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FucT-VIII shRNA (m) Lentiviral Particles: sc-45758-V

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. α -(1,6)-fucosyltransferase or Fucosyltransferase 8 (FucT-VIII) catalyzes the addition of fucose in α 1-6 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: α 1-6 fucosyltransferase (α 1-6 FucT) from human gastric cancer MKN45 cells. *J. Biochem.* 121: 626-632.
2. 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.
3. Javaud, C., et al. 2000. Ancestral exonic organization of FUT8, the gene encoding the α 6-fucosyltransferase, reveals successive peptide domains which suggest a particular three-dimensional core structure for the Mol. Biol. Evol. 17: 1661-1672.
4. Yamaguchi, Y., et al. 2000. Genomic structure and promoter analysis of the human α 1, 6-fucosyltransferase gene (FUT8). *Glycobiology* 10: 637-643.
5. Takahashi, T., et al. 2000. A sequence motif involved in the donor substrate binding by α 1,6-fucosyltransferase: the role of the conserved arginine residues. *Glycobiology* 10: 503-510.

CHROMOSOMAL LOCATION

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

PRODUCT

FucT-VIII shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see FucT-VIII siRNA (m): sc-45758 and FucT-VIII shRNA Plasmid (m): sc-45758-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

See our web site at www.scbt.com or our catalog for detailed protocols and support products.

APPLICATIONS

FucT-VIII shRNA (m) Lentiviral Particles is recommended for the inhibition of FucT-VIII expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.