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SANTA CRUZ BIOTECHNOLOGY, INC.

FX siRNA (h): sc-45306

BACKGROUND

GDP-L-fucose synthetase (FX protein), also designated Tissue-specific transplantation antigen P35B or GDP-keto-6-deoxymannose 3,5-epimerase, 4-reductase, belongs to the fucose synthetase family. FX is a red cell NADP(H)- binding protein that is important in leukocyte adhesion and trafficking processes. The FX protein, together with GDP-mannose 4,6-dehydratase, can convert GDP-mannose to GDP-L-fucose by catalyzing the two-step epimerase and reductase reactions. GDP-L-fucose is the substrate of several fucosyltransferases that function in the expression of many glycoconjugates such as blood group ABH antigens and developmental adhesion antigens. Defects in the gene encoding for the FX protein cause leukocyte adhesion deficiency (LAD).

REFERENCES

1. Lenzerini, L., et al. 1981. Genetic variation in the quantitative levels of an NADP (H)-binding protein (FX) in human erythrocytes. Blood 57: 209-217.
2. Camardella, L., et al. 1995. Primary structure of human erythrocyte nicotinamide adenine dinucleotide phosphate (NADP[H])-binding protein FX: identification with the mouse tum- transplantation antigen P35B. Blood 85: 264-267.
3. Sullivan, F.X., et al. 1998. Molecular cloning of human GDP-mannose 4,6-dehydratase and reconstitution of GDP-fucose biosynthesis *in vitro*. J. Biol. Chem. 273: 8193-8202.
4. Ohyama, C., et al. 1998. Molecular cloning and expression of GDP-D-mannose-4,6-dehydratase, a key enzyme for fucose metabolism defective in Lec13 cells. J. Biol. Chem. 273: 14582-14587.
5. Korner, C., et al. 1999. Decreased availability of GDP-L-fucose in a patient with LAD II with normal GDP-D-mannose dehydratase and FX protein activities. J. Leukoc. Biol. 66: 95-98.

CHROMOSOMAL LOCATION

Genetic locus: TSTA3 (human) mapping to 8q24.3.

PRODUCT

FX 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 FX shRNA Plasmid (h): sc-45306-SH and FX shRNA (h) Lentiviral Particles: sc-45306-V as alternate gene silencing products.

For independent verification of FX (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-45306A, sc-45306B and sc-45306C.

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

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

GENE EXPRESSION MONITORING

FX (43.1): sc-100531 is recommended as a control antibody for monitoring of FX 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 FX gene expression knockdown using RT-PCR Primer: FX (h)-PR: sc-45306-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.