



SphK2 siRNA (h): sc-39225

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.

CHROMOSOMAL LOCATION

Genetic locus: SPHK2 (human) mapping to 19q13.33.

PRODUCT

SphK2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see SphK2 shRNA Plasmid (h): sc-39225-SH and SphK2 shRNA (h) Lentiviral Particles: sc-39225-V as alternate gene silencing products.

For independent verification of SphK2 (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-39225A, sc-39225B and sc-39225C.

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

SphK2 siRNA (h) is recommended for the inhibition of SphK2 expression in human cells.

PROTOCOLS

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

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

SphK2 (9C5E1): sc-517192 is recommended as a control antibody for monitoring of SphK2 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 SphK2 gene expression knockdown using RT-PCR Primer: SphK2 (h)-PR: sc-39225-PR (20 μ l, 431 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Gao, P. and Smith, C.D. 2011. Ablation of sphingosine kinase-2 inhibits tumor cell proliferation and migration. *Mol. Cancer Res.* 9: 1509-1519.
- Kalari, S., et al. 2012. Sphingosine kinase 1 is required for mesothelioma cell proliferation: role of histone acetylation. *PLoS ONE* 7: e45330.
- Fu, P., et al. 2016. Role of sphingosine kinase 1 and S1P transporter Spns2 in HGF-mediated lamellipodia formation in lung endothelium. *J. Biol. Chem.* 291: 27187-27203.
- Ghosh, S., et al. 2018. TNF α mediated ceramide generation triggers cisplatin induced apoptosis in B16F10 melanoma in a PKC δ independent manner. *Oncotarget* 9: 37627-37646.
- Fohmann, I., et al. 2023. Sphingosine kinase 1/S1P receptor signaling axis is essential for cellular uptake of *Neisseria meningitidis* in brain endothelial cells. *PLoS Pathog.* 19: e1011842.

RESEARCH USE

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