

HoxD3 siRNA (h): sc-38698

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. HoxD3, a homeobox transcription factor that promotes angiogenesis and collagen synthesis, is up-regulated during normal wound repair and may provide a means to directly improve collagen deposition, angiogenesis and closure in poorly healing wounds in diabetics. One study found that six of seven observed melanoma cell lines expressed the HoxD3 gene, whereas normal melanocytes did not. When overexpressed, HoxD3 upregulates Integrin $\beta 3$ expression in human erythroleukemia HEL cells and lung cancer A549 cells and enhances their motility and invasiveness. HoxD3 may also enhance the invasive and metastatic potential of cancer cells through TGF β -dependent and independent pathways.

REFERENCES

- 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.
- Gellon, G., et al. 1998. Shaping animal body plans in development and evolution by modulation of Hox expression patterns. *Bioessays* 20: 116-122.
- Okubo, Y., et al. 2002. Transduction of HOXD3-antisense into human melanoma cells results in decreased invasive and motile activities. *Clin. Exp. Metastasis* 19: 503-511.
- Miyazaki, Y.J., et al. 2002. HoxD3 enhances motility and invasiveness through the TGF β -dependent and -independent pathways in A549 cells. *Oncogene* 21: 798-808.
- Hansen, S.L., et al. 2003. HoxD3 accelerates wound healing in diabetic mice. *Am. J. Pathol.* 163: 2421-2431.

CHROMOSOMAL LOCATION

Genetic locus: HOXD3 (human) mapping to 2q31.1.

PRODUCT

HoxD3 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 HoxD3 shRNA Plasmid (h): sc-38698-SH and HoxD3 shRNA (h) Lentiviral Particles: sc-38698-V as alternate gene silencing products.

For independent verification of HoxD3 (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-38698A, sc-38698B and sc-38698C.

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

HoxD3 siRNA (h) is recommended for the inhibition of HoxD3 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

HoxD3 (4AY): sc-130378 is recommended as a control antibody for monitoring of HoxD3 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 HoxD3 gene expression knockdown using RT-PCR Primer: HoxD3 (h)-PR: sc-38698-PR (20 μ l, 537 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.