

HXX I siRNA (r): sc-270659

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. Four hexokinase isoenzymes have been identified, including hexokinase I (HXX I), hexokinase II (HXX II), hexokinase III (HXX III) and hexokinase IV (HXX IV, also designated glucokinase or GCK). Hexokinases I-III each contain an N-terminal cluster of hydrophobic amino acids. Glucokinase lacks the N-terminal hydrophobic cluster. The hydrophobic cluster is thought to be necessary for membrane binding. This is substantiated by the finding that glucokinase has lower affinity for glucose than do the other hexokinases. HXX I has been shown to be expressed in brain, kidney and heart tissues as well as in hepatoma cell lines. HXX II is involved in the uptake and utilization of glucose by adipose and skeletal tissues. Of the hexokinases, HXX III has the highest affinity for glucose. Glucokinase is expressed in pancreatic beta cells where it functions as a glucose sensor, determining the "set point" for Insulin secretion.

REFERENCES

1. Katzen, H.M. and Schimke, R.T. 1965. Multiple forms of hexokinase in the rat: tissue distribution, age dependency and properties. *Proc. Natl. Acad. Sci. USA* 54: 1218-1225.
2. Arora, K.K., et al. 1990. Glucose phosphorylation in tumor cells. Cloning, sequencing and overexpression in active form of a fulllength cDNA encoding a mitochondrial bindable form of hexokinase. *J. Biol. Chem.* 265: 6481-6488.
3. Stoeffel, M., et al. 1992. Human glucokinase gene: isolation, characterization and identification of two missense mutations linked to early-onset non-insulin-dependent (type 2) diabetes mellitus. *Proc. Natl. Acad. Sci. USA* 89: 7698-7702.
4. Deeb, S.S., et al. 1993. Human hexokinase II: sequence and homology to other hexokinases. *Biochem. Biophys. Res. Comm.* 197: 68-74.
5. Palma, F., et al. 1996. Purification and characterization of the carboxyl-domain of human hexokinase type III expressed as fusion protein. *Mol. Cell. Biochem.* 155: 23-29.

CHROMOSOMAL LOCATION

Genetic locus: Hk1 (rat) mapping to 20q11.

PRODUCT

HXX I siRNA (r) is a pool of 2 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 HXX I shRNA Plasmid (r): sc-270659-SH and HXX I shRNA (r) Lentiviral Particles: sc-270659-V as alternate gene silencing products.

For independent verification of HXX I (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270659A and sc-270659B.

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

HXX I siRNA (r) is recommended for the inhibition of HXX I expression in rat 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

HXX I (G-1): sc-46695 is recommended as a control antibody for monitoring of HXX I 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 HXX I gene expression knockdown using RT-PCR Primer: HXX I (r)-PR: sc-270659-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.

PROTOCOLS

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