SANTA CRUZ BIOTECHNOLOGY, INC.

AGXT siRNA (h): sc-94932

BACKGROUND

AGXT (alanine-glyoxylate aminotransferase), also known as AGT, AGT1, AGXT1, PH1, SPAT (serine—pyruvate aminotransferase) or TLH6, is a 392 amino acid protein belonging to the class-V pyridoxal-phosphate-dependent aminotransferase family. Encoded by a gene that maps to human chromosome 2q37.3, AGXT consists of a homodimer subunit structure and uses pyridoxal phosphate as a cofactor. Localized mainly in peroxisome, AGXT is expressed in liver. AGXT participates in alanine-glyoxylate transaminase activity, amino acid and protein binding, protein homodimerization, pyridoxal phosphate binding, serine-pyruvate transaminase activity and transferase roles. AGXT is linked to hyperoxaluria primary type 1 (HP1), a rare autosomal recessive disease characterized by heightened excretion of oxalate and glycolate, and build up of insoluble calcium oxalate in urinary tract and kidney.

REFERENCES

1. Noguchi, T., et al. 1978. Characteristics of hepatic alanine-glyoxylate aminotransferase in different mammalian species. Biochem. J. 169: 113-122.
2. Danpure, C.J., et al. 1986. Peroxisomal alanine:glyoxylate aminotransferase deficiency in primary hyperoxaluria type I. FEBS Lett. 201: 20-24.
3. Cooper, P.J., et al. 1988. Immunocytochemical localization of human hepatic alanine: glyoxylate aminotransferase in control subjects and patients with primary hyperoxaluria type 1. J. Histochem. Cytochem. 36: 1285-1294.
4. Purdue, P.E., et al. 1991. Characterization and chromosomal mapping of a genomic clone encoding human alanine:glyoxylate aminotransferase. Genomics 10: 34-42.
5. Danpure, C.J., et al. 1993. Enzymological and mutational analysis of a complex primary hyperoxaluria type 1 phenotype involving alanine:glyoxylate aminotransferase peroxisome-to-mitochondrion mistargeting and intraperoxisomal aggregation. Am. J. Hum. Genet. 53: 417-432.
6. Danpure, C.J. 1993. Primary hyperoxaluria type 1 and peroxisome-to-mitochondrion mistargeting of alanine:glyoxylate aminotransferase. Biochimie 75: 309-315.

CHROMOSOMAL LOCATION

Genetic locus: AGXT (human) mapping to 2q37.3.

PRODUCT

AGXT 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 AGXT shRNA Plasmid (h): sc-94932-SH and AGXT shRNA (h) Lentiviral Particles: sc-94932-V as alternate gene silencing products.

For independent verification of AGXT (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-94932A, sc-94932B and sc-94932C.

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

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

AGXT (173J2B): sc-517624 is recommended as a control antibody for monitoring of AGXT 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 AGXT gene expression knockdown using RT-PCR Primer: AGXT (h)-PR: sc-94932-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.