SANTA CRUZ BIOTECHNOLOGY, INC.

GART siRNA (m): sc-145331

BACKGROUND

Purines are critical for energy metabolism, cell signaling and cell reproduction and also function as precursors for coenzymes, energy transfer molecules, regulatory factors and proteins involved in RNA and DNA synthesis. GART (GAR transformylase), also referred to as AIRS, GARS, PAIS, PGFT, PRGS or GARTF, is 1,010 amino acids in length and is a key folate-dependent trifunctional enzyme with phosphoribosylglycinamide formyltransferase, phosphoribosylglycinamide synthetase and AICAR (phosphoribosylaminoimidazole synthetase) activity required for *de novo* purine biosynthesis. Cancer cells require considerable amounts of purines to sustain their accelerated growth and GART is, therefore, a target for cancer chemotherapy. GART is highly conserved in vertebrates. Two isoforms of GART are expressed due to alternative splicing events.

REFERENCES

1. Smith, G.K., et al. 1982. Direct transfer of one-carbon units in the transformylations of *de novo* purine biosynthesis. Biochemistry 21: 2870-2874.
2. Deacon, R., et al. 1985. Role of folate dependent transformylases in synthesis of purine in bone marrow of man and in bone marrow and liver of rats. J. Clin. Pathol. 38: 1349-1352.
3. Daubner, S.C., et al. 1986. Structural and mechanistic studies on the HeLa and chicken liver proteins that catalyze glycinamide ribonucleotide synthesis and formylation and aminoimidazole ribonucleotide synthesis. Biochemistry 25: 2951-2957.
4. Brodsky, G., et al. 1997. The human GARS-AIRS-GART gene encodes two proteins which are differentially expressed during human brain development and temporally overexpressed in cerebellum of individuals with Down syndrome. Hum. Mol. Genet. 6: 2043-2050.
5. Nixon, A.E., et al. 1997. Assembly of an active enzyme by the linkage of two protein modules. Proc. Natl. Acad. Sci. USA 94: 1069-1073.
6. Poch, M.T., et al. 1998. The human trifunctional enzyme of *de novo* purine biosynthesis: heterologous expression, purification, and preliminary characterization. Protein Expr. Purif. 12: 17-24.
7. Liu, C., et al. 2000. The unexpected catalytic properties of a heterodimer of GAR transformylase. Bioorg. Chem. 28: 316-323.

CHROMOSOMAL LOCATION

Genetic locus: Gart (mouse) mapping to 16 C3.3.

PRODUCT

GART 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GART shRNA Plasmid (m): sc-145331-SH and GART shRNA (m) Lentiviral Particles: sc-145331-V as alternate gene silencing products.

For independent verification of GART (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-145331A, sc-145331B and sc-145331C.

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

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

GART (D-4): sc-166379 is recommended as a control antibody for monitoring of GART 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 GART gene expression knockdown using RT-PCR Primer: GART (m)-PR: sc-145331-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.