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SphK1 shRNA (m) Lentiviral Particles: sc-45446-V

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.
- 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.

CHROMOSOMAL LOCATION

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

PRODUCT

SphK1 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see SphK1 siRNA (m): sc-45446 and SphK1 shRNA Plasmid (m): sc-45446-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

SphK1 shRNA (m) Lentiviral Particles is recommended for the inhibition of SphK1 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.