

NANP siRNA (m): sc-149821

BACKGROUND

Sialic acids are a family of 9-carbon 2-keto-3-deoxy sugars that are found on the ends of glycoproteins and glycolipids and play important roles in recognition events within the cell. Playing an important role in cell-cell and protein-protein recognition, N-acetylneuraminic acid is the main form of sialic acid in vertebrates. NANP (N-acetylneuraminic acid-9-phosphatase), also known as HDHD4 (haloacid dehalogenase-like hydrolase domain-containing protein 4), is a 248 amino acid protein that belongs to the haloacid dehalogenase (HAD) family and is responsible for dephosphorylating Neu5Ac-9-phosphate to form N-acetylneuraminic acid. Characteristic of the HAD phosphatase family, the catalytic activity of NANP is dependent upon the presence of magnesium and is inhibited by vanadate and calcium.

REFERENCES

1. Van Rinsum, J., et al. 1984. Subcellular localization and tissue distribution of sialic acid-forming enzymes. N-acetylneuraminic acid-9-phosphate synthase and N-acetylneuraminic acid-9-phosphatase. *Biochem. J.* 223: 323-328.
2. Lawrence, S.M., et al. 2000. Cloning and expression of the human N-acetylneuraminic acid phosphate synthase gene with 2-keto-3-deoxy-D-glycero-D-galacto-nononic acid biosynthetic ability. *J. Biol. Chem.* 275: 17869-17877.
3. Chen, H., et al. 2002. Purification and characterization of N-acetylneuraminic acid-9-phosphate synthase from rat liver. *Glycobiology* 12: 65-71.
4. Hao, J., et al. 2005. Cloning, expression, and characterization of sialic acid synthases. *Biochem. Biophys. Res. Commun.* 338: 1507-1514.
5. Hao, J., et al. 2006. Elimination of 2-keto-3-deoxy-D-glycero-D-galacto-nonulosonic acid 9-phosphate synthase activity from human N-acetylneuraminic acid 9-phosphate synthase by a single mutation. *Biochem. J.* 397: 195-201.
6. Glasner, M.E., et al. 2006. Evolution of enzyme superfamilies. *Curr. Opin. Chem. Biol.* 10: 492-497.
7. Maliekal, P., et al. 2006. Identification of the sequence encoding N-acetylneuraminic acid-9-phosphate phosphatase. *Glycobiology* 16: 165-172.

CHROMOSOMAL LOCATION

Genetic locus: Nanp (mouse) mapping to 2 G3.

PRODUCT

NANP 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 NANP shRNA Plasmid (m): sc-149821-SH and NANP shRNA (m) Lentiviral Particles: sc-149821-V as alternate gene silencing products.

For independent verification of NANP (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-149821A, sc-149821B and sc-149821C.

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

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

NANP (D-8): sc-374637 is recommended as a control antibody for monitoring of NANP 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 NANP gene expression knockdown using RT-PCR Primer: NANP (m)-PR: sc-149821-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.