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Diagnostik & molekulare Diagnostik



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G6Pase- α siRNA (m): sc-145294



The Power to Question

BACKGROUND

Glucose-6-phosphatase (G6Pase), is a multicomponent enzyme system that hydrolyzes glucose-6-phosphate (G6P) in the final step of gluconeogenesis and gluconeolysis. G6Pase localizes to the endoplasmic reticulum, and while liver, kidney, and intestine are the only tissues that express the first identified isoform, G6Pase- α , a second form, designated G6Pase- β , contributes to blood glucose homeostasis in a wider range of tissues. Glucocorticoids stimulate the expression of the G6Pase gene while Insulin rapidly inhibits expression via the thymine-rich Insulin response element located within the promoter of the G6Pase gene. Due to its necessary involvement in normal glucose metabolism, G6Pase plays an integral role in Diabetes and glycogen storage diseases (GSDs). The presence of different isoforms may explain the ability of some individuals with GSDs to still produce glucose, despite their lack of functional G6Pase- α .

REFERENCES

- 1. Goh, B.H., et al. 2003. Evidence for the expression of both the hydrolase and translocase components of hepatic glucose-6-phosphatase in murine pancreatic islets. Biochem. Biophys. Res. Commun. 307: 935-941.
- 2. Guionie, O., et al. 2003. Identification and characterization of a new human glucose-6-phosphatase isoform. FEBS Lett. 551: 159-164.
- 3. Shieh, J.J., et al. 2003. A glucose-6-phosphate hydrolase, widely expressed outside the liver, can explain age-dependent resolution of hypoglycemia in glycogen storage disease type 1α . J. Biol. Chem. 278: 47098-47103.
- Barthel, A., et al. 2003. Novel aspects in the mechanisms of steroid diabetes and the regulation of hepatic glucose production by Insulin and steroids. Med. Klin. 98: 283-286.
- Thiel, G., et al. 2005. cAMP response element binding protein (CREB) activates transcription via two distinct genetic elements of the human glucose-6-phosphatase gene. BMC Mol. Biol. 6: 2.

CHROMOSOMAL LOCATION

Genetic locus: G6pc (mouse) mapping to 11 D.

PRODUCT

G6Pase- α 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. Suit-able for 50-100 transfections. Also see G6Pase- α shRNA Plasmid (m): sc-145294-SH and G6Pase- α shRNA (m) Lentiviral Particles: sc-145294-V as alternate gene silencing products.

For independent verification of G6Pase- α (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-145294A, sc-145294B and sc-145294C.

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

G6Pase- α siRNA (m) is recommended for the inhibition of G6Pase- α 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 μM in 66 μl . 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-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

G6Pase- α (H-4): sc-398155 is recommended as a control antibody for monitoring of G6Pase- α 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-lgG κ BP-HRP: sc-516102 or m-lgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz MarkerTM Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-lgG κ BP-FITC: sc-516140 or m-lgG 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 G6Pase- α gene expression knockdown using RT-PCR Primer: G6Pase- α (m)-PR: sc-145294-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.

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