SANTA CRUZ BIOTECHNOLOGY, INC.

Acid Ceramidase siRNA (h): sc-105032

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

Acid Ceramidase catalyzes the degradation of ceramide in normal tissues, and deficiency leads to accumulation of ceramide in tissues, a hallmark of Farber disease. Effected individuals experience early onset joint problems and neurological problems, owing to mutations in the Acid Ceramidase gene. Bioinformatic analysis of gene expression also reveals Acid Ceramidase to be among the five most important genes associated with melanoma. In addition to ceramide hydrolysis, purified Acid Ceramidase also exhibits the ability to catalyze ceramide synthesis, utilizing [14C] lauric acid and sphingosine as substrates. Interestingly, pH regulates which reaction is favored; for hydrolysis the optimum pH is 4.5, whereas the reverse reaction favors a pH of 5.5, further supporting a complex and central role for Acid Ceramidase in sphingolipid metabolism.

REFERENCES

1. Koch, J., et al. 1996. Molecular cloning and characterization of a full-length complementary DNA encoding human Acid Ceramidase. Identification of the first molecular lesion causing Farber disease. J. Biol. Chem. 271: 33110-33115.
2. Strelow, A., et al. 2000. Overexpression of Acid Ceramidase protects from tumor necrosis factor-induced cell death. J. Exp. Med. 192: 601-612.
3. Bernardo, K., et al. 2001. Purification, characterization, and biosynthesis of human Acid Ceramidase. J. Biol. Chem. 270: 11098-11102.
4. Linke, T., et al. 2001. Interfacial regulation of Acid Ceramidase activity. Stimulation of ceramide degradation by lysosomal lipids and sphingolipid activator proteins. J. Biol. Chem. 276: 5760-5768.

CHROMOSOMAL LOCATION

Genetic locus: ASAH1 (human) mapping to 8p22.

PRODUCT

Acid Ceramidase siRNA (h) 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 Acid Ceramidase shRNA Plasmid (h): sc-105032-SH and Acid Ceramidase shRNA (h) Lentiviral Particles: sc-105032-V as alternate gene silencing products.

For independent verification of Acid Ceramidase (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-105032A, sc-105032B and sc-105032C.

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

Acid Ceramidase siRNA (h) is recommended for the inhibition of Acid Ceramidase expression in human 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

Acid Ceramidase (23): sc-136275 is recommended as a control antibody for monitoring of Acid Ceramidase 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 Acid Ceramidase gene expression knockdown using RT-PCR Primer: Acid Ceramidase (h)-PR: sc-105032-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Stefanovic, M., et al. 2016. Targeting glucosylceramide synthase upregulation reverts sorafenib resistance in experimental hepatocellular carcinoma. Oncotarget 7: 8253-8267.
2. Munk, R., et al. 2021. Acid Ceramidase promotes senescent cell survival. Aging 13: 15750-15769.

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.