

UGCG siRNA (m): sc-45405

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

1. Hakomori, S., et al. 1990. Bifunctional role of glycosphingolipids. Modulators for transmembrane signaling and mediators for cellular interactions. *J. Biol. Chem.* 265: 18713-18716.
2. 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.
3. Paul, P., et al. 1996. Purification and characterization of UDP-glucose: ceramide glucosyltransferase from rat liver Golgi membranes. *J. Biol. Chem.* 271: 2287-2293.
4. Watanabe, R., et al. 1998. Upregulation of glucosylceramide synthase expression and activity during human keratinocyte differentiation. *J. Biol. Chem.* 273: 9651-9655.
5. Liu, Y.Y., et al. 1999. Expression of glucosylceramide synthase, converting ceramide to glucosylceramide, confers adriamycin resistance in human breast cancer cells. *J. Biol. Chem.* 274: 1140-1146.

CHROMOSOMAL LOCATION

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

PRODUCT

UGCG siRNA (m) 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 UGCG shRNA Plasmid (m): sc-45405-SH and UGCG shRNA (m) Lentiviral Particles: sc-45405-V as alternate gene silencing products.

For independent verification of UGCG (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-45405A, sc-45405B and sc-45405C.

PROTOCOLS

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

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

UGCG siRNA (m) is recommended for the inhibition of UGCG expression in mouse cells.

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

UGCG (1E5): sc-293235 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 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 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° C and the extension temperature should be 68-72° C.

RESEARCH USE

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