SANTA CRUZ BIOTECHNOLOGY, INC.

Alk-SMase siRNA (h): sc-60151

BACKGROUND

Sphingomyelin (SM) is a lipid that is found in the membranous myelin sheath surrounding nerve cell axons. Sphingomyelin is made up of sphingosine and fatty acids and potentially plays a role in signal transduction. SM hydrolysis is involved in colonic tumorigenesis and cholesterol absorption, and it is also a source of various lipid messengers. It is triggered in a bile salt-dependent manner by intestinal alkaline sphingomyelinase (Alk-SMase), which is expressed in the intestinal mucosa and human bile. Alk-SMase is an ectoenzyme related to the NPP (nucleotide phosphodiesterase) family with five potential N-glycosylation sites and integral membrane domains at each termini. Trypsin cleaves Alk-SMase at its C-terminus, thereby releasing it from the mucosa and enhancing its activity. In the colon, Alk-SMase displays anti-proliferative and anti-inflammatory properties by generating ceramide, reducing the formation of lysophosphatidic acid and inactivating platelet-activating factor. Alk-SMase is downregulated in human long-standing ulcerative colitis and colonic adenocarcinoma, and mutations in the Alk-SMase gene may lead to colon cancer.

REFERENCES

1. Liu, J.J., et al. 2002. *In vitro* effects of fat, FA, and cholesterol on sphingomyelin hydrolysis induced by rat intestinal alkaline sphingomyelinase. Lipids 37: 469-474.
2. Wu, J., et al. 2004. Pancreatic trypsin cleaves intestinal alkaline sphingomyelinase from mucosa and enhances the sphingomyelinase activity. Am. J. Physiol. Gastrointest. Liver Physiol. 287: G967-G973.
3. Wu, J., et al. 2004. Identification of one exon deletion of intestinal alkaline sphingomyelinase in colon cancer HT-29 cells and a differentiation-related expression of the wild-type enzyme in Caco-2 cells. Carcinogenesis 25: 1327-1333.
4. Wu, J., et al. 2005. Acid sphingomyelinase is induced by butyrate but does not initiate the anticancer effect of butyrate in HT-29 and Hep G2 cells. J. Lipid Res. 46: 1944-1952.
5. Wu, J., et al. 2005. Cloning of alkaline sphingomyelinase from rat intestinal mucosa and adjusting of the hypothetical protein XP_221184 in GenBank. Biochim. Biophys. Acta 1687: 94-102.

CHROMOSOMAL LOCATION

Genetic locus: ENPP7 (human) mapping to 17q25.3.

PRODUCT

Alk-SMase siRNA (h) is a pool of 2 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 Alk-SMase shRNA Plasmid (h): sc-60151-SH and Alk-SMase shRNA (h) Lentiviral Particles: sc-60151-V as alternate gene silencing products.

For independent verification of Alk-SMase (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-60151A and sc-60151B.

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

Alk-SMase siRNA (h) is recommended for the inhibition of Alk-SMase 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Alk-SMase gene expression knockdown using RT-PCR Primer: Alk-SMase (h)-PR: sc-60151-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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.