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SANTA CRUZ BIOTECHNOLOGY, INC.

SphK1 siRNA (h): sc-44114

BACKGROUND

Sphingosine kinase (SphK or SphK1) 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 SphK1 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/SphK1 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 TNFα. Furthermore, SPP/SphK1 may play an important role in neuronal survival by regulating activation of SAPKs and caspases. SphK1 is widely expressed with highest levels in adult liver, kidney, heart and skeletal muscle; however, activation of SphK1 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. TNFα-induced sphingosine 1-phosphate inhibits apoptosis through a phosphatidylinositol 3-kinase/Akt pathway in human hepatocytes. J. Immunol. 167: 173-180.

CHROMOSOMAL LOCATION

Genetic locus: SPHK1 (human) mapping to 17q25.1.

PRODUCT

SphK1 siRNA (h) is a target-specific 19-25 nt siRNA 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 SphK1 shRNA Plasmid (h): sc-44114-SH and SphK1 shRNA (h) Lentiviral Particles: sc-44114-V as alternate gene silencing 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

SphK1 siRNA (h) is recommended for the inhibition of SphK1 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

SphK1 (G-11): sc-365401 is recommended as a control antibody for monitoring of SphK1 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 SphK1 gene expression knockdown using RT-PCR Primer: SphK1 (h)-PR: sc-44114-PR (20 µl, 425 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Cho, S.Y., et al. 2011. Sphingosine kinase 1 pathway is involved in melatonin-induced HIF-1α inactivation in hypoxic PC-3 prostate cancer cells. J. Pineal Res. 51: 87-93.
2. Gao, P. and Smith, C.D. 2011. Ablation of sphingosine kinase-2 inhibits tumor cell proliferation and migration. Mol. Cancer Res. 9: 1509-1519.
3. Oh, Y.T., et al. 2017. DR5 suppression induces sphingosine-1-phosphate-dependent TRAF2 polyubiquitination, leading to activation of JNK/AP-1 and promotion of cancer cell invasion. Cell Commun. Signal. 15: 18.
4. Costales, M.G., et al. 2019. A designed small molecule inhibitor of a non-coding RNA sensitizes HER2 negative cancers to herceptin. J. Am. Chem. Soc. 141: 2960-2974.

RESEARCH USE

For research use only, not for use in diagnostic procedures.