SANTA CRUZ BIOTECHNOLOGY, INC.

GLUD2 siRNA (h): sc-90970

BACKGROUND

GLUD1 (glutamate dehydrogenase 1), also known as GDH, GDH1 or GLUD, and GLUD2 (glutamate dehydrogenase 2), also known as GDH2 or GLUDP1, are both mitochondrial matrix enzymes belonging to the Glu/Leu/Phe/Val dehydrogenases family. Exisiting as homohexamers, GLUD1 catalyzes the oxidative deamination of glutamate to α-ketoglutarate and ammonia while GLUD2 is involved in the recycling of glutamate during neurotransmission. 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. GLUD2 is expressed in testis and retina, with lower levels found in brain.

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. HI/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. Mastorodemos, V., et al. 2009. Human GLUD1 and GLUD2 glutamate dehydrogenase localize to mitochondria and endoplasmic reticulum. Biochem. Cell Biol. 87: 505-516.
4. Pajic, T., et al. 2009. Glutamate dehydrogenase activity in lymphocytes of B-cell chronic lymphocytic leukaemia patients. Clin. Biochem. 42: 1677-1684.
5. Kapoor, R., et al. 2009. Hyperinsulinism-hyperammonaemia (HI/HA) syndrome: novel mutations in the GLUD1 gene and genotype-phenotype correlations. Eur. J. Endocrinol. 161: 731-735.

CHROMOSOMAL LOCATION

Genetic locus: GLUD2 (human) mapping to Xq24.

PRODUCT

GLUD2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GLUD2 shRNA Plasmid (h): sc-90970-SH and GLUD2 shRNA (h) Lentiviral Particles: sc-90970-V as alternate gene silencing products.

For independent verification of GLUD2 (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-90970A, sc-90970B and sc-90970C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

GLUD2 siRNA (h) is recommended for the inhibition of GLUD2 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 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-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

GLUD2 (3C2): sc-293459 is recommended as a control antibody for monitoring of GLUD2 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 GLUD2 gene expression knockdown using RT-PCR Primer: GLUD2 (h)-PR: sc-90970-PR (20 µl). 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.