SANTA CRUZ BIOTECHNOLOGY, INC.

Nkx-6.3 siRNA (h): sc-77590

BACKGROUND

Members of the Nkx family of homeodomain proteins are key regulators of growth and development in several tissues, including brain, heart and pancreas. The Nkx-6 family is involved in the patterning of the pancreas and central nervous system and consists of three proteins: Nkx-6.1, Nkx-6.2 and Nkx-6.3. Nkx-6.1 is responsible for cellular differentiation in the ventral neural tube and spinal meninges in response to Shh. Nkx-6.2 is also expressed during neural tube development by neural progenitor cells. During development, Nkx-6.2 regulates interneuron fates by repressing the expression of Dbx1, a class I homeodomain transcription repressor. Nkx-6.3 is a 265 amino acid homeobox protein that shows selective expression in the duodenal and glandular endoderm, in contrast to Nkx-6.1 and Nkx-6.2 that are broadly expressed in the ventral positions of the developing CNS. Nkx-6.3 is required in differentiation of gastrin-producing G-cells in the stomach and antrum. There are two isoforms of Nkx-6.3 that are produced as a result of alternative splicing events.

REFERENCES

1. Pedersen, J.K., et al. 2005. Endodermal expression of Nkx-6 genes depends differentially on Pdx1. Dev. Biol. 288: 487-501.
2. Nelson, S.B., et al. 2005. Expression of Nkx-6 genes in the hindbrain and gut of the developing mouse. J. Histochem. Cytochem. 53: 787-790.
3. Alanentalo, T., et al. 2006. Cloning and analysis of Nkx-6.3 during CNS and gastrointestinal development. Gene Expr. Patterns 6: 162-170.
4. Zhao, S., et al. 2007. Cloning and developmental expression of the *Xenopus* Nkx-6 genes. Dev. Genes Evol. 217: 477-483.
5. Hafler, B.P., et al. 2008. Expression and function of Nkx-6.3 in vertebrate hindbrain. Brain Res. 1222: 42-50.
6. Klinck, R., et al. 2008. Specificity of four monoclonal anti-NKx-6.1 antibodies. J. Histochem. Cytochem. 56: 415-424.
7. Choi, M.Y., et al. 2008. Requirement of the tissue-restricted homeodomain transcription factor Nkx-6.3 in differentiation of gastrin-producing G cells in the stomach antrum. Mol. Cell. Biol. 28: 3208-3218.

CHROMOSOMAL LOCATION

Genetic locus: NKX6-3 (human) mapping to 8p11.21.

PRODUCT

Nkx-6.3 siRNA (h) is a pool of 2 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-6.3 shRNA Plasmid (h): sc-77590-SH and Nkx-6.3 shRNA (h) Lentiviral Particles: sc-77590-V as alternate gene silencing products.

For independent verification of Nkx-6.3 (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-77590A and sc-77590B.

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-6.3 siRNA (h) is recommended for the inhibition of Nkx-6.3 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-6.3 (A-9): sc-390665 is recommended as a control antibody for monitoring of Nkx-6.3 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-6.3 gene expression knockdown using RT-PCR Primer: Nkx-6.3 (h)-PR: sc-77590-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.