SANTA CRUZ BIOTECHNOLOGY, INC.

SphK2 siRNA (m): sc-39226

BACKGROUND

Sphingosine kinase (SphK) is a key enzyme catalyzing the phosphorylation of sphingosine to form sphingosine 1-phosphate (SPP or S1P). SPP is a bioactive lipid that exerts multiple biological effects in a large variety of cell types, acting as either an intracellular messenger or an extracellular ligand coupled to EDG-family receptors. Competitive inhibitors of SphK block formation of SPP and selectively inhibit cellular proliferation induced by a variety of factors. One potent inhibitor of SphK1 activity is DMS (N,N-dimethylsphingosine). SPP/SphK has been implicated as a signaling pathway that regulates diverse cellular functions, including cell growth, proliferation and survival. Specifically, SphK1 is involved in the signaling pathway(s) that protects human hepatocytes from the apoptotic action of TNF-α. Furthermore, SPP/SphK may play an important role in neuronal survival by regulating activation of SAPKs and caspases. SphK is widely expressed with highest levels in adult liver, kidney, heart and skeletal muscle, however activation of SphK disengages cells from their liver-specific phenotype. SphK1 is highly homologous with SphK2, another member of a growing class of sphingolipid kinases. Expression of SphK2 mRNA exhibits a markedly different tissue distribution than that of SphK1 and appears at a later stage in embryonic development.

REFERENCES

1. Xia, P., et al. 2000. An oncogenic role of sphingosine kinase. Curr. Biol. 10: 1527-1530.
2. Liu, H., et al. 2000. Molecular cloning and functional characterization of a novel mammalian sphingosine kinase type 2 isoform. J. Biol. Chem. 275: 19513-19520.
3. Osawa, Y., et al. 2001. Sphingosine kinase regulates hepatoma cell differentiation: roles of hepatocyte nuclear factor and retinoid receptor. Biochem. Biophys. Res. Commun. 286: 673-677.
4. Edsall, L.C., et al. 2001. Sphingosine kinase expression regulates apoptosis and caspase activation in PC12 cells. J. Neurochem. 76: 1573-1584.
5. Osawa, Y., et al. 2001. TNF-α-induced sphingosine 1-phosphate inhibits apoptosis through a phosphatidylinositol 3-kinase/Akt pathway in human hepatocytes. J. Immunol. 167: 173-180.
6. Meacci, E., et al. 2002. Sphingosine 1-phosphate evokes calcium signals in C2C12 myoblasts via EDG-3 and EDG-5 receptors. Biochem. J. 362: 349-357.

CHROMOSOMAL LOCATION

Genetic locus: Sphk2 (mouse) mapping to 7 B4.

PRODUCT

SphK2 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 SphK2 shRNA Plasmid (m): sc-39226-SH and SphK2 shRNA (m) Lentiviral Particles: sc-39226-V as alternate gene silencing products.

For independent verification of SphK2 (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-39226A, sc-39226B and sc-39226C.

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

SphK2 siRNA (m) is recommended for the inhibition of SphK2 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 SphK2 gene expression knockdown using RT-PCR Primer: SphK2 (m)-PR: sc-39226-PR (20 µl, 506 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Fu, P., et al. 2021. NOX4 mediates *Pseudomonas aeruginosa*-induced nuclear reactive oxygen species generation and chromatin remodeling in lung epithelium. Antioxidants 10: 477.
2. Di Pietro, P., et al. 2022. Targeting the ASMase/S1P pathway protects from sortilin-evoked vascular damage in hypertension. J. Clin. Invest. 132: e146343.

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.