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SANTA CRUZ BIOTECHNOLOGY, INC.

N-SMase siRNA (m): sc-43574

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 Mg^{2+} sensitive enzyme that can be activated by a host of physiologically relevant and structurally diverse molecules like tumor necrosis factor a (TNFα), oxidized human low density lipoproteins (Ox-LDL) and several growth factors.

REFERENCES

1. Chatterjee, S. 1999. Neutral sphingomyelinase: past, present and future. Chem. Phys. Lipids 102: 79-96.
2. Chan, E.C., et al. 2000. Purification and characterization of neutral sphingomyelinase from *Helicobacter pylori*. Biochemistry 39: 4838-4845.
3. 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.
4. Marchesini, N., et al. 2003. Biochemical properties of mammalian neutral sphingomyelinase 2 and its role in sphingolipid metabolism. J. Biol. Chem. 278: 13775-13783.
5. 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 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 N-SMase shRNA Plasmid (m): sc-43574-SH and N-SMase shRNA (m) Lentiviral Particles: sc-43574-V as alternate gene silencing products.

For independent verification of N-SMase (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-43574A, sc-43574B and sc-43574C.

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.

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

N-SMase siRNA (m) is recommended for the inhibition of N-SMase 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

N-SMase (B-1): sc-377135 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 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 N-SMase gene expression knockdown using RT-PCR Primer: N-SMase (m)-PR: sc-43574-PR (20 µl, 570 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.