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ALX4 shRNA (h) Lentiviral Particles: sc-44976-V

BACKGROUND

ALX4 (aristaless-like homeobox 4) is a DNA-binding transcription factor involved in skull and limb development. It is a nuclear protein with expression restricted to bone. Defects in ALX4 are the cause of parietal foramina 2 (PFM2), also known as foramina parietalia permagna. PFM2 is an autosomal dominant disease characterized by oval defects of the parietal bones caused by deficient ossification around the parietal notch, which is normally obliterated during the fifth fetal month. PFM2 is also a clinical feature of Potocki-Shaffer syndrome. BMP induces *Msx-2* and ALX4 expression in calvarial mesenchyme tissue. Northern blot analysis demonstrates that expression of both the human and mouse ALX4 genes is restricted to bone.

REFERENCES

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4. Asbreuk, C.H., et al. 2002. Survey for paired-like homeodomain gene expression in the hypothalamus: restricted expression patterns of Rx ALX4 and gooseoid. *Neuroscience* 114: 883-889.
5. Boras, K., et al. 2002. ALX4 binding to LEF-1 regulates N-CAM promoter activity. *J. Biol. Chem.* 277: 1120-1127.
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CHROMOSOMAL LOCATION

Genetic locus: ALX4 (human) mapping to 11p11.2.

PRODUCT

ALX4 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 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 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see ALX4 siRNA (h): sc-44976 and ALX4 shRNA Plasmid (h): sc-44976-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

ALX4 shRNA (h) Lentiviral Particles is recommended for the inhibition of ALX4 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 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

ALX4 (N-12): sc-22066 is recommended as a control antibody for monitoring of ALX4 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 ALX4 gene expression knockdown using RT-PCR Primer: ALX4 (h)-PR: sc-44976-PR (20 μ l, 434 bp). 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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