SANTA CRUZ BIOTECHNOLOGY, INC.

N-SMase2 siRNA (m): sc-62656

BACKGROUND

N-SMase2 (neutral sphingomyelinase 2), also known as NSMASE2 or SMPD3 (sphingomyelin phosphodiesterase 3), is a ubiquitously expressed 655 amino acid member of the magnesium-dependent phosphohydrolase protein family. Localized to the membrane of the Golgi apparatus, N-SMase2 functions to catalyze the hydrolysis of sphingomyelin to form ceramide and phosphocholine—two proteins that mediate cell growth arrest and apoptosis. N-SMase2 is enzymatically activated by unsaturated fatty acids and phosphatidylserine and, through regulation of ceramide synthesis, is involved in growth suppression and postnatal development. Expression of N-SMase2 is upregulated during the G_0/G_1 phases of the cell cycle and optimal N-SMase2 activity occurs at a slightly basic pH of 7.5. N-SMase2 deficiency is the cause of chondrodysplasia, a genetic disorder characterized by impaired bone growth that leads to short stature, bowlegs and underdeveloped joints.

REFERENCES

1. Hofmann, K., et al. 2000. Cloning and characterization of the mammalian brain-specific, Mg^{2+}-dependent neutral sphingomyelinase. Proc. Natl. Acad. Sci. USA 97: 5895-5900.
2. Marchesini, N., et al. 2003. Biochemical properties of mammalian neutral sphingomyelinase 2 and its role in sphingolipid metabolism. J. Biol. Chem. 278: 13775-13783.
3. Stoffel, W., et al. 2005. Neutral sphingomyelinase 2 (smpd3) in the control of postnatal growth and development. Proc. Natl. Acad. Sci. USA 102: 4554-4559.

CHROMOSOMAL LOCATION

Genetic locus: Smpd3 (mouse) mapping to 8 D3.

PRODUCT

N-SMase2 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-SMase2 shRNA Plasmid (m): sc-62656-SH and N-SMase2 shRNA (m) Lentiviral Particles: sc-62656-V as alternate gene silencing products.

For independent verification of N-SMase2 (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-62656A, sc-62656B and sc-62656C.

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-SMase2 siRNA (m) is recommended for the inhibition of N-SMase2 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-SMase2 (G-6): sc-166637 is recommended as a control antibody for monitoring of N-SMase2 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-SMase2 gene expression knockdown using RT-PCR Primer: N-SMase2 (m)-PR: sc-62656-PR (20 µl, 419 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Supinski, G.S., et al. 2015. Neutral sphingomyelinase 2 is required for cytokine-induced skeletal muscle calpain activation. Am. J. Physiol. Lung Cell. Mol. Physiol. 309: L614-L624.
2. Zhang, H., et al. 2023. Electrical stimulation increases the secretion of cardioprotective extracellular vesicles from cardiac mesenchymal stem cells. Cells 12: 875.

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.