SANTA CRUZ BIOTECHNOLOGY, INC.

ALX1 siRNA (h): sc-96179

BACKGROUND

ALX homeobox proteins are members of the paired homeobox family, which play a role in regulating cell development and pattern formation during embryonic stages. ALX1 (ALX homeobox protein 1), also known as CART-1 (cartilage homeoprotein-1), is a 326 amino acid protein specific to cervix and cartilage tissues. As well as having a homeobox domain, ALX1 also contains an OAR domain, which has been suggested to be important for DNA binding or protein-protein interactions and transactivation. First characterized from a rat chondrosarcoma tumor cell line, ALX1 is a homeobox transcription factor that regulates downstream target genes and has specifically shown to act as a transcriptional repressor for rat prolactin *in vivo*. Homozygous ALX1 deficient mice are born with acrania and meroanencephaly, suggesting ALX1 function in the development of the neural tube. It has also been suggested that ALX1 cooperates with ALX4 in limb development and craniofacial bone formation.

REFERENCES

1. Zhao, G.Q., et al. 1993. Cartilage homeoprotein 1, a homeoprotein selectively expressed in chondrocytes. Proc. Natl. Acad. Sci. USA 90: 8633-8637.
2. Atkinson, B.L., et al. 1996. Elucidation of homeoprotein Cart-1 function during *in vitro* chondrogenesis of C3H10T1/2 micromass cultures. Ann. N.Y. Acad. Sci. 785: 206-208.
3. Gordon, D.F., et al. 1996. Human Cart-1: structural organization, chromosomal localization, and functional analysis of a cartilage-specific homeodomain cDNA. DNA Cell Biol. 15: 531-541.
4. Zhao, Q., et al. 1996. Prenatal folic acid treatment suppresses acrania and meroanencephaly in mice mutant for the Cart1 homeobox gene. Nat. Genet. 13: 275-283.
5. Cai, R.L. 1998. Human CART1, a paired-class homeodomain protein, activates transcription through palindromic binding sites. Biochem. Biophys. Res. Commun. 250: 305-311.
6. Qu, S., et al. 1999. Physical and genetic interactions between Alx4 and Cart1. Development 126: 359-369.
7. Brouwer, A., et al. 2003. The OAR/aristaless domain of the homeodomain protein Cart1 has an attenuating role *in vivo*. Mech. Dev. 120: 241-252.

CHROMOSOMAL LOCATION

Genetic locus: ALX1 (human) mapping to 12q21.31.

PRODUCT

ALX1 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 ALX1 shRNA Plasmid (h): sc-96179-SH and ALX1 shRNA (h) Lentiviral Particles: sc-96179-V as alternate gene silencing products.

For independent verification of ALX1 (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-96179A, sc-96179B and sc-96179C.

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

ALX1 siRNA (h) is recommended for the inhibition of ALX1 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

ALX1 (G-14): sc-161338 is recommended as a control antibody for monitoring of ALX1 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 ALX1 gene expression knockdown using RT-PCR Primer: ALX1 (h)-PR: sc-96179-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.