydratase maps to 10q22. *Genomics* 19: 365-368.
3. Kluge, C., et al. 1996. Chromosomal localization, genomic structure and characterization of the human gene and a retropseudogene for 6-pyruvoyl-tetrahydropterin synthase. *Eur. J. Biochem.* 240: 477-484.
4. Laufs, S., et al. 1998. Retrovirus-mediated double transduction of the GTPCH and PTPS genes allows 6-pyruvoyltetrahydropterin synthase-deficient human fibroblasts to synthesize and release tetrahydrobiopterin. *J. Neurochem.* 71: 33-40.
5. Linscheid, P., et al. 1998. Regulation of 6-pyruvoyltetrahydropterin synthase activity and messenger RNA abundance in human vascular endothelial cells. *Circulation* 98: 1703-1706.

CHROMOSOMAL LOCATION

Genetic locus: Pts (mouse) mapping to 9 A5.3.

PRODUCT

PTPS 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 PTPS shRNA Plasmid (m): sc-155952-SH and PTPS shRNA (m) Lentiviral Particles: sc-155952-V as alternate gene silencing products.

For independent verification of PTPS (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-155952A, sc-155952B and sc-155952C.

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

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

PTPS (D-8): sc-514628 is recommended as a control antibody for monitoring of PTPS 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 PTPS gene expression knockdown using RT-PCR Primer: PTPS (m)-PR: sc-155952-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.

PROTOCOLS

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