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NPC2 siRNA (m): sc-44816

BACKGROUND

Niemann-Pick disease, type C2 (NPC2), also known as epididymal secretory protein, is a secreted protein mapping against gene 14q24.3. NPC2 regulates the lipid composition of sperm membranes during maturation in the epididymis. Mutations in the NPC2 gene may cause Nieman-Pick type C2 disease and frontal lobe atrophy. Nieman-Pick type C2 is a fatal hereditary disease characterized by defective lysosome release of cholesterol. The disease is caused by HE1 deficiency, a lysosomal protein proven to be undetectable in fibroblasts from NPC2 patients. This differentiates NPC2 from NPC1, as NPC1 has HE1 protein present.

REFERENCES

1. Naureckiene, S., et al. 2000. Identification of HE1 as the second gene of Niemann-Pick C disease. *Science* 290: 2298-2301.
2. Vanier, M.T. 2003. Niemann-Pick disease type C. *Clin. Am. J. Hum. Genet.* 64: 269-281.
3. Frolov, A. 2003. NPC1 and NPC2 regulate cellular cholesterol homeostasis through generation of low density lipoprotein cholesterol-derived oxysterols. *J. Biol. Chem.* 278: 25517-25525.
4. Ko, D.C., et al. 2003. The integrity of a cholesterol-binding pocket in Niemann-Pick C2 protein is necessary to control lysosome cholesterol levels. *Proc. Natl. Acad. Sci. USA* 100: 2518-2525.
5. Sleat, D.E. 2004. Genetic evidence for nonredundant functional cooperativity between NPC1 and NPC2 in lipid transport. *Proc. Natl. Acad. Sci. USA* 101: 5886-5891.
6. Mutka, A.L. 2004. Secretion of sterols and the NPC2 protein from primary astrocytes. *J. Biol. Chem.* 279: 48654-48662.
7. Deisz, R.A., et al. 2005. Pathological cholesterol metabolism fails to modify electrophysiological properties of afflicted neurones in Niemann-Pick disease type C. *Neuroscience* 130: 867-873.

CHROMOSOMAL LOCATION

Genetic locus: *Npc2* (mouse) mapping to 12 D1.

PRODUCT

NPC2 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 NPC2 shRNA Plasmid (m): sc-44816-SH and NPC2 shRNA (m) Lentiviral Particles: sc-44816-V as alternate gene silencing products.

For independent verification of NPC2 (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-44816A, sc-44816B and sc-44816C.

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

NPC2 siRNA (m) is recommended for the inhibition of NPC2 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

NPC2 (D-3): sc-166449 is recommended as a control antibody for monitoring of NPC2 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 NPC2 gene expression knockdown using RT-PCR Primer: NPC2 (m)-PR: sc-44816-PR (20 μ l, 568 bp). 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.