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SMS2 shRNA (h) Lentiviral Particles: sc-44429-V

BACKGROUND

The SMS (sphingomyelin synthase) family is a group of integral membrane proteins that includes SMS1 (sphingomyelin synthase 1) and SMS2 (sphingomyelin synthase 2). SMS1 is located in the Golgi apparatus, whereas SMS2 resides primarily at the plasma membrane. Both are bidirectional lipid cholinephosphotransferases which convert phosphatidylcholine (PC) and ceramide to sphingomyelin (SM) and diacylglycerol (DAG) and vice versa, the direction of which depends on the relative concentrations of ceramide and diacylglycerol as phosphocholine acceptors. Therefore, sphingomyelin synthases are thought to be involved in both cell death and survival. Tricyclodecan-9-yl-xanthogenate (D609), a selective tumor cytotoxic agent, inhibits SMS activity, contributing to tumor cell cytotoxicity. SMS proteins are expressed in liver, muscle, heart, brain, stomach and kidney. SMS1 is expressed as four alternatively spliced mRNAs (SMS1 α 1, SMS1 α 2, SMS1 β and SMS1 γ) that translate into three different proteins (SMS1 α , SMS1 β and SMS1 γ), which differ in their tissue distribution and function.

REFERENCES

1. Luberto, C., et al. 1998. Sphingomyelin synthase, a potential regulator of intracellular levels of ceramide and diacylglycerol during SV40 transformation. Does sphingomyelin synthase account for the putative phosphatidylcholine-specific J. Biol. Chem. 273: 14550-14559.
2. Huitema, K., et al. 2004. Identification of a family of animal sphingomyelin synthases. EMBO. J. 23: 33-44.
3. Yamaoka, S., et al. 2004. Expression cloning of a human cDNA restoring sphingomyelin synthesis and cell growth in sphingomyelin synthase-defective lymphoid cells. J. Biol. Chem. 279: 18688-18693.
4. SWISS-PROT/TrEMBL (Q86VZ5). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

Genetic locus: SGMS2 (human) mapping to 4q25.

PRODUCT

SMS2 shRNA (h) 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 SMS2 siRNA (h): sc-44429 and SMS2 shRNA Plasmid (h): sc-44429-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

SMS2 shRNA (h) Lentiviral Particles is recommended for the inhibition of SMS2 expression in human 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

SMS2 (N-13): sc-34048 is recommended as a control antibody for monitoring of SMS2 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor SMS2 gene expression knockdown using RT-PCR Primer: SMS2 (h)-PR: sc-44429-PR (20 μ l). 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.