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N-SMase shRNA (m) Lentiviral Particles: sc-43574-V

BACKGROUND

Sphingomyelin and its metabolic products are now known to have second messenger functions in a variety of cellular signaling pathways. At the epicenter of the sphingomyelin cell signaling pathway is a family of phospholipases called sphingomyelinases. These enzymes cleave sphingomyelin to produce ceramide and phosphocholine. Ceramide in turn serves as a lipid second messenger that induces a variety of cell regulatory phenomenon such as programmed cell death (apoptosis), cell differentiation, cell proliferation, and sterol homeostasis. Neutral sphingomyelinase (N-SMase) is a Mg²⁺ sensitive enzyme that can be activated by a host of physiologically relevant and structurally diverse molecules like tumor necrosis factor α (TNF α), oxidized human low density lipoproteins (Ox-LDL) and several growth factors.

REFERENCES

- Chatterjee, S. 1999. Neutral sphingomyelinase: past, present and future. *Chem. Phys. Lipids* 102: 79-96.
- Chan, E.C., et al. 2000. Purification and characterization of neutral sphingomyelinase from *Helicobacter pylori*. *Biochemistry* 39: 4838-4845.
- Luberto, C., et al. 2002. Inhibition of tumor necrosis factor-induced cell death in MCF7 by a novel inhibitor of neutral sphingomyelinase. *J. Biol. Chem.* 277: 41128-41139.
- Marchesini, N., et al. 2003. Biochemical properties of mammalian neutral sphingomyelinase 2 and its role in sphingolipid metabolism. *J. Biol. Chem.* 278: 13775-13783.
- Chen, S., et al. 2006. Amyloid β peptide increases DP5 expression via activation of neutral sphingomyelinase and JNK in oligodendrocytes. *J. Neurochem.* 97: 631-640.

CHROMOSOMAL LOCATION

Genetic locus: Smpd2 (mouse) mapping to 10 B2.

PRODUCT

N-SMase 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 x 10⁶ 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 N-SMase siRNA (m): sc-43574 and N-SMase shRNA Plasmid (m): sc-43574-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

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

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 10⁶ 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

N-SMase (R-20): sc-26215 is recommended as a control antibody for monitoring of N-SMase 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor N-SMase gene expression knockdown using RT-PCR Primer: N-SMase (m)-PR: sc-43574-PR (20 μ l, 570 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.