SANTA CRUZ BIOTECHNOLOGY, INC.

HoxD1 siRNA (m): sc-38697

BACKGROUND

The Hox (homeobox) genes play an important role in the development and design of anterior-posterior body axes in animals. Although Hox proteins can bind to DNA as monomers, dimerization with PBX homeoproteins can significantly increase the DNA binding activity of these transcription factors. The HoxD9 gene is involved in the development and patterning of the forelimb and axial skeleton. Transcriptional activation of HoxD9 has been shown to be enhanced by HMG1 (high mobility group protein 1) and antagonized by HoxD8, suggesting that Hox protein function depends on both DNA-protein and protein-protein interactions. The HOX genes are known to regulate a number of cell adhesion molecules (CAMs), with HoxD9 specifically increasing levels of L-CAM transcripts. In presomitic mesoderm, HoxD1 displays dynamic stripes of expression. In the three stages of diencephalon development, HoxD1 is strongly expressed in the first two stages and downregulated in the third stage.

REFERENCES

1. Zappavigna, V., et al. 1994. Specificity of Hox protein function depends on DNA-protein and protein-protein interactions, both mediated by the homeo domain. Genes Dev. 8: 732-744.
2. Goomer, R.S., et al. 1994. Regulation *in vitro* of an L-CAM enhancer by homeobox genes HoxD9 and HNF-1. Proc. Natl. Acad. Sci. USA 91: 7985-7989.
3. Zappavigna, V., et al. 1996. HMG1 interacts with Hox proteins and enhances their DNA binding and transcriptional activation. EMBO J. 15: 4981-4991.
4. Fromental-Ramain, C., et al. 1996. Specific and redundant functions of the paralogous HoxA9 and HoxD9 genes in forelimb and axial skeleton patterning. Development 122: 461-472.
5. Phelan, M.L., et al. 1997. Distinct Hox N-terminal arm residues are responsible for specificity of DNA recognition by Hox monomers and Hox.PBX heterodimers. J. Biol. Chem. 272: 8635-8643.
6. Gellon, G., et al. 1998. Shaping animal body plans in development and evolution by modulation of Hox expression patterns. Bioessays 20: 116-122.

CHROMOSOMAL LOCATION

Genetic locus: Hoxd1 (mouse) mapping to 2 C3.

PRODUCT

HoxD1 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 HoxD1 shRNA Plasmid (m): sc-38697-SH and HoxD1 shRNA (m) Lentiviral Particles: sc-38697-V as alternate gene silencing products.

For independent verification of HoxD1 (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-38697A, sc-38697B and sc-38697C.

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

HoxD1 siRNA (m) is recommended for the inhibition of HoxD1 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

HoxD1 (H-6): sc-365853 is recommended as a control antibody for monitoring of HoxD1 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 HoxD1 gene expression knockdown using RT-PCR Primer: HoxD1 (m)-PR: sc-38697-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.