

Ketohexokinase siRNA (h): sc-60878

BACKGROUND

The hexokinases utilize Mg-ATP as a phosphoryl donor to catalyze the first step of intracellular glucose metabolism, the conversion of glucose to glucose-6-phosphate. Ketohexokinase (hepatic fructokinase) belongs to the carbohydrate kinase pfkB family and requires potassium. It functions in the metabolism of dietary fructose in mammals, catalyzing the conversion of fructose to fructose-1-phosphate. Ketohexokinase is expressed most abundantly in kidney, liver, pancreas and spleen, while lower levels are seen in muscle, eye and brain. Mutations in KHK, the gene encoding for Ketohexokinase, cause fructosuria, a benign defect of intermediary metabolism characterized by the excretion of fructose in the urine.

REFERENCES

1. Khachadurian, A.K. 1964. Nonalimentary fructosuria. *Pediatrics* 32: 455-457.
2. Boesiger, P., et al. 1995. Changes of liver metabolite concentrations in adults with disorders of fructose metabolism after intravenous fructose by ³¹P magnetic resonance spectroscopy. *Pediatr. Res.* 36: 436-440.
3. Bonthron, D.T., et al. 1995. Molecular basis of essential fructosuria: molecular cloning and mutational analysis of human ketohexokinase (fructokinase). *Hum. Mol. Genet.* 3: 1627-1631.
4. Hayward, B.E., et al. 1998. Structure and alternative splicing of the ketohexokinase gene. *Eur. J. Biochem.* 257: 85-91.
5. Funari, V.A., et al. 2005. Genes required for fructose metabolism are expressed in Purkinje cells in the cerebellum. *Brain Res. Mol. Brain Res.* 142: 115-122.
6. Fabbro, C. et al. 2005. Analysis of regulatory regions of Emilin1 gene and their combinatorial contribution to tissue-specific transcription. *J. Biol. Chem.* 280: 15749-15760.

CHROMOSOMAL LOCATION

Genetic locus: KHK (human) mapping to 2p23.3.

PRODUCT

Ketohexokinase 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 Ketohexokinase shRNA Plasmid (h): sc-60878-SH and Ketohexokinase shRNA (h) Lentiviral Particles: sc-60878-V as alternate gene silencing products.

For independent verification of Ketohexokinase (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-60878A, sc-60878B and sc-60878C.

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

Ketohexokinase siRNA (h) is recommended for the inhibition of Ketohexokinase 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

Ketohexokinase (B-6): sc-377411 is recommended as a control antibody for monitoring of Ketohexokinase 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 Ketohexokinase gene expression knockdown using RT-PCR Primer: Ketohexokinase (h)-PR: sc-60878-PR (20 μ l). 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.