

GLUD1 siRNA (h): sc-90633

BACKGROUND

GLUD1 (glutamate dehydrogenase 1), also known as GDH, GDH1 or GLUD, is a 558 amino acid mitochondrial matrix enzyme belonging to the Glu/Leu/Phe/Val dehydrogenases family. Existing as a homohexamer, GLUD1 catalyzes the oxidative deamination of glutamate to α -ketoglutarate and ammonia. GLUD1 is critical for regulating amino acid-induced Insulin secretion and is allosterically activated by ADP and inhibited by GTP and ATP. Mutations in the gene encoding GLUD1 causes hyperInsulinism-hyperammonemia syndrome (HHS), which is an inherited condition characterized by high Insulin and ammonia levels in the blood. GLUD1 may also be involved in learning and memory reactions by increasing the turnover of the excitatory neurotransmitter glutamate.

REFERENCES

1. Stanley, C.A., et al. 2000. Molecular basis and characterization of the hyperInsulinism/hyperammonemia syndrome: predominance of mutations in exons 11 and 12 of the glutamate dehydrogenase gene. *HL/HA Contributing Investigators. Diabetes* 49: 667-673.
2. Tanizawa, Y., et al. 2002. Unregulated elevation of glutamate dehydrogenase activity induces glutamine-stimulated Insulin secretion: identification and characterization of a GLUD1 gene mutation and Insulin secretion studies with MIN6 cells overexpressing the mutant glutamate dehydrogenase. *Diabetes* 51: 712-717.
3. de Lonlay, P., et al. 2005. Dominantly inherited hyperInsulinaemic hypoglycaemia. *J. Inherit. Metab. Dis.* 28: 267-276.
4. Mastorodemos, V., et al. 2009. Human GLUD1 and GLUD2 glutamate dehydrogenase localize to mitochondria and endoplasmic reticulum. *Biochem. Cell Biol.* 87: 505-516.
5. Kanavouras, K., et al. 2009. Mutations in human GLUD2 glutamate dehydrogenase affecting basal activity and regulation. *J. Neurochem.* 109: 167-173.
6. Kapoor, R.R., et al. 2009. Advances in the diagnosis and management of hyperinsulinemic hypoglycemia. *Nat. Clin. Pract. Endocrinol. Metab.* 5: 101-112.

CHROMOSOMAL LOCATION

Genetic locus: GLUD1 (human) mapping to 10q23.2.

PRODUCT

GLUD1 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 GLUD1 shRNA Plasmid (h): sc-90633-SH and GLUD1 shRNA (h) Lentiviral Particles: sc-90633-V as alternate gene silencing products.

For independent verification of GLUD1 (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-90633A, sc-90633B and sc-90633C.

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

GLUD1 siRNA (h) is recommended for the inhibition of GLUD1 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

GLUD1/2 (C-10): sc-515542 is recommended as a control antibody for monitoring of GLUD1 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 GLUD1 gene expression knockdown using RT-PCR Primer: GLUD1 (h)-PR: sc-90633-PR (20 μ l, 438 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.