SANTA CRUZ BIOTECHNOLOGY, INC.

GlcNAc kinase siRNA (h): sc-75136

BACKGROUND

GlcNAc kinase, also known as GNK or NAGK (N-acetylglucosamine kinase), is a 344 amino acid homodimeric protein that is ubiquitously expressed. Belonging to the sugar kinase/Hsp70/Actin superfamily and the eukaryotic-type N-acetylglucosamine kinase family, GlcNAc kinase converts endogenous N-acetylglucosamine (GlcNAc), a major component of complex carbohydrates, from lysosomal degradation or nutritional sources into GlcNAc 6-phosphate. GlcNAc kinase is considered a salvage enzyme of amino sugar metabolism in mammals and predominately produces the β anomer of phosphorylated sugars. It is suggested that GlcNAc kinase has ManNAc kinase activity.

REFERENCES

1. Mattia, E., et al. 1982. Induction of germ tube formation by N-acetyl-D-glucosamine in *Candida albicans:* uptake of inducer and germinative response. J. Bacteriol. 152: 555-562.
2. Meglasson, M.D., et al. 1983. Chromatographic resolution and kinetic characterization of glucokinase from islets of Langerhans. Proc. Natl. Acad. Sci. USA 80: 85-89.
3. Hinderlich, S., et al. 1998. Purification and characterization of N-acetylglucosamine kinase from rat liver—comparison with UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase. Eur. J. Biochem. 252: 133-139.
4. Hinderlich, S., et al. 2000. Molecular cloning and characterization of murine and human N-acetylglucosamine kinase. Eur. J. Biochem. 267: 3301-3308.
5. Yamada-Okabe, T., et al. 2001. Identification and characterization of the genes for N-acetylglucosamine kinase and N-acetylglucosamine-phosphate deacetylase in the pathogenic fungus Candida albicans. Eur. J. Biochem. 268: 2498-2505.
6. Perez-Arellano, I., et al. 2006. Mapping active site residues in glutamate-5-kinase. The substrate glutamate and the feed-back inhibitor proline bind at overlapping sites. FEBS Lett. 580: 6247-6253.

CHROMOSOMAL LOCATION

Genetic locus: NAGK (human) mapping to 2p13.3.

PRODUCT

GlcNAc kinase 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 GlcNAc kinase shRNA Plasmid (h): sc-75136-SH and GlcNAc kinase shRNA (h) Lentiviral Particles: sc-75136-V as alternate gene silencing products.

For independent verification of GlcNAc kinase (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-75136A, sc-75136B and sc-75136C.

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

GlcNAc kinase siRNA (h) is recommended for the inhibition of GlcNAc kinase 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

GlcNAc kinase (G-5): sc-390499 is recommended as a control antibody for monitoring of GlcNAc kinase 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 GlcNAc kinase gene expression knockdown using RT-PCR Primer: GlcNAc kinase (h)-PR: sc-75136-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.