SANTA CRUZ BIOTECHNOLOGY, INC.

Chondroitinase siRNA (m): sc-72903

BACKGROUND

Chondroitinase, also known as GALNS (galactosamine (N-acetyl)-6-sulfate sulfatase), GAS or MPS4A, is a 522 amino acid protein that localizes to the lysosome and functions as an exohydrolase that is essential for the degradation of glycosaminoglycans, keratan sulfate and chondroitin 6-sulfate. Using calcium as a cofactor, Chondroitinase, which exists as a disulfide linked oligomer, catalyzes the hydrolysis of the 6-sulfate group on target substrates. Defects in the gene encoding Chondroitinase are the cause of mucopolysaccharidosis type 4A (MPS4A), an autosomal recessive lysosomal storage disease that is characterized by the intracellular accumulation of keratan sulfate and chondroitin-6-sulfate and is associated with dental anomalies, short stature and, in some cases, death in the second or third decade of life.

REFERENCES

1. Bielicki, J., et al. 1991. Human liver N-acetylgalactosamine 6-sulphatase. Purification and characterization. Biochem. J. 279: 515-520.
2. Masue, M., et al. 1991. N-acetylgalactosamine-6-sulfate sulfatase in human placenta: purification and characteristics. J. Biochem. 110: 965-970.
3. Fukuda, S., et al. 1992. Mucopolysaccharidosis type IVA. N-acetylgalactosamine-6-sulfate sulfatase exonic point mutations in classical Morquio and mild cases. J. Clin. Invest. 90: 1049-1053.
4. Baker, E., et al. 1993. The morquio A syndrome (mucopolysaccharidosis IVA) gene maps to 16q24.3. Am. J. Hum. Genet. 52: 96-98.
5. Bielicki, J., et al. 1995. Expression, purification and characterization of recombinant human N-acetylgalactosamine-6-sulphatase. Biochem. J. 311: 333-339.
6. Sukegawa, K., et al. 2000. Biochemical and structural analysis of missense mutations in N-acetylgalactosamine-6-sulfate sulfatase causing mucopolysaccharidosis IVA phenotypes. Hum. Mol. Genet. 9: 1283-1290.
7. Tomatsu, S., et al. 2005. Mutation and polymorphism spectrum of the GALNS gene in mucopolysaccharidosis IVA (Morquio A). Hum. Mutat. 26: 500-512.
8. Carraresi, L., et al. 2008. GALNS gene expression profiling in Morquio A patients' fibroblasts. Clin. Chim. Acta 397: 72-76.

CHROMOSOMAL LOCATION

Genetic locus: Galns (mouse) mapping to 8 E1.

PRODUCT

Chondroitinase 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 Chondroitinase shRNA Plasmid (m): sc-72903-SH and Chondroitinase shRNA (m) Lentiviral Particles: sc-72903-V as alternate gene silencing products.

For independent verification of Chondroitinase (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-72903A, sc-72903B and sc-72903C.

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

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

Chondroitinase (G-4): sc-390713 is recommended as a control antibody for monitoring of Chondroitinase 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 Chondroitinase gene expression knockdown using RT-PCR Primer: Chondroitinase (m)-PR: sc-72903-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.