SANTA CRUZ BIOTECHNOLOGY, INC.

Nkx-2.3 siRNA (h): sc-38725

BACKGROUND

Nkx homeodomain proteins are members of a large family of vertebrate transcription factors that have a strong homology to the NK genes in *Drosophila*. Nkx proteins are involved in several aspects of cell type specification and maintenance of differentiated tissue functions. Nkx-2.3 contains a homeobox, a conserved NK2 domain, a C-terminal GIRAW motif and an N-terminal homology domain that is conserved among all NK2 genes. Expression of Nkx-2.3 is activated later in differentiated myocardial cells, and the protein is primarily detected in gut mesoderm, distinct regions of the brachial arches, the tongue epithelium and some domains in the developing jaws. Nkx-2.3 is necessary to function as a transcriptional activator during the earliest stages of heart formation. Overexpression of Nkx-2.3 leads to an enlarged heart due to a thickening of the myocardium caused by an increase in the overall number of myocardial cells.

REFERENCES

1. Harvey, R.P. 1996. NK-2 homeobox genes and heart development. Dev. Biol. 178: 203-216.
2. Ray, M.K., et al. 1996. Transcriptional regulation of a mouse Clara cell-specific protien (mCC10) gene by the Nkx transcription factor family members thyroid transciption factor 1 and cardiac muscle-specific homeobox protein (CSX). Mol. Cell. Biol. 16: 2056-2064.
3. Buchberger, A., et al. 1997. Chick Nkx-2.3 represents a novel family member of vertebrate homologues to the *Drosophila* homeobox gene tinman: differential expression of cNkx-2.3 and cNkx-2.5 during heart and gut development. Mech. Dev. 56: 151-163.
4. Cleaver, O.B., et al. 1997. Overexpression of the tinman-related genes XNkx-2.5 and XNkx-2.3 in *Xenopus* embryos results in myocardial hyperplasia. Development 122: 3549-3556.
5. Fu, Y., et al. 1999. Vertebrate tinman homologues XNkx-2.3 and XNkx-2.5 are required for heart formation in a functionally redundant manner. Development 125: 4439-4449.
6. Pabst, O., et al. 1999. Targeted disruption of the homeobox transcription factor Nkx2-3 in mice results in postnatal lethality and abnormal development of small intestine and spleen. Development 126: 2215-2225.

CHROMOSOMAL LOCATION

Genetic locus: NKX2-3 (human) mapping to 10q24.2.

PRODUCT

Nkx-2.3 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 Nkx-2.3 shRNA Plasmid (h): sc-38725-SH and Nkx-2.3 shRNA (h) Lentiviral Particles: sc-38725-V as alternate gene silencing products.

For independent verification of Nkx-2.3 (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-38725A, sc-38725B and sc-38725C.

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

Nkx-2.3 siRNA (h) is recommended for the inhibition of Nkx-2.3 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

Nkx-2.3 (4F4): sc-517182 is recommended as a control antibody for monitoring of Nkx-2.3 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 Nkx-2.3 gene expression knockdown using RT-PCR Primer: Nkx-2.3 (h)-PR: sc-38725-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.