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NPC2 shRNA (m) Lentiviral Particles: sc-44816-V

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. Genet.* 64: 269-281.
3. 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.
4. 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.
5. Mutka, A.L. 2004. Secretion of sterols and the NPC2 protein from primary astrocytes. *J. Biol. Chem.* 279: 48654-48662.
6. 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.

CHROMOSOMAL LOCATION

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

PRODUCT

NPC2 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see NPC2 siRNA (m): sc-44816 and NPC2 shRNA Plasmid (m): sc-44816-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

NPC2 shRNA (m) Lentiviral Particles is recommended for the inhibition of NPC2 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.