SANTA CRUZ BIOTECHNOLOGY, INC.

GLCNE siRNA (m): sc-60694

BACKGROUND

The bifunctional enzyme UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE/Mnk), or GLCNE, regulates and initiates biosynthesis of N-acetylneuraminic acid (NeuAc), a precursor of sialic acids. GLCNE is required for normal sialylation in hematopoietic cells. Sialylation is implicated in cell adhesion, signal transduction, tumorigenicity and metastatic behavior of malignant cells. It is upregulated after PKC-dependent phosphorylation and is most abundantly expressed in liver and placenta. It is also expressed, to a lesser extent, in heart, brain, lung, kidney, skeletal muscle and pancreas. Defects in GLCNE are the cause of sialuria, inclusion body myopathy 2 (IBM2) and Nonaka myopathy (NM) or distal myopathy with rimmed vacuoles (DMRV). Sialuria is an autosomal dominant disorder caused by a lack of feedback inhibition of GLCNE by CMP-NeuAc, resulting in overproduction of NeuAc. It is characterized by an accumulation of free sialic acid in the cytoplasm and large quantities of neuraminic acid in the urine. Both IBM2 and NM/DMRV are autosomal recessive neuromuscular disorders characterized by adult onset, distal and proximal muscle weakness (especially in the legs) and a typical muscle pathology including filamentous inclusions and rimmed vacuoles.

REFERENCES

1. Amouri, R., et al. 2005. Allelic heterogeneity of GNE gene mutation in two Tunisian families with autosomal recessive inclusion body myopathy. Neuromuscul. Disord. 15: 361-363.
2. Bork, K., et al. 2005. The intracellular concentration of sialic acid regulates the polysialylation of the neural cell adhesion molecule. FEBS Lett. 579: 5079-5083.
3. Krause, S., et al. 2005. Localization of UDP-GlcNAc 2-epimerase/ManAc kinase (GNE) in the Golgi complex and the nucleus of mammalian cells. Exp. Cell Res. 304: 365-379.
4. Nonaka, I., et al. 2005. Distal myopathy with rimmed vacuoles and hereditary inclusion body myopathy. Curr. Neurol. Neurosci. Rep. 5: 61-65.
5. Ro, L.S., et al. 2005. Phenotypic variability in a Chinese family with rimmed vacuolar distal myopathy. J. Neurol. Neurosurg. Psychiatr. 76: 752-755.
6. Salama, I., et al. 2005. No overall hyposialylation in hereditary inclusion body myopathy myoblasts carrying the homozygous M712T GNE mutation. Biochem. Biophys. Res. Commun. 328: 221-226.

CHROMOSOMAL LOCATION

Genetic locus: Gne (mouse) mapping to 4 B1.

PRODUCT

GLCNE 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 GLCNE shRNA Plasmid (m): sc-60694-SH and GLCNE shRNA (m) Lentiviral Particles: sc-60694-V as alternate gene silencing products.

For independent verification of GLCNE (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-60694A, sc-60694B and sc-60694C.

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

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

GLCNE (H-10): sc-376057 is recommended as a control antibody for monitoring of GLCNE 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 GLCNE gene expression knockdown using RT-PCR Primer: GLCNE (m)-PR: sc-60694-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.