

Nkx-6.1 siRNA (m): sc-38732

BACKGROUND

Members of the Nkx family of homeodomain proteins are key regulators of growth and development in several tissues, including brain, heart and pancreas. During neural development, sonic hedgehog (Shh) is known to control cell fate and mitogenesis, which is correlated with Shh dose-dependent expression of several genes, including Nkx-6.1. Specifically, Nkx-6.1 is responsible for cellular differentiation in the ventral neural tube and spinal meninges in response to Shh. In the pancreas, Nkx-6.1 is exclusively expressed in the islets of Langerhans in differentiating and mature β cells, which produce Insulin. The presence of Pdx-1 is required for the expression of Nkx-6.1 as well as other pancreatic β cell specific genes, including Insulin, Glut2 and IAPP. Subsequently, Nkx-6.1 binds to the DNA consensus sequence, TTAATTAC, to direct the repression of specific genes in β cells.

REFERENCES

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2. Briscoe, J., et al. 1999. Homeobox gene Nkx-2.2 and specification of neuronal identity by graded Sonic hedgehog signalling. *Nature* 398: 622-667.
3. Jorgensen, M.C., et al. 1999. Cloning and DNA-binding properties of the rat pancreatic β cell-specific factor Nkx-6.1. *FEBS Lett.* 461: 287-294.
4. Cai, J., et al. 2000. Evidence for the differential regulation of Nkx-6.1 expression in the ventral spinal cord and foregut by Shh-dependent and -independent mechanisms. *Genesis* 27: 6-11.
5. Hessabi, B., et al. 2000. The homeodomain of Nkx-2.2 carries two cooperatively acting nuclear localization signals. *Biochem. Biophys. Res. Commun.* 270: 695-700.
6. Mirmira, R.G., et al. 2000. β cell differentiation factor Nkx-6.1 contains distinct DNA binding interference and transcriptional repression domains. *J. Biol. Chem.* 275: 14743-14751.
7. Pabst, O., et al. 2000. NKX2 gene expression in neuroectoderm but not in mesodermally derived structures depends on sonic hedgehog in mouse embryos. *Dev. Genes Evol.* 210: 47-50.

CHROMOSOMAL LOCATION

Genetic locus: Nkx6-1 (mouse) mapping to 5 E4.

PRODUCT

Nkx-6.1 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Nkx-6.1 shRNA Plasmid (m): sc-38732-SH and Nkx-6.1 shRNA (m) Lentiviral Particles: sc-38732-V as alternate gene silencing products.

For independent verification of Nkx-6.1 (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-38732A, sc-38732B and sc-38732C.

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-6.1 siRNA (m) is recommended for the inhibition of Nkx-6.1 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 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-6.1 (5B8): sc-130385 is recommended as a control antibody for monitoring of Nkx-6.1 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 MarkerTM 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-6.1 gene expression knockdown using RT-PCR Primer: Nkx-6.1 (m)-PR: sc-38732-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.