

GALC siRNA (h): sc-60669

BACKGROUND

GALC (galactosylceramidase) is a lysosomal enzyme that hydrolyzes galactose ester bonds in various galactolipids, including galactosylceramide, galactosylsphingosine, lactosylceramide and monogalactosyldiglyceride. Galactolipids contain glucose and/or galactose, and are found in the brain and other nerve tissue, especially the myelin sheath. Galactosylceramide is a major lipid in myelin, kidney and epithelial cells of the small intestine and colon. Mutations in the GALC gene that compromise protein function correlate to Krabbe disease (globoid cell leukodystrophy, GLD). GLD is an autosomal recessive condition that affects approximately 1 in 150,000 infants and results in progressive destruction of the nervous system. The "twitcher" mouse is a model system for GLD; the genotype is a premature stop codon (W339X) in the galactosylceramidase (GALC) gene that abolishes enzymatic activity. Alternate transcriptional splice variants, encoding different isoforms, have been characterized.

REFERENCES

1. Kondo, Y., et al. 2005. Galactocerebroside-deficient oligodendrocytes maintain stable central myelin by exogenous replacement of the missing enzyme in mice. *Proc. Natl. Acad. Sci. USA* 102: 18670-18675.
2. Rafi, M.A., et al. 2005. AAV-mediated expression of galactocerebroside symptoms and extended life span in murine models of globoid cell leukodystrophy. *Mol. Ther.* 11: 734-744.
3. Lin, D., et al. 2005. AAV2/5 vector expressing galactocerebroside ameliorates CNS disease in the murine model of globoid-cell leukodystrophy more efficiently than AAV2. *Mol. Ther.* 12: 422-430.
4. Meng, X.L., et al. 2005. GALC transduction leads to morphological improvement of the twitcher oligodendrocytes *in vivo*. *Mol. Genet. Metab.* 84: 332-343.
5. Luzi, P., et al. 2005. Biochemical and pathological evaluation of long-lived mice with globoid cell leukodystrophy after bone marrow transplantation. *Mol. Genet. Metab.* 86: 150-159.

CHROMOSOMAL LOCATION*

Genetic locus: GALC (human) mapping to 14q31.3.

PRODUCT

GALC siRNA (h) 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 GALC shRNA Plasmid (h): sc-60669-SH and GALC shRNA (h) Lentiviral Particles: sc-60669-V as alternate gene silencing products.

For independent verification of GALC (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-60669A, sc-60669B and sc-60669C.

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.

APPLICATION

GALC siRNA (h) is recommended for the inhibition of GALC expression in human 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

GALC (2D1): sc-293200 is recommended as a control antibody for monitoring of GALC 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 GALC gene expression knockdown using RT-PCR Primer: GALC (h)-PR: sc-60669-PR (20 μ l, 437 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.