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SANTA CRUZ BIOTECHNOLOGY, INC.

N-SMase3 siRNA (m): sc-149774

BACKGROUND

Sphingomyelin and its metabolic products are well 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, two proteins that mediate cell growth arrest and apoptosis. N-SMase3 (neutral sphingomyelinase 3), also known as NSMASE3, nSMase-3 or SMPD4, is an 827 amino acid single-pass membrane protein that localizes to the endoplasmic reticulum and Golgi apparatus. Widely expressed in most tissues, with highest levels in heart and skeletal muscle, N-SMase3 is a member of the magnesium-dependent phosphohydrolase protein family. Enhanced in the presence of phosphatidylserine and tumor necrosis factor (TNF) and inhibited by scyphostatin, N-SMase3 exhibits optimal activity at pH 7. N-SMase3 is considered to play an important role in tumorigenesis and cellular stress response.

REFERENCES

1. Ella, K.M., et al. 1997. Characterization of a sphingomyelinase activity in *Saccharomyces cerevisiae*. Arch. Biochem. Biophys. 340: 101-110.
2. Chatterjee, S. 1999. Neutral sphingomyelinase: past, present and future. Chem. Phys. Lipids 102: 79-96.
3. Chan, E.C., et al. 2000. Purification and characterization of neutral sphingomyelinase from *Helicobacter pylori*. Biochemistry 39: 4838-4845.
4. 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.
5. Okamoto, Y., et al. 2002. Bcl-x_L interrupts oxidative activation of neutral sphingomyelinase. FEBS Lett. 530: 104-108.

CHROMOSOMAL LOCATION

Genetic locus: Smpd4 (mouse) mapping to 16 A3.

PRODUCT

N-SMase3 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-SMase3 shRNA Plasmid (m): sc-149774-SH and N-SMase3 shRNA (m) Lentiviral Particles: sc-149774-V as alternate gene silencing products.

For independent verification of N-SMase3 (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-149774A, sc-149774B and sc-149774C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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-SMase3 siRNA (m) is recommended for the inhibition of N-SMase3 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor N-SMase3 gene expression knockdown using RT-PCR Primer: N-SMase3 (m)-PR: sc-149774-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.