SANTA CRUZ BIOTECHNOLOGY, INC.

Nkx-2.2 siRNA (h): sc-38723

BACKGROUND

Members of the NK-2 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-2.1, Nkx-2.2 and Nkx-2.9. Specifically, the Nkx-2.2 protein is responsible for directing ventral neuronal patterning in response to graded Shh signaling. In the pancreas, Nkx-2.2 is expressed in α, β and pancreatic polypeptide (PP) cells, but not in δ cells. Nkx-2.2 expression is required for differentiation of pancreatic β cells, which produce Insulin. Homozygous null mutations of the Nkx-2.2 gene in mice lead to severe hyperglycemia and death shortly after birth, which suggests that Nkx-2.2 may be an important therapeutic target for pancreatic diseases, including diabetes and cancer.

REFERENCES

1. Sussel, L., et al. 1998. Mice lacking the homeodomain transcription factor Nkx-2.2 have diabetes due to arrested differentiation of pancreatic β cells. Development 125: 2213-2221.
2. Briscoe, J., et al. 1999. Homeobox gene Nkx-2.2 and specification of neuronal identity by graded Sonic hedgehog signalling. Nature 398: 622-627.
3. St-Onge, L., et al. 1999. Pancreas development and diabetes. Curr. Opin. Genet. Dev. 9: 295-300.
4. 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.
5. Hynes, M., et al. 2000. The seven-transmembrane receptor smoothened cell-autonomously induces multiple ventral cell types. Nat. Neurosci. 3: 41-46.
6. Pabst, O., et al. 2000. Nkx-2 gene expression in neuroectoderm but not in mesendodermally derived structures depends on sonic hedgehog in mouse embryos. Dev. Genes Evol. 210: 47-50.
7. Smith, R., et al. 2006. Expression profiling of EWS/FLI identifies Nkx-2.2 as a critical target gene in Ewing's sarcoma. Cancer Cell 9: 405-416.

CHROMOSOMAL LOCATION

Genetic locus: NKX2-2 (human) mapping to 20p11.22.

PRODUCT

Nkx-2.2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Nkx-2.2 shRNA Plasmid (h): sc-38723-SH and Nkx-2.2 shRNA (h) Lentiviral Particles: sc-38723-V as alternate gene silencing products.

For independent verification of Nkx-2.2 (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-38723A, sc-38723B and sc-38723C.

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-2.2 siRNA (h) is recommended for the inhibition of Nkx-2.2 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 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-2.2 (D-4): sc-398951 is recommended as a control antibody for monitoring of Nkx-2.2 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-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-2.2 gene expression knockdown using RT-PCR Primer: Nkx-2.2 (h)-PR: sc-38723-PR (20 µl, 594 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.