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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic)

GD3 Synthase shRNA (h) Lentiviral Particles: sc-44586-V

BACKGROUND

GD3 Synthase (GD3S, SIAT8, ST8Sial, ST8 α -N-acetyl-neuraminidase α -2,8-sialyltransferase 1) is a type II membrane protein that catalyzes the transfer of sialic acid from CMP-sialic acid to GM3 to produce gangliosides GD3 and GT3. Gangliosides are membrane-bound glycosphingolipids containing sialic acid. Ganglioside GD3 is known to be important for cell adhesion and growth of cultured malignant cells. GD3 Synthase is found in the Golgi apparatus and is a member of glycosyltransferase family 29. GD3 Synthase can down-regulate MMP-9 promoter activity in response to TNF- α by association with NF κ B and activation protein-1 (AP-1) sites in the MMP-9 promoter. GD3 Synthase has an apoptotic effect on ECV304 cells through downregulation of Bcl-2 expression via dephosphorylation of AKT and CREB.

REFERENCES

- Martina, J.A., et al. 1998. Influence of N-glycosylation and N-glycan trimming on the activity and intracellular traffic of GD3 synthase. *J. Biol. Chem.* 273: 3725-3731.
- Kawai, H., et al. 1998. Embryonic stem cells with a disrupted GD3 synthase gene undergo neuronal differentiation in the absence of β -series gangliosides. *J. Biol. Chem.* 273: 19634-19638.
- Birkle, S., et al. 2000. Down-regulation of GD3 ganglioside and its O-acetylated derivative by stable transfection with antisense vector against GD3-synthase gene expression in hamster melanoma cells: effects on cellular growth, melanogenesis, and dendricity. *J. Neurochem.* 74: 547-554.
- Fukumoto, S., et al. 2000. GD3 synthase gene expression in PC12 cells results in the continuous activation of TrkA and ERK1/2 and enhanced proliferation. *J. Biol. Chem.* 275: 5832-5838.

CHROMOSOMAL LOCATION

Genetic locus: ST8SIA1 (human) mapping to 12p12.1.

PRODUCT

GD3 Synthase 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 GD3 Synthase siRNA (h): sc-44586 and GD3 Synthase shRNA Plasmid (h): sc-44586-SH as alternate gene silencing products.

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.

APPLICATIONS

GD3 Synthase shRNA (h) Lentiviral Particles is recommended for the inhibition of GD3 Synthase 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

GD3 Synthase (B-11): sc-390123 is recommended as a control antibody for monitoring of GD3 Synthase 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 GD3 Synthase gene expression knockdown using RT-PCR Primer: GD3 Synthase (h)-PR: sc-44586-PR (20 μ l, 586 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.

SPROTOCOLS

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