

GCK siRNA (h): sc-35458

BACKGROUND

Glucokinase (also designated hexokinase IV, HXKIV or GCK) plays a key role in the regulation of glucose-induced Insulin secretion. GCK is expressed in pancreatic β cells where it functions as the major glucose sensor of the body, determining the "set point" for Insulin secretion. GCK is also expressed in the liver, where it catalyzes the first committed step in the disposal of glucose. Phosphorylation of glucose by glucokinase appears to be the rate-limiting step for glucose catabolism. A lack of glucokinase activity leads to reduced Insulin secretion and hyperglycemia, and has been implicated as a cause for maturity onset diabetes of the youth (MODY). In fact, heterozygous point mutations in the gene encoding GCK have been detected in individuals suffering from MODY.

REFERENCES

1. De Vos, A., et al. 1995. Human and rat β cells differ in glucose transporter but not in glucokinase gene expression. *J. Clin. Invest.* 96: 2489-2495.
2. Hosokawa, H., et al. 1995. Upregulated hexokinase activity in isolated islets from diabetic 90% pancreatectomized rats. *Diabetes* 44: 1328-1333.
3. Grupe, A., et al. 1995. Transgenic knockouts reveal a critical requirement for pancreatic β cell glucokinase in maintaining glucose homeostasis. *Cell* 83: 69-78.
4. Liang, Y., et al. 1995. Variable effects of maturity-onset-diabetes-of-youth (MODY)-associated glucokinase mutations on substrate interactions and stability of the enzyme. *Biochem. J.* 309: 167-173.
5. Bali, D., et al. 1995. Animal model for maturity-onset diabetes of the young generated by disruption of the mouse glucokinase gene. *J. Biol. Chem.* 270: 21464-21467.
6. Tu, J., et al. 1996. Glucose regulates the maximal velocities of glucokinase and glucose utilization in the immature fetal rat pancreatic islet. *Diabetes* 45: 1068-1075.
7. Heimberg, H., et al. 1996. The glucose sensor protein glucokinase is expressed in glucagon-producing α cells. *Proc. Natl. Acad. Sci. USA* 93: 7036-7041.
8. Ferre, T., et al. 1996. Correction of diabetic alterations by glucokinase. *Proc. Natl. Acad. Sci. USA* 93: 7225-7230.

CHROMOSOMAL LOCATION

Genetic locus: GCK (human) mapping to 7p13.

PRODUCT

GCK 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 GCK shRNA Plasmid (h): sc-35458-SH and GCK shRNA (h) Lentiviral Particles: sc-35458-V as alternate gene silencing products.

For independent verification of GCK (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-35458A, sc-35458B and sc-35458C.

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

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

GCK (G-6): sc-17819 is recommended as a control antibody for monitoring of GCK 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 GCK gene expression knockdown using RT-PCR Primer: GCK (h)-PR: sc-35458-PR (20 μ l, 311 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.

PROTOCOLS

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