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SphK1 siRNA (m): sc-45446

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 TNF α . 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

- Xia, P., et al. 2000. An oncogenic role of sphingosine kinase. *Curr. Biol.* 10: 1527-1530.
- 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.

CHROMOSOMAL LOCATION

Genetic locus: Sphk1 (mouse) mapping to 11 E2.

PRODUCT

SphK1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SphK1 shRNA Plasmid (m): sc-45446-SH and SphK1 shRNA (m) Lentiviral Particles: sc-45446-V as alternate gene silencing products.

For independent verification of SphK1 (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-45446A, sc-45446B and sc-45446C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

SphK1 siRNA (m) is recommended for the inhibition of SphK1 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 μ M in 66 μ l. 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 (m)-PR: sc-45446-PR (20 μ l, 526 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Prakash, H., et al. 2010. Sphingosine kinase-1 (SphK-1) regulates *Mycobacterium smegmatis* infection in macrophages. *PLoS ONE* 5: e10657.
- Zhang, Y., et al. 2014. Elevated sphingosine-1-phosphate promotes sickling and sickle cell disease progression. *J. Clin. Invest.* 124: 2750-2761.

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.