

AGPS siRNA (m): sc-140906

BACKGROUND

AGPS (alkyldihydroxyacetonephosphate synthase), also known as alkyglycerone-phosphate synthase and AAG5 (aging-associated gene 5 protein), is a 658 amino acid enzyme that is required for glycerolipid metabolism and ether lipid biosynthesis. Localized to the inner aspect of the peroxisomal membrane, AGPS is likely part of a heterotrimeric complex that is also composed of GNPAT and a modified form of GNPAT. Containing one FAD-binding PCMH-type domain, AGPS utilizes FAD as a cofactor in the synthesis of alkyl-glycerone 3-phosphate and a long-chain acid anion from 1-acteyl-glycerone 3-phosphate and a long-chain alcohol. Defects in the gene encoding AGPS results in rhizomelic chondrodysplasia punctata type 3, a disease characterized by vertebral disorders, severe mental retardation, cutaneous lesions, cataracts and rhizomelic shortening of the humerus and femur.

REFERENCES

1. de Vet, E.C., et al. 1997. Nucleotide sequence of human alkyl-dihydroxy-acetonephosphate synthase cDNA reveals the presence of a peroxisomal targeting signal 2. *Biochim. Biophys. Acta* 1346: 25-29.
2. de Vet, E.C., et al. 1998. Alkyl-dihydroxyacetonephosphate synthase. Fate in peroxisome biogenesis disorders and identification of the point mutation underlying a single enzyme deficiency. *J. Biol. Chem.* 273: 10296-10301.
3. Biermann, J., et al. 1999. Alkyl-dihydroxyacetone phosphate synthase and dihydroxyacetone phosphate acyltransferase form a protein complex in peroxisomes. *Eur. J. Biochem.* 261: 492-499.
4. de Vet, E.C. and van den Bosch, H. 2000. Alkyl-dihydroxyacetonephosphate synthase. *Cell Biochem. Biophys.* 32: 117-121.
5. Thai, T.P., et al. 2001. Impaired membrane traffic in defective ether lipid biosynthesis. *Hum. Mol. Genet.* 10: 127-136.

CHROMOSOMAL LOCATION

Genetic locus: Agps (mouse) mapping to 2 C3.

PRODUCT

AGPS 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. Suitable for 50-100 transfections. Also see AGPS shRNA Plasmid (m): sc-140906-SH and AGPS shRNA (m) Lentiviral Particles: sc-140906-V as alternate gene silencing products.

For independent verification of AGPS (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-140906A, sc-140906B and sc-140906C.

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.

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

AGPS siRNA (m) is recommended for the inhibition of AGPS 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 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

AGPS (A-2): sc-374201 is recommended as a control antibody for monitoring of AGPS 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 AGPS gene expression knockdown using RT-PCR Primer: AGPS (m)-PR: sc-140906-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.