SANTA CRUZ BIOTECHNOLOGY, INC.

RFT1 siRNA (h): sc-78393

BACKGROUND

RFT1, also known as CDG1N, FLJ25945 or DKFZp667J092, is a 541 amino acid multi-pass transmembrane protein and flippase enzyme which likely assists in assembly of N-linked oligosaccharides. RFT1 also catalyzes the translocation of Man(5)GlcNAc (2)-PP-Dol through the endoplasmic reticulum membrane from the cytoplasmic side to the lumenal side, which is a necessary step in the N-glycosylation pathway. Defects of RFT1 lead to autosomal recessive type 1N congenital disorders of glycosylation (or CDG1N) whose effects include immunodeficiency, disorders of the nervous system during development, hypotonia, coagulation disorders, psychomotor retardation and dysmorphic features. RFT1 has multiple transmembrane domains and is encoded by a gene which maps to human chromosome 3p21.1.

REFERENCES

1. Stibler, H., et al. 1998. Isoforms and levels of transferrin, antithrombin, α_1-antitrypsin and thyroxine-binding globulin in 48 patients with carbohydrate-deficient glycoprotein syndrome type I. Scand. J. Clin. Lab. Invest. 58: 55-61.
2. Helenius, J., et al. 2002. Translocation of lipid-linked oligosaccharides across the ER membrane requires Rft1 protein. Nature 415: 447-450.
3. Leroy, J.G. 2006. Congenital disorders of N-glycosylation including diseases associated with O- as well as N-glycosylation defects. Pediatr. Res. 60: 643-656.
4. Haeuptle, M.A., et al. 2008. Human RFT1 deficiency leads to a disorder of N-linked glycosylation. Am. J. Hum. Genet. 82: 600-606.
5. Online Mendelian Inheritance in Man, OMIM™. 2008. Johns Hopkins University, Baltimore, MD. MIM Number: 612015. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: RFT1 (human) mapping to 3p21.1.

PRODUCT

RFT1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see RFT1 shRNA Plasmid (h): sc-78393-SH and RFT1 shRNA (h) Lentiviral Particles: sc-78393-V as alternate gene silencing products.

For independent verification of RFT1 (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-78393A, sc-78393B and sc-78393C.

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

RFT1 siRNA (h) is recommended for the inhibition of RFT1 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 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor RFT1 gene expression knockdown using RT-PCR Primer: RFT1 (h)-PR: sc-78393-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.