SANTA CRUZ BIOTECHNOLOGY, INC.

AGL siRNA (h): sc-88368

BACKGROUND

AGL (amylo-1,6-glucosidase, 4-α-glucotransferase), also known as GDE (glycogen debranching enzyme), is a 1,532 amino acid protein that exists as three alternatively spliced isoforms which are expressed in kidney, liver, heart and muscle in an isoform-specific manner. Exhibiting multifunctional enzyme capabilities, AGL contains two catalytic active sites, one of which acts as an 4-α-glucotransferase and the other of which acts as an amylo-1,6-glucosidase during glycogen degradation. Defects in the gene encoding AGL are the cause of glycogen storage disease type 3 (GSD3), also known as Forbes disease. GSD3 is a metabolic disorder that is characterized by the presence of abnormal glycogen due to a lack of AGL activity. Symptoms of GSD3 generally include hypoglycemia, variable myopathy, hepatomegaly and short stature.

REFERENCES

1. Ding, J.H., et al. 1990. Immunoblot analyses of glycogen debranching enzyme in different subtypes of glycogen storage disease type III. J. Pediatr. 116: 95-100.
2. Yang, B.Z., et al. 1992. Molecular cloning and nucleotide sequence of cDNA encoding human muscle glycogen debranching enzyme. J. Biol. Chem. 267: 9294-9299.
3. Shen, J., et al. 1996. Mutations in exon 3 of the glycogen debranching enzyme gene are associated with glycogen storage disease type III that is differentially expressed in liver and muscle. J. Clin. Invest. 98: 352-357.
4. Orho, M., et al. 1998. Mutations in the liver glycogen synthase gene in children with hypoglycemia due to glycogen storage disease type 0. J. Clin. Invest. 102: 507-515.
5. Horinishi, A., et al. 2002. Mutational and haplotype analysis of AGL in patients with glycogen storage disease type III. J. Hum. Genet. 47: 55-59.
6. Sakoda, H., et al. 2005. Glycogen debranching enzyme association with β-subunit regulates AMP-activated protein kinase activity. Am. J. Physiol. Endocrinol. Metab. 289: E474-E481.

CHROMOSOMAL LOCATION

Genetic locus: AGL (human) mapping to 1p21.2.

PRODUCT

AGL 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 AGL shRNA Plasmid (h): sc-88368-SH and AGL shRNA (h) Lentiviral Particles: sc-88368-V as alternate gene silencing products.

For independent verification of AGL (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-88368A, sc-88368B and sc-88368C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

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

AGL (B-11): sc-518176 is recommended as a control antibody for monitoring of AGL 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 AGL gene expression knockdown using RT-PCR Primer: AGL (h)-PR: sc-88368-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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