

Nkx-3.2 siRNA (h): sc-38729

BACKGROUND

The homeobox gene Nkx-3.2, also designated Bapx1, is the human homolog of *Drosophila* bagpipe, which, in conjunction with tinman, determines cell fate in the dorsal mesoderm. In mammalian species, Nkx-3.2 is a key transcription factor that regulates the development of smooth muscle tissues and skeletal structures. Specifically, Nkx-3.2 regulates spleen development and the development of the axial skeleton. It is also considered as an early marker for prechondrogenic cells. The human Nkx-3.2 gene maps to chromosome 4p15.33, a region containing loci for several skeletal diseases. Nkx-3.2 null mice exhibit skeletal dysplasia, asplenia, and gastroduodenal malformation, with abnormal development of the vertebral column and cranial bones of mesodermal origin. During axial cartilage formation, Nkx-3.2 inhibits the actions of Shh, a factor that interferes with the prechondrogenic effects of the BMPs.

REFERENCES

1. Azpiroz, N., et al. 1993. tinman and bagpipe: two homeo box genes that determine cell fates in the dorsal mesoderm of *Drosophila*. *Genes Dev.* 7: 1325-1340.
2. Lettice, L.A., et al. 1999. The mouse bagpipe gene controls development of axial skeleton, skull, and spleen. *Proc. Natl. Acad. Sci. USA* 96: 9695-9700.
3. Tribioli, C., et al. 1999. The murine Bapx1 homeobox gene plays a critical role in embryonic development of the axial skeleton and spleen. *Development* 126: 5699-5711.
4. Akazawa, H., et al. 2000. Targeted disruption of the homeobox transcription factor Bapx1 results in lethal skeletal dysplasia with asplenia and gastroduodenal malformation. *Genes Cells* 5: 499-513.
5. Murtaugh, L.C., et al. 2001. The chick transcriptional repressor Nkx-3.2 acts downstream of Shh to promote BMP-dependent axial chondrogenesis. *Dev. Cell* 1: 411-422.
6. Nishida, W., et al. 2002. A triad of serum response factor and the GATA and NK families governs the transcription of smooth and cardiac muscle genes. *J. Biol. Chem.* 277: 7308-7317.

CHROMOSOMAL LOCATION

Genetic locus: NKX3-2 (human) mapping to 4p15.33.

PRODUCT

Nkx-3.2 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 Nkx-3.2 shRNA Plasmid (h): sc-38729-SH and Nkx-3.2 shRNA (h) Lentiviral Particles: sc-38729-V as alternate gene silencing products.

For independent verification of Nkx-3.2 (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-38729A, sc-38729B and sc-38729C.

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

Nkx-3.2 siRNA (h) is recommended for the inhibition of Nkx-3.2 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

Nkx-3.2 (H-4): sc-514166 is recommended as a control antibody for monitoring of Nkx-3.2 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 Nkx-3.2 gene expression knockdown using RT-PCR Primer: Nkx-3.2 (h)-PR: sc-38729-PR (20 μ l). 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.