

POFUT1 siRNA (h): sc-76184

BACKGROUND

Glycosyltransferases that mediate the regio- and stereoselective transfer of sugars, such as the fucosyltransferases, determine cell surface-carbohydrate profiles, which is an essential interface for biological recognition processes. Fucosyltransferases catalyze the covalent association of fucose to different positional linkages in sugar acceptor molecules. POFUT1 (protein O-fucosyltransferase 1), also known as FUT12, O-FUT or O-FucT-1, is a 388 amino acid protein that localizes to the endoplasmic reticulum and belongs to the fucosyltransferase subfamily of glycosyltransferases. Highly expressed in pancreas, kidney, lung, heart, brain, liver, placenta and skeletal muscle, POFUT1 uses manganese to catalyze the attachment (specifically the O-glycosidic linkage) of fucose to a conserved serine or threonine residue on a protein acceptor. Via its catalytic activity, POFUT1 plays an important role in notch signaling, as notch ligands can serve as POFUT1 substrates. Two isoforms of POFUT1 exist due to alternative splicing events.

REFERENCES

1. Wang, Y., et al. 1996. Identification of a GDP-L-fucose: polypeptide fucosyltransferase and enzymatic addition of O-linked fucose to EGF domains. *Glycobiology* 6: 837-842.
2. Wang, Y., et al. 1998. Purification and characterization of a GDP-fucose: polypeptide fucosyltransferase from Chinese hamster ovary cells. *J. Biol. Chem.* 273: 8112-8118.
3. Wang, Y., et al. 2001. Modification of epidermal growth factor-like repeats with O-fucose. Molecular cloning and expression of a novel GDP-fucose protein O-fucosyltransferase. *J. Biol. Chem.* 276: 40338-40345.
4. Panin, V.M., et al. 2002. Notch ligands are substrates for protein O-fucosyltransferase-1 and Fringe. *J. Biol. Chem.* 277: 29945-29952.
5. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607491. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: POFUT1 (human) mapping to 20q11.21.

PRODUCT

POFUT1 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 POFUT1 shRNA Plasmid (h): sc-76184-SH and POFUT1 shRNA (h) Lentiviral Particles: sc-76184-V as alternate gene silencing products.

For independent verification of POFUT1 (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-76184A, sc-76184B and sc-76184C.

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

POFUT1 siRNA (h) is recommended for the inhibition of POFUT1 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

POFUT1 (F-7): sc-271026 is recommended as a control antibody for monitoring of POFUT1 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 POFUT1 gene expression knockdown using RT-PCR Primer: POFUT1 (h)-PR: sc-76184-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.