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SANTA CRUZ BIOTECHNOLOGY, INC.

FucT-VIII siRNA (m): sc-45758

BACKGROUND

Fucosyltransferases catalyze the covalent association of fucose to different positional linkages in sugar acceptor molecules. The carbohydrate moieties generated and covalently attached to cell surfaces are necessary to ensure a surface contour that satisfies physiological roles, which are reliant on adhesion molecules such as Selectins. Hematopoietic lineages rely on Fucosyltransferases to confer a surface carbohydrate phenotype, which mediates proper cell adhesion molecule recruitment and cell trafficking. α1,6-fucosyltransferase or Fucosyltransferase 8 (FucT-VIII) catalyzes the addition of fucose in α1-6 linkage to the innermost GlcNAc residue of an N-linked oligosaccharide.

REFERENCES

1. Yanagidani, S., et al. 1997. Purification and cDNA cloning of GDP-L-Fuc:N-acetyl-β-D-glucosaminide: α1-6 fucosyltransferase (α1-6 FucT) from human gastric cancer MKN45 cells. J. Biochem. 121: 626-632.
2. Takahashi, T., et al. 2000. A sequence motif involved in the donor substrate binding by α1,6-fucosyltransferase: the role of the conserved arginine residues. Glycobiology 10: 503-510.
3. Yamaguchi, Y., et al. 2000. Genomic structure and promoter analysis of the human α1,6-fucosyltransferase gene (FUT8). Glycobiology 10: 637-643.
4. White, K.E., et al. 2000. Molecular cloning of a novel human UDP-GalNAc: polypeptide N-acetylgalactosaminyltransferase, GalNAc-T8, and analysis as a candidate autosomal dominant hypophosphatemic rickets (ADHR) gene. Gene 246: 347-356.
5. Javaud, C., et al. 2000. Ancestral exonic organization of FUT8, the gene encoding the α6-fucosyltransferase, reveals successive peptide domains which suggest a particular three-dimensional core structure for the α6-fucosyltransferase family. Mol. Biol. Evol. 17: 1661-1672.

CHROMOSOMAL LOCATION

Genetic locus: Fut8 (mouse) mapping to 12 C3.

PRODUCT

FucT-VIII 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 FucT-VIII shRNA Plasmid (m): sc-45758-SH and FucT-VIII shRNA (m) Lentiviral Particles: sc-45758-V as alternate gene silencing products.

For independent verification of FucT-VIII (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-45758A, sc-45758B and sc-45758C.

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 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

FucT-VIII siRNA (m) is recommended for the inhibition of FucT-VIII 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

FucT-VIII (B-10): sc-271244 is recommended as a control antibody for monitoring of FucT-VIII 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 FcRH2 gene expression knockdown using RT-PCR Primer: FcRH2 (h)-PR: sc-45686-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.