SANTA CRUZ BIOTECHNOLOGY, INC.

GAMT siRNA (m): sc-145323

BACKGROUND

In the creatine biosynthesis pathway, glycine is converted to guanidinoacetate by amidinotransferase, and guanidinoacetate is then converted to creatine by Guanidinoacetate N-methyltransferase (GAMT). GAMT, a methyltransferase, uses S-adenosylmethionine as the methyl donor for this reaction. Methyltransferases are a type of transferase enzyme which transfers a methyl group to nucleic bases in DNA or amino acids in protein. Encoding a 236 amino acid protein, the human GAMT gene maps to chromosome 19p13.3. Defects in the GAMT gene leads to GAMT deficiency, which is associated with guanidinoacetate accumulation and decreased levels of creatine excretion in brain. Such biochemical changes are thought to lead to various neurological syndromes and muscular hypotonia.

REFERENCES

1. Stöckler, S., et al. 1994. Creatine deficiency in the brain: a new, treatable inborn error of metabolism. Pediatr. Res. 36: 409-413.
2. Isbrandt, D. and von Figura, K. 1995. Cloning and sequence analysis of human guanidinoacetate N-methyltransferase cDNA. Biochim. Biophys. Acta 1264: 265-267.
3. Jenne, D.E., et al. 1997. The human guanidinoacetate methyltransferase (GAMT) gene maps to a syntenic region on 19p13.3, homologous to band C of mouse chromosome 10, but GAMT is not mutated in jittery mice. Biochem. Biophys. Res. Commun. 238: 723-727.
4. Schulze, A., et al. 1997. Creatine deficiency syndrome caused by guanidinoacetate methyltransferase deficiency: diagnostic tools for a new inborn error of metabolism. J. Pediatr. 131: 626-631.
5. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 601240. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
6. Wang, Y., et al. 2006. Tyrosine phosphorylated Par3 regulates epithelial tight junction assembly promoted by EGFR signaling. EMBO J. 25: 5058-5070.

CHROMOSOMAL LOCATION

Genetic locus: Gamt (mouse) mapping to 10 C1.

PRODUCT

GAMT siRNA (m) is a pool of 2 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 GAMT shRNA Plasmid (m): sc-145323-SH and GAMT shRNA (m) Lentiviral Particles: sc-145323-V as alternate gene silencing products.

For independent verification of GAMT (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-145323A and sc-145323B.

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

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

GAMT (G-9): sc-398960 is recommended as a control antibody for monitoring of GAMT 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 GAMT gene expression knockdown using RT-PCR Primer: GAMT (m)-PR: sc-145323-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.