

ALX4 siRNA (h): sc-44976

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

1. Qu, S., et al. 1999. Physical and genetic interactions between ALX4 and CART 1. *Development* 126: 359-369.
2. Beverdam, A., et al. 2001. Severe nasal clefting and abnormal embryonic apoptosis in ALX3/ALX4 double mutant mice. *Development* 128: 3975-3986.
3. Mavrogiannis, L.A., et al. 2001. Haploinsufficiency of the human homeobox gene ALX4 causes skull ossification defects. *Nat. Genet.* 27: 17-18.
4. Asbreuk, C.H., et al. 2002. Survey for paired-like homeodomain gene expression in the hypothalamus: restricted expression patterns of *Rx* ALX4 and goosecoid. *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.
6. Rice, R., et al. 2003. Progression of calvarial bone development requires FOXC1 regulation of *Msx-2* and ALX4. *Dev. Biol.* 262: 75-87.
7. Antonopoulou, I., et al. 2004. ALX4 and *Msx-2* play phenotypically similar and additive roles in skull vault differentiation. *J. Anat.* 204: 487-499.
8. SWISS-PROT/TrEMBL (Q9H161). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>

CHROMOSOMAL LOCATION

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

PRODUCT

ALX4 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ALX4 shRNA Plasmid (h): sc-44976-SH and ALX4 shRNA (h) Lentiviral Particles: sc-44976-V as alternate gene silencing products.

For independent verification of ALX4 (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-44976A, sc-44976B and sc-44976C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

ALX4 siRNA (h) is recommended for the inhibition of ALX4 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 μ M in 66 μ l. 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

ALX4 (KAB4): sc-33643 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 reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 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° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.