SANTA CRUZ BIOTECHNOLOGY, INC.

GalNAc-T6 siRNA (m): sc-75101

BACKGROUND

The UDP-N-acetyl-α-D-galactosamine:polypeptide N-acetylgalactosaminyl-transferase (GalNAc-T) family of enzymes are substrate-specific proteins that catalyze the transfer of GalNAc (N-acetylgalactosaminyl) to serine and threonine residues onto various proteins, thereby initiating mucin-type O-linked glycosylation in the Golgi apparatus. GalNAc-T6, also known as GALNT6 (polypeptide N-acetylgalactosaminyltransferase 6), is a 622 amino acid single-pass type II membrane protein that localizes to the Golgi and, like other GalNAc proteins, contains a stem region and a C-terminal ricin/lectin-like domain. Highly expressed in trachea, fibroblasts and placenta with lower expression in brain and pancreas, GalNAc-T6 catalyzes the first reaction in O-linked oligosaccharide biosynthesis, namely the transfer of an N-acetyl-D-galactosamine residue to a protein acceptor. GalNAc-T6 uses calcium and manganese as cofactors and is thought to participate in the synthesis of oncofetal fibronectin. Additionally, GalNAc-T6 may serve as a potential marker for breast cancer.

REFERENCES

1. Bennett, E.P., et al. 1999. Cloning and characterization of a close homologue of human UDP-N-acetyl-α-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase-T3, designated GalNAc-T6. Evidence for genetic but not functional redundancy. J. Biol. Chem. 274: 25362-25370.
2. Porowska, H., et al. 1999. Activity of partially purified UDP-N-acetyl-α-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase with different peptide acceptors. Acta Biochim. Pol. 46: 365-370.
3. Schwientek, T., et al. 2002. Functional conservation of subfamilies of putative UDP-N-acetylgalactosamine: polypeptide N-acetylgalactosaminyl-transferases in *Drosophila*, *Caenorhabditis elegans*, and mammals. One subfamily composed of l(2)35Aa is essential in *Drosophila*. J. Biol. Chem. 277: 22623-22638.
4. Argüeso, P., et al. 2003. The cell-layer- and cell-type-specific distribution of GalNAc-transferases in the ocular surface epithelia is altered during keratinization. Invest. Ophthalmol. Vis. Sci. 44: 86-92.

CHROMOSOMAL LOCATION

Genetic locus: Galnt6 (mouse) mapping to 15 F1.

PRODUCT

GalNAc-T6 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 GalNAc-T6 shRNA Plasmid (m): sc-75101-SH and GalNAc-T6 shRNA (m) Lentiviral Particles: sc-75101-V as alternate gene silencing products.

For independent verification of GalNAc-T6 (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-75101A, sc-75101B and sc-75101C.

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

GalNAc-T6 siRNA (m) is recommended for the inhibition of GalNAc-T6 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

GalNAc-T6 (Y5J): sc-100755 is recommended as a control antibody for monitoring of GalNAc-T6 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 GalNAc-T6 gene expression knockdown using RT-PCR Primer: GalNAc-T6 (m)-PR: sc-75101-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.