SANTA CRUZ BIOTECHNOLOGY, INC.

neutral ceramidase siRNA (m): sc-75909

BACKGROUND

Neutral ceramidase, also known as ASAH2 (N-acylsphingosine amidohydrolase (non-lysosomal ceramidase) 2), HNAC1, BCDase or NCDase, is a 780 amino acid single-pass type II membrane protein that exists as a precursor which is proteolytically cleaved to produce a soluble active peptide. Expressed ubiquitously with highest levels present in heart, intestine, kidney and skeletal muscle, neutral ceramidase functions to catalyze the hydrolysis of sphingolipid ceramide into sphingosine and free fatty acid, a reaction that occurs at an optimal pH of 6.5-8.5 and is essential for the regulation of sphingolipid signaling metabolites. Additionally, neutral ceramidase plays a role in apoptotic suppression, as well as in the digestion of dietary sphingolipids within intestinal tissue. Multiple isoforms of neutral ceramidase exist due to alternative splicing events.

REFERENCES

1. Tani, M., et al. 2000. Molecular cloning of the full-length cDNA encoding mouse neutral ceramidase. A novel but highly conserved gene family of neutral/alkaline ceramidases. J. Biol. Chem. 275: 11229-11234.
2. El Bawab, S., et al. 2000. Molecular cloning and characterization of a human mitochondrial ceramidase. J. Biol. Chem. 275: 21508-21513.
3. Mitsutake, S., et al. 2001. Purification, characterization, molecular cloning, and subcellular distribution of neutral ceramidase of rat kidney. J. Biol. Chem. 276: 26249-26259.
4. Osawa, Y., et al. 2005. Roles for C16-ceramide and sphingosine 1-phosphate in regulating hepatocyte apoptosis in response to tumor necrosis factor-α. J. Biol. Chem. 280: 27879-27887.
5. Tani, M., et al. 2005. Mechanisms of sphingosine and sphingosine 1-phosphate generation in human platelets. J. Lipid Res. 46: 2458-2467.

CHROMOSOMAL LOCATION

Genetic locus: Asah2 (mouse) mapping to 19 C1.

PRODUCT

neutral ceramidase 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 neutral ceramidase shRNA Plasmid (m): sc-75909-SH and neutral ceramidase shRNA (m) Lentiviral Particles: sc-75909-V as alternate gene silencing products.

For independent verification of neutral ceramidase (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-75909A, sc-75909B and sc-75909C.

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

neutral ceramidase siRNA (m) is recommended for the inhibition of neutral ceramidase 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 neutral ceramidase gene expression knockdown using RT-PCR Primer: neutral ceramidase (m)-PR: sc-75909-PR (20 µl). 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.