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. Okamoto, Y., et al. 2002. Bcl-x_L interrupts oxidative activation of neutral sphingomyelinase. FEBS Lett. 530: 104-108.
5. Marchesini, N., et al. 2003. Biochemical properties of mammalian neutral sphingomyelinase 2 and its role in sphingolipid metabolism. J. Biol. Chem. 278: 13775-13783.
6. 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.
7. Adamy, C., et al. 2007. Neutral sphingomyelinase inhibition participates to the benefits of N-acetylcysteine treatment in post-myocardial infarction failing heart rats. J. Mol. Cell. Cardiol. 43: 344-533.
8. Yabu, T., et al. 2008. Identification of Mg^{2+}-dependent neutral sphingomyelinase 1 as a mediator of heat stress-induced ceramide generation and apoptosis. J. Biol. Chem. 283: 29971-29982.

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.

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.

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.