

NDST3 siRNA (h): sc-40765

BACKGROUND

The N-deacetylation and N-sulfation of N-acetylglucosamine residues in heparan sulfate and heparin initiate a set of biochemical reactions, which lead to the synthesis of oligosaccharide sequences that have specific ligand binding properties. These reactions are catalyzed by the monomeric enzymes GlcNAc N-deacetylase/N-sulfotransferases (NDSTs), which have two catalytic activities. Multiple NDST isozymes have been identified, each having unique tissue distribution and enzymatic properties. Phylogenetic data suggests that NDST1-4 evolved from a common ancestral gene, which diverged to give rise to two subtypes, NDST1/2 and NDST3/4. NDST1, which maps to human chromosome 5q33.1, shares the most homology with NDST2, which maps to human chromosome 10q22.2. The least conserved amino acids between these two enzymes are found in the N-terminus/putative transmembrane regions. The human NDST3 and NDST4 genes are closely linked on chromosome 4, mapping to chromosome 4q26, respectively. RT-PCR analysis of various mouse tissues reveals a restricted pattern of NDST3 and NDST4 mRNA expression when compared with that of NDST1 and NDST2, which are abundantly and ubiquitously expressed.

REFERENCES

- Dixon, J., Loftus, S.K., Gladwin, A.J., Scambler, P.J., Wasmuth, J.J. and Dixon, M.J. 1995. Cloning of the human heparan sulfate-N-deacetylase/N-sulfotransferase gene from the Treacher Collins syndrome candidate region at 5q32-q33.1. *Genomics* 26: 239-244.
- Humphries, D.E., Lanciotti, J. and Karlinsky, J.B. 1998. cDNA cloning, genomic organization and chromosomal localization of human heparan glucosaminyl N-deacetylase/N-sulphotransferase-2. *Biochem. J.* 332: 303-307.
- Aikawa, J. and Esko, J.D. 1999. Molecular cloning and expression of a third member of the heparan sulfate/heparin GlcNAc N-deacetylase/N-sulfotransferase family. *J. Biol. Chem.* 274: 2690-2695.
- Aikawa, J., Grobe, K., Tsujimoto, M. and Esko, J.D. 2001. Multiple isozymes of heparan sulfate/heparin GlcNAc N-deacetylase/GlcN N-sulfotransferase. Structure and activity of the fourth member, NDST4. *J. Biol. Chem.* 276: 5876-5882.

CHROMOSOMAL LOCATION

Genetic locus: NDST3 (human) mapping to 4q26.

PRODUCT

NDST3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NDST3 shRNA Plasmid (h): sc-40765-SH and NDST3 shRNA (h) Lentiviral Particles: sc-40765-V as alternate gene silencing products.

For independent verification of NDST3 (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-40765A, sc-40765B and sc-40765C.

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

NDST3 siRNA (h) is recommended for the inhibition of NDST3 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 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

NDST3 (JK-3): sc-100789 is recommended as a control antibody for monitoring of NDST3 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 NDST3 gene expression knockdown using RT-PCR Primer: NDST3 (h)-PR: sc-40765-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.