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NARC-1 shRNA (m) Lentiviral Particles: sc-45483-V

BACKGROUND

Proprotein convertase subtilisin/kexin type 9 (PCSK9), also known as NARC-1, is a 692 amino acid protein that belongs to the peptidase S8 family and contains one peptidase S8 domain. Important in the regulation of plasma cholesterol homeostasis, PCSK9 binds to low-density lipoprotein receptor family members LDLR, very low-density lipoprotein receptor (VLDLR) and apolipoprotein receptor 2 (ApoER2) and promotes their degradation in intracellular acidic compartments. PCSK9 also plays a role in neuronal differentiation and apoptosis. PCSK9 is expressed in Schwann cells, neuro-epithelioma, colon carcinoma, and hepatic and pancreatic cell lines. PCSK9 levels in the brain are highest in the cerebellum during perinatal development, with ischemia causing increased levels in the adult brain. Defects in the gene encoding this protein causes the autosomal dominant disorder familial hypercholesterolemia 3 (HCHOLA3).

REFERENCES

1. Damgaard, D., et al. 2004. No genetic linkage or molecular evidence for involvement of the PCSK9, ARH or CYP7A1 genes in the familial hypercholesterolemia phenotype in a sample of Danish families without pathogenic mutations in the LDL receptor and apoB genes. *Atherosclerosis* 177: 415-422.
2. Maxwell, K.N., et al. 2004. Adenoviral-mediated expression of Pcsk9 in mice results in a low-density lipoprotein receptor knockout phenotype. *Proc. Natl. Acad. Sci. USA* 101: 7100-7105.
3. Allard, D., et al. 2005. Novel mutations of the PCSK9 gene cause variable phenotype of autosomal dominant hypercholesterolemia. *Hum. Mutat.* 26: 497.
4. Lallane, F., et al. 2005. Wildtype PCSK9 inhibits LDL clearance but does not affect apoB-containing lipoprotein production in mouse and cultured cells. *J. Lipid Res.* 46: 1312-1319.
5. SWISS-PROT/TrEMBL (Q80W65). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: Pcsk9 (mouse) mapping to 4 C7.

PRODUCT

NARC-1 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 NARC-1 siRNA (m): sc-45483 and NARC-1 shRNA Plasmid (m): sc-45483-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.

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.

APPLICATIONS

PCSK9 siRNA (m) is recommended for the inhibition of PCSK9 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

PCSK9 (H-160): sc-66996 is recommended as a control antibody for monitoring of PCSK9 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NARC-1 gene expression knockdown using RT-PCR Primer: NARC-1 (m)-PR: sc-45483-PR (20 μ l, 600 bp). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ 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.

PROTOCOLS

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