

β -1,4-GalNAc-T2 siRNA (h): sc-93560

BACKGROUND

β -1,4-N-acetyl-galactosaminyl transferase 2 (β -1,4-GalNAc-T2) is a 566 amino acid protein belonging to the glycosyltransferase 2 family. Localized to the membrane of the Golgi apparatus, β -1,4-GalNAc-T2 participates in the synthesis of the Sd(a) antigen, a carbohydrate determinant expressed on erythrocytes, colonic mucosa and other tissues. During Sd(a) production, β -1,4-GalNAc-T2 transfers a β -1,4-linked GalNAc to the galactose residue of an α -2,3-sialylated chain. β -1,4-GalNAc-T2 also catalyzes the last step in the biosynthesis of the CAD antigen. β -1,4-GalNAc-T2 is widely expressed, with the highest expression in colon and lesser expression in kidney, stomach, ileum and rectum. Mutations in the gene encoding β -1,4-GalNAc-T2 have been linked to Type I von Willebrand disease (VWD), the most common bleeding disorder in humans, characterized by reduced levels of plasma von Willebrand factor. Two named isoforms of β -1,4-GalNAc-T2 exist as a result of alternative splicing events.

REFERENCES

1. Smith, P.L., et al. 1994. Molecular cloning of a murine N-acetylgalactosamine transferase cDNA that determines expression of the T lymphocyte-specific CT oligosaccharide differentiation antigen. *J. Biol. Chem.* 269: 15162-15171.
2. Dohi, T., et al. 1996. Detection of N-acetylgalactosaminyltransferase mRNA which determines expression of Sda blood group carbohydrate structure in human gastrointestinal mucosa and cancer. *Int. J. Cancer* 67: 626-631.
3. Mohlke, K.L., et al. 1996. A novel modifier gene for plasma von Willebrand factor level maps to distal mouse chromosome 11. *Proc. Natl. Acad. Sci. USA* 93: 15352-15357.
4. Hakomori, S. 1999. Antigen structure and genetic basis of histo-blood groups A, B and O: their changes associated with human cancer. *Biochim. Biophys. Acta* 1473: 247-266.
5. Mohlke, K.L., et al. 1999. Mvwf, a dominant modifier of murine von Willebrand factor, results from altered lineage-specific expression of a glycosyltransferase. *Cell* 96: 111-120.

CHROMOSOMAL LOCATION

Genetic locus: B4GALNT2 (human) mapping to 17q21.32.

PRODUCT

β -1,4-GalNAc-T2 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 β -1,4-GalNAc-T2 shRNA Plasmid (h): sc-93560-SH and β -1,4-GalNAc-T2 shRNA (h) Lentiviral Particles: sc-93560-V as alternate gene silencing products.

For independent verification of β -1,4-GalNAc-T2 (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-93560A, sc-93560B and sc-93560C.

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

β -1,4-GalNAc-T2 siRNA (h) is recommended for the inhibition of β -1,4-GalNAc-T2 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

β -1,4-GalNAc-T2 (G-1): sc-393370 is recommended as a control antibody for monitoring of β -1,4-GalNAc-T2 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 β -1,4-GalNAc-T2 gene expression knockdown using RT-PCR Primer: β -1,4-GalNAc-T2 (h)-PR: sc-93560-PR (20 μ l). 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.