SANTA CRUZ BIOTECHNOLOGY, INC.

NAGS siRNA (h): sc-93810

BACKGROUND

The function of the urea cycle is to remove excess nitrogen from the body. Six distinct enzymes comprise the urea cycle, and urea cycle disorders (UCD) are a direct results of deficiency in any one of those enzymes. N-acetylglutamate synthase (NAGS) catalyzes the conversion of N-acetylglutamate (NAG) from glutamate and acetylcoenzyme A. NAG is an obligatory activator of carbamylphosphate I (CPSI), the first and rate limiting enzyme of ureagenesis. Therefore, deficiency of NAGS results in severe hyperammonemia. 21 mutations have been described in humans, 10 of which are associated with acute neonatal hyperammonemia, and the remainder found in patients with late onset disease. Treatment with N-carbamylglutamate (NCG) can ameliorate hyperammonemia for inherited and secondary NAGS deficiency. Expression of NAGS occurs in the liver, small intestine and kidney.

REFERENCES

1. Caldovic, L., et al. 2002. Cloning and expression of the human N-acetylglutamate synthase gene. Biochem. Biophys. Res. Commun. 299: 581-586.
2. Häberle, J., et al. 2003. Mutation analysis in patients with N-acetylglutamate synthase deficiency. Hum. Mutat. 21: 593-597.
3. Morizono, H., et al. 2004. Mammalian N-acetylglutamate synthase. Mol. Genet. Metab. 81: S4-S11.
4. Caldovic, L., et al. 2006. Biochemical properties of recombinant human and mouse N-acetylglutamate synthase. Mol. Genet. Metab. 87: 226-232.
5. Caldovic, L., et al. 2007. Mutations and polymorphisms in the human N-acetylglutamate synthase (NAGS) gene. Hum. Mutat. 28: 754-759.
6. Nordenström, A., et al. 2007. A trial with N-carbamylglutamate may not detect all patients with NAGS deficiency and neonatal onset. J. Inherit. Metab. Dis. 30: 400.
7. Deignan, J.L., et al. 2008. Contrasting features of urea cycle disorders in human patients and knockout mouse models. Mol. Genet. Metab. 93: 7-14.
8. Tuchman, M., et al. 2008. N-carbamylglutamate markedly enhances ureagenesis in N-acetylglutamate deficiency and propionic acidemia as measured by isotopic incorporation and blood biomarkers. Pediatr. Res. 64: 213-217.

CHROMOSOMAL LOCATION

Genetic locus: NAGS (human) mapping to 17q21.31.

PRODUCT

NAGS siRNA (h) 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 NAGS shRNA Plasmid (h): sc-93810-SH and NAGS shRNA (h) Lentiviral Particles: sc-93810-V as alternate gene silencing products.

For independent verification of NAGS (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-93810A and sc-93810B.

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

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

NAGS (E-8): sc-515127 is recommended as a control antibody for monitoring of NAGS 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 NAGS gene expression knockdown using RT-PCR Primer: NAGS (h)-PR: sc-93810-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.