SANTA CRUZ BIOTECHNOLOGY, INC.

PNPO siRNA (h): sc-76182

BACKGROUND

PNPO (pyridoxamine 5'-phosphate oxidase), also known as PDXPO, FLJ10535 or pyridoxine-5'-phosphate oxidase, is a 261 amino acid protein belonging to the pyridoxamine 5'-phosphate oxidase family. Encoded by a gene that maps to human chromosome 17q21.32, PNPO catalyzes the oxidation of either pyridoxine 5'-phosphate (PNP) or pyridoxamine 5'-phosphate (PMP) into pyridoxal 5'-phosphate (PLP). PNPO is composed of seven exons and six introns, with all exon/intron junctions containing the GT/AG consensus splicing site. Characteristic of housekeeping genes, PNPO contains Sp1-binding sites and CpG islands in its regulatory region and lacks TATA-like sequences. PNPO binds a single FMN per subunit. Developmentally regulated in both liver and brain, PNPO is also found in skeletal muscle and kidney, with very weak expression detected in lung. Mutations in PNPO may cause PNPO-related neonatal epileptic encephalopathy and may be associated with schizophrenia.

REFERENCES

1. Ngo, E.O., et al. 1998. Absence of pyridoxine-5'-phosphate oxidase (PNPO) activity in neoplastic cells: isolation, characterization, and expression of PNPO cDNA. Biochemistry 37: 7741-7748.
2. Bräutigam, C., et al. 2002. Clinical and laboratory findings in twins with neonatal epileptic encephalopathy mimicking aromatic L-amino acid decarboxylase deficiency. Neuropediatrics 33: 113-117.
3. Musayev, F.N., et al. 2003. Structure and properties of recombinant human pyridoxine 5'-phosphate oxidase. Protein Sci. 12: 1455-1463.
4. Kang, J.H., et al. 2004. Genomic organization, tissue distribution and deletion mutation of human pyridoxine 5'-phosphate oxidase. Eur. J. Biochem. 271: 2452-2461.
5. Mills, P.B., et al. 2005. Neonatal epileptic encephalopathy caused by mutations in the PNPO gene encoding pyridox(am)ine 5'-phosphate oxidase. Hum. Mol. Genet. 14: 1077-1086.
6. Hoffmann, G.F., et al. 2007. Pyridoxal 5'-phosphate may be curative in early-onset epileptic encephalopathy. J. Inherit. Metab. Dis. 30: 96-99.

CHROMOSOMAL LOCATION

Genetic locus: PNPO (human) mapping to 17q21.32.

PRODUCT

PNPO siRNA (h) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PNPO shRNA Plasmid (h): sc-76182-SH and PNPO shRNA (h) Lentiviral Particles: sc-76182-V as alternate gene silencing products.

For independent verification of PNPO (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-76182A, sc-76182B and sc-76182C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

PNPO siRNA (h) is recommended for the inhibition of PNPO expression in human 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 µM in 66 µl. 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

PNPO (E-8): sc-393561 is recommended as a control antibody for monitoring of PNPO 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 PNPO gene expression knockdown using RT-PCR Primer: PNPO (h)-PR: sc-76182-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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