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UGCG shRNA (m) Lentiviral Particles: sc-45405-V

BACKGROUND

Glucosylceramide synthase (GCS), also designated ceramide glucosyltransferase, belongs to the glycosyltransferase 2 family. It is a widely expressed integral membrane protein encoded by UGCG. The enzyme can be found in the plasma membrane of all eukaryotic cells, and a significant concentration of glucosylceramide synthase activity has been reported in the Golgi complex. Glucosylceramide synthase catalyzes the first glycosylation step in glycosphingolipid biosynthesis and functions as a glucosyltransferase and flippase in the transfer of glucose to ceramide. Glucosylceramide synthase operates in cell recognition, cell proliferation and differentiation, immune recognition and signal transduction. The regulation of ceramide levels through glucosylceramide synthase has been associated with the induction of apoptosis and notable research implicates this relationship with drug-induced apoptosis in a variety of cell types.

REFERENCES

- Hakomori, S., et al. 1990. Bifunctional role of glycosphingolipids. Modulators for transmembrane signaling and mediators for cellular interactions. *J. Biol. Chem.* 265: 18713-18716.
- Ichikawa, S., et al. 1996. Expression cloning of a cDNA for human ceramide glucosyltransferase that catalyzes the first glycosylation step of glycosphingolipid synthesis. *Proc. Natl. Acad. Sci. USA* 93: 4638-4643.
- Paul, P., et al. 1996. Purification and characterization of UDP-glucose: ceramide glucosyltransferase from rat liver Golgi membranes. *J. Biol. Chem.* 271: 2287-2293.
- Watanabe, R., et al. 1998. Up-regulation of glucosylceramide synthase expression and activity during human keratinocyte differentiation. *J. Biol. Chem.* 273: 9651-9655.

CHROMOSOMAL LOCATION

Genetic locus: Ugcg (mouse) mapping to 4 B3.

PRODUCT

UGCG 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 UGCG siRNA (m): sc-45405 and UGCG shRNA Plasmid (m): sc-45405-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

UGCG shRNA (m) Lentiviral Particles is recommended for the inhibition of UGCG 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

UGCG (E-14): sc-33869 is recommended as a control antibody for monitoring of UGCG 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 UGCG gene expression knockdown using RT-PCR Primer: UGCG (m)-PR: sc-45405-PR (20 μ l, 534 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.