SANTA CRUZ BIOTECHNOLOGY, INC.

FXYD3 siRNA (m): sc-60666

BACKGROUND

The mammalian FXYD family maintains Na^+ and K^+ gradients between the intracellular and extracellular milieus of cells in processes such as renal Na^+-reabsorption, muscle contraction and neuronal excitability. FXYDs are single-span membrane proteins that share a 35 amino acid signature domain, beginning with the sequence PFXYD and containing seven invariant and six conserved amino acids. Members of the FXYD family include FXYD1 (PLM, phospholemman), FXYD2 (the γ subunit of the Na/K-ATPase), FXYD3 (Mat8, mammary tumor protein), FXYD4 (CHIF) and FXYD5 (RIC). FXYD3, a 67 amino acid protein, may act as a chloride channel or as a chloride channel regulator. It is expressed in a subset of human breast tumors and shows partial homology to FXYD1. FXYD3 has a putative 20 amino acid leader sequence and a putative transmembrane domain (with 2 cysteine residues). It contains no consensus phosphorylation sites in the cytoplasmic domain.

REFERENCES

1. Morrison, B.W. and Leder, P. 1994. Neu and Ras initiate murine mammary tumors that share genetic markers generally absent in c-Myc and Int-2-initiated tumors. Oncogene 9: 3417-3426.
2. Morrison, B.W., et al. 1995. Mat-8, a novel phospholemman-like protein expressed in human breast tumors, induces a chloride conductance in *Xenopus* oocytes. J. Biol. Chem. 270: 2176-2182.
3. Sweadner, K.J. and Rael, E. 2000. The FXYD gene family of small ion transport regulators or channels: cDNA sequence, protein signature sequence, and expression. Genomics 68: 41-56.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 604996. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Arimochi, J., et al. 2005. Stable expression and visualization of Mat-8 (FXYD-3) tagged with a fluorescent protein in Chinese hamster ovary (CHO)-K1 cells. Biotechnol. Lett. 27: 1017-1024.
6. Crambert, G., et al. 2005. FXYD3 (Mat8), a new regulator of Na,K-ATPase. Mol. Biol. Cell 16: 2363-2371.

CHROMOSOMAL LOCATION

Genetic locus: Fxyd3 (mouse) mapping to 7 B1.

PRODUCT

FXYD3 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 FXYD3 shRNA Plasmid (m): sc-60666-SH and FXYD3 shRNA (m) Lentiviral Particles: sc-60666-V as alternate gene silencing products.

For independent verification of FXYD3 (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-60666A, sc-60666B and sc-60666C.

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

FXYD3 siRNA (m) is recommended for the inhibition of FXYD3 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

FXYD3 (B-3): sc-393639 is recommended as a control antibody for monitoring of FXYD3 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 FXYD3 gene expression knockdown using RT-PCR Primer: FXYD3 (m)-PR: sc-60666-PR (20 µl). 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.

PROTOCOLS

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