

GalNAc-T14 siRNA (h): sc-75092

BACKGROUND

The UDP-N-acetyl- α -D-galactosamine:polypeptide N-acetylgalactosaminyltransferase (GalNAc-T) family of enzymes are substrate-specific proteins that catalyze the transfer of GalNAc (N-acetylgalactosaminyl) to serine and threonine residues on various proteins, thereby initiating mucin-type O-linked glycosylation in the Golgi apparatus. GalNAc-T14 (UDP-N-acetyl- α -D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 14) is a 552 amino acid single-pass type II membrane protein that localizes to the Golgi apparatus and contains one Ricin B-type lectin domain. Existing as multiple alternatively spliced isoforms that are highly expressed in fetal and adult kidney, GalNAc-T14 uses calcium and manganese as cofactors to catalyze the initial reaction in O-linked oligosaccharide biosynthesis, namely the transfer of GalNAc to select residues to target proteins.

REFERENCES

1. Elhammer, A.P., et al. 1999. The acceptor specificity of UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases. *Glycoconj. J.* 16: 171-180.
2. Wang, H., et al. 2003. Cloning and characterization of a novel UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferase, pp-GalNAc-T14. *Biochem. Biophys. Res. Commun.* 300: 738-744.
3. Ten Hagen, K.G., et al. 2003. All in the family: the UDP-GalNAc:polypeptide N-acetylgalactosaminyltransferases. *Glycobiology* 13: 1R-16R.
4. Wu, C., et al. 2007. N-Acetylgalactosaminyltransferase 14, a novel Insulin-like growth factor binding protein-3 binding partner. *Biochem. Biophys. Res. Commun.* 357: 360-365.
5. Wagner, K.W., et al. 2007. Death-receptor O-glycosylation controls tumor-cell sensitivity to the proapoptotic ligand Apo2L/TRAIL. *Nat. Med.* 13: 1070-1077.

CHROMOSOMAL LOCATION

Genetic locus: GALNT14 (human) mapping to 2p23.1.

PRODUCT

GalNAc-T14 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GalNAc-T14 shRNA Plasmid (h): sc-75092-SH and GalNAc-T14 shRNA (h) Lentiviral Particles: sc-75092-V as alternate gene silencing products.

For independent verification of GalNAc-T14 (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-75092A, sc-75092B and sc-75092C.

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.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

GalNAc-T14 siRNA (h) is recommended for the inhibition of GalNAc-T14 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 μ M in 66 μ l. 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-T14 (A-8): sc-393051 is recommended as a control antibody for monitoring of GalNAc-T14 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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-T14 gene expression knockdown using RT-PCR Primer: GalNAc-T14 (h)-PR: sc-75092-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.