SANTA CRUZ BIOTECHNOLOGY, INC.

GalNAc-TL5 siRNA (m): sc-145316

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-TL5 (polypeptide GalNAc transferase 15), also known as GALNTL5, is a 443 amino acid single-pass type II membrane protein belonging to the glycosyltransferase 2 family and GalNAc-T subfamily. Localizing to Golgi apparatus, GalNAc-TL4 utilizes manganese and calcium as cofactors and is expressed in testis. GalNAc-TL5 may assist with the transfer of an N-acetyl-D-galactosamine residue to a serine or threonine residue on protein receptors and likely catalyzes the initial reaction in O-linked oligosaccharide biosynthesis. Unlike other members of the GalNAc-T subfamily, GalNAc-TL5 does not contain a C-terminal ricin B-type lectin domain. GalNAc-TL5 contains two conserved domains located in its glycosyltransferase region. The N-terminal domain, also known as domain A or GT1 motif, may be involved in manganese coordination and substrate binding while the C-terminal domain, also known as domain B or Gal/GalNAc-T motif, is likely involved in catalytic reactions and UDP-Gal binding. GalNAc-TL5 exists as two alternatively spliced isoforms.

REFERENCES

1. Gooi, H.C., et al. 1985. Differing reactions of monoclonal anti-A antibodies with oligosaccharides related to blood group A. J. Biol. Chem. 260: 13218-13224.
2. Hayes, B.K. and Varki, A. 1993. The biosynthesis of oligosaccharides in intact Golgi preparations from rat liver. Analysis of N-linked and O-linked glycans labeled by UDP-[6-3H]N-acetylgalactosamine. J. Biol. Chem. 268: 16170-16178.
3. 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.

CHROMOSOMAL LOCATION

Genetic locus: Galntl5 (mouse) mapping to 5 A3.

PRODUCT

GalNAc-TL5 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-TL5 shRNA Plasmid (m): sc-145316-SH and GalNAc-TL5 shRNA (m) Lentiviral Particles: sc-145316-V as alternate gene silencing products.

For independent verification of GalNAc-TL5 (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-145316A, sc-145316B and sc-145316C.

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-TL5 siRNA (m) is recommended for the inhibition of GalNAc-TL5 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-TL5 (F-5): sc-398569 is recommended as a control antibody for monitoring of GalNAc-TL5 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-TL5 gene expression knockdown using RT-PCR Primer: GalNAc-TL5 (m)-PR: sc-145316-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.