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FOXC1 shRNA (h) Lentiviral Particles: sc-43766-V

BACKGROUND

The forkhead transcription factor genes FOXC1 (Mf1) and FOXC2 (Mfh1) interact with the Notch signaling pathway and are required for the pre patterning of anterior and posterior domains in the presumptive somites through a putative Notch/Delta/Mesp regulatory loop. The genes have similar, dose-dependent functions, and compensate for each other in the early development of the heart, blood vessels and somites. Both FOXC1 and FOXC2 are expressed in the mesenchyme from which the ocular drainage structures derive. FOXC1 and FOXC2 also interact in kidney and heart development. Mutations in the FOXC1 gene result in Axenfeld-Rieger malformations of the anterior segment of the eye and lead to an increased susceptibility of glaucoma, including juvenile glaucoma. Functional regions in FOXC1 are required for nuclear localization and transcriptional regulation. Specifically, two regions in the FOXC1 forkhead domain, one rich in basic amino acid residues, and a second, highly conserved among all FOX proteins, are necessary for nuclear localization of the FOXC1 protein.

REFERENCES

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4. Kume, T., Jiang, H., Topczewska, J.M. and Hogan, B.L. 2001. The murine winged helix transcription factors, FOXC1 and FOXC2, are both required for cardiovascular development and somitogenesis. *Genes Dev.* 15: 2470-2482.

CHROMOSOMAL LOCATION

Genetic locus: FOXC1 (human) mapping to 6p25.3.

PRODUCT

FOXC1 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 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 lentiviral transducing particles per milliliter in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see FOXC1 siRNA (h): sc-43766 and FOXC1 shRNA Plasmid (h): sc-43766-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.

APPLICATIONS

FOXC1 shRNA (h) Lentiviral Particles is recommended for the inhibition of FOXC1 expression in human cells.

SUPPORT REAGENTS

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

GENE EXPRESSION MONITORING

FOXC1 (N-19): sc-21394 is recommended as a control antibody for monitoring of FOXC1 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 donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (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 donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor FOXC1 gene expression knockdown using RT-PCR Primer: FOXC1 (h)-PR: sc-43766-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.

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