SANTA CRUZ BIOTECHNOLOGY, INC.

AGAT siRNA (h): sc-90190

BACKGROUND

AGAT, also known as glycine amidinotransferase (L-arginine:glycine amidinotransferase), GATM or transamidinase, is a 423 amino acid protein belonging to the amidinotransferase family. Encoded by a gene that maps to human chromosome 15q21.1, AGAT exists as three alternatively spliced isoforms and consists of a homodimer, with equilibrium between monomeric and dimeric forms favoring a monomer subunit structure. AGAT localizes to mitochondrial inner membranes, peripheral membranes and cytoplasm. Biallelically expressed in placenta and fetal tissues, AGAT is also expressed in brain, heart, liver, lung, salivary gland and skeletal muscle tissue, with high expression in kidney. AGAT is elevated in the myocardium during heart failure and is decreased in inter-uterine growth restriction (IUGR)-associated placenta. AGAT catalyzes biosynthesis of guanidinoacetate, the immediate precursor of creatine, which plays a vital role in energy metabolism in muscle tissues. AGAT defects are associated with arginine:glycine amidinotransferase deficiency, an autosomal recessive disorder characterized by developmental delay or regression, mental retardation, severe disturbance of expressive and cognitive speech and severe depletion of creatine/phosphocreatine in brain. AGAT may be linked to embryonic and central nervous system development and may function in heart failure response by elevating local creatine synthesis.

REFERENCES

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4. Humm, A., et al. 1997. Structure and reaction mechanism of L-arginine: glycine amidinotransferase. Biol. Chem. 378: 193-197.
5. Humm, A., et al. 1997. Crystal structure and mechanism of human L-arginine:glycine amidinotransferase: a mitochondrial enzyme involved in creatine biosynthesis. EMBO J. 16: 3373-3385.
6. Fritsche, E., et al. 1997. Substrate binding and catalysis by L-arginine: glycine amidinotransferase—a mutagenesis and crystallographic study. Eur. J. Biochem. 247: 483-490.
7. Zhao, H., et al. 2001. Expression of Xenopus L-arginine:glycine amidinotransferase (XAT) during early embryonic development. Dev. Genes Evol. 211: 358-360.
8. Cullen, M.E., et al. 2006. Myocardial expression of the arginine:glycine amidinotransferase gene is elevated in heart failure and normalized after recovery: potential implications for local creatine synthesis. Circulation 114: I16-I20.

PROTOCOLS

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

CHROMOSOMAL LOCATION

Genetic locus: GATM (human) mapping to 15q21.1.

PRODUCT

AGAT 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 AGAT shRNA Plasmid (h): sc-90190-SH and AGAT shRNA (h) Lentiviral Particles: sc-90190-V as alternate gene silencing products.

For independent verification of AGAT (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-90190A, sc-90190B and sc-90190C.

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

AGAT siRNA (h) is recommended for the inhibition of AGAT 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor AGAT gene expression knockdown using RT-PCR Primer: AGAT (h)-PR: sc-90190-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.