SANTA CRUZ BIOTECHNOLOGY, INC.

NPC1L1 siRNA (m): sc-61226

BACKGROUND

Niemann-Pick disease type C (NPC) is an autosomal recessive disease characterized by the accumulation of unesterified cholesterol in the endosomal/lysosomal system, which results in progressive neurodegeneration and death. Niemann-Pick C1-like protein 1 precusor, or NPC1L1, is a membrane protein involved in the uptake of cholesterol at the intestinal enterocyte across the plasma membrane. NPC1L1 is widely expressed and is the target of ezetimibe, a drug involved in the inhibition of cholesterol absorption. In human, mouse and rat, small intestine tissue shows the highest level of NPC1L1 expression; expression in other tissues includes gallbladder, liver, testis and stomach. The NPC1L1 gene contains 20 exons, with an unusually large 1,526 bp exon 2, and spans approximately 29 kb. The presumed promoter region of the gene harbors a sterol-regulatory element (SRE) for SRE-binding protein, further suggesting that NPC1L1 may play a role in subcellular cholesterol homeostasis.

REFERENCES

1. Davies, J.P., et al. 2005. Inactivation of NPC1L1 causes multiple lipid transport defects and protects against diet-induced hypercholesterolemia. J. Biol. Chem. 280: 12710-12720.
2. Garcia-Calvo, M., et al. 2005. The target of ezetimibe is Niemann-Pick C1-like 1 (NPC1L1). Proc. Natl. Acad. Sci. USA 102: 8132-8137.
3. Hegele, R.A., et al. 2005. NPC1L1 haplotype is associated with inter-individual variation in plasma low-density lipoprotein response to ezetimibe. Lipids Health Dis. 4: 16.
4. Iyer, S.P., et al. 2005. Characterization of the putative native and recombinant rat sterol transporter Niemann-Pick C1-like 1 (NPC1L1) protein. Biochim. Biophys. Acta 1722: 282-292.
5. van der Veen, J.N., et al. 2005. Reduced cholesterol absorption upon PPARδ activation coincides with decreased intestinal expression of NPC1L1. J. Lipid Res. 46: 526-534.
6. Wang, J., et al. 2005. Compound heterozygosity for two non-synonymous polymorphisms in NPC1L1 in a non-responder to ezetimibe. Clin. Genet. 67: 175-177.

CHROMOSOMAL LOCATION

Genetic locus: Npc1l1 (mouse) mapping to 11 A1.

PRODUCT

NPC1L1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NPC1L1 shRNA Plasmid (m): sc-61226-SH and NPC1L1 shRNA (m) Lentiviral Particles: sc-61226-V as alternate gene silencing products.

For independent verification of NPC1L1 (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-61226A, sc-61226B and sc-61226C.

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

NPC1L1 siRNA (m) is recommended for the inhibition of NPC1L1 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 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

NPC1L1 (G-1): sc-166802 is recommended as a control antibody for monitoring of NPC1L1 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 NPC1L1 gene expression knockdown using RT-PCR Primer: NPC1L1 (m)-PR: sc-61226-PR (20 µl). 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.