

GALK1 siRNA (m): sc-60672

BACKGROUND

Galactose kinase (GALK1), also often designated galactokinase, is important in the first step of the galactose metabolism pathway. GALK1, which belongs to the GHMP kinase family of proteins, is a crucial enzyme for galactose metabolism. Defects in the gene encoding for galactose kinase, GALK1, can cause galactosemia II, an autosomal recessive disorder characterized by congenital cataracts during infancy, often within the first two weeks of life. In the adult population it can cause presenile cataracts that are secondary to accumulation of galactitol in the lens of the eye.

REFERENCES

1. Hunter, M., et al. 2001. Novel mutations in the GALK1 gene in patients with galactokinase deficiency. *Hum. Mutat.* 17: 77-78.
2. Okano, Y., et al. 2001. A genetic factor for age-related cataract: identification and characterization of a novel galactokinase variant, "Osaka", in Asians. *Am. J. Hum. Genet.* 68: 1036-1042.
3. Timson, D.J. and Reece, R.J. 2003. Functional analysis of disease-causing mutations in human galactokinase. *Eur. J. Biochem.* 270: 1767-1774.
4. Holden, H.M., et al. 2004. Galactokinase: structure, function and role in type II galactosemia. *Cell. Mol. Life Sci.* 61: 2471-2484.
5. Ross, K.L., et al. 2004. Differential roles of the Leloir pathway enzymes and metabolites in defining galactose sensitivity in yeast. *Mol. Genet. Metab.* 83: 103-116.
6. Sangiulio, F., et al. 2004. Biochemical characterization of two GALK1 mutations in patients with galactokinase deficiency. *Hum. Mutat.* 23: 396.
7. Thoden, J.B., et al. 2005. Molecular structure of human galactokinase: implications for type II galactosemia. *J. Biol. Chem.* 280: 9662-9670.

CHROMOSOMAL LOCATION

Genetic locus: Galk1 (mouse) mapping to 11 E2.

PRODUCT

GALK1 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 GALK1 shRNA Plasmid (m): sc-60672-SH and GALK1 shRNA (m) Lentiviral Particles: sc-60672-V as alternate gene silencing products.

For independent verification of GALK1 (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-60672A, sc-60672B and sc-60672C.

RESEARCH USE

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

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

GALK1 siRNA (m) is recommended for the inhibition of GALK1 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

GALK1 (A-2): sc-393404 is recommended as a control antibody for monitoring of GALK1 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 GALK1 gene expression knockdown using RT-PCR Primer: GALK1 (m)-PR: sc-60672-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.