SANTA CRUZ BIOTECHNOLOGY, INC.

Nkx-2.5 siRNA (m): sc-36076

BACKGROUND

Nkx-2.5, which is also designated cardiac specific homeobox protein (Csx), is a homeodomain-containing nuclear transcription protein of the Nkx-2 gene family. These transcriptional activators, which include thyroid transcription factor-1 (TTF-1), regulate the expression of tissue specific genes and are required for maintaining the differentiated phenotypes of various lineages. Nkx-2.5 is a homolog to the tinman protein expressed in *Drosophila*, and is essential for normal cardiovascular development. Expression of Nkx-2.5 during cardiomyogenesis is required for cardiac septation, in which a single atrium and ventricle are separated into four chambers. During embryonic development, Nkx-2.5 is also expressed in the foregut, thyroid, spleen and stomach, while in the adult expression is predominantly restricted to the heart. Mutations that disrupt Nkx-2.5 can result in atrial-septal defects, embryonic lethality and congenital heart disease.

REFERENCES

1. Guazzi, S., et al. 1990. Thyroid nuclear factor 1 (TTF-1) contains a homeodomain and displays a novel DNA binding specificity. EMBO J. 9: 3631-3639.
2. Komuro, I., et al. 1993. Csx: a murine homeobox-containing gene specifically expressed in the developing heart. Proc. Natl. Acad. Sci. USA 90: 8145-8149.
3. Lints, T.J., et al. 1993. Nkx-2.5: a novel murine homeobox gene expressed in early heart progenitor cells and their myogenic descendants. Development 119: 419-431.

CHROMOSOMAL LOCATION

Genetic locus: Nkx2-5 (mouse) mapping to 17 A3.3.

PRODUCT

Nkx-2.5 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Nkx-2.5 shRNA Plasmid (m): sc-36076-SH and Nkx-2.5 shRNA (m) Lentiviral Particles: sc-36076-V as alternate gene silencing products.

For independent verification of Nkx-2.5 (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-36076A, sc-36076B and sc-36076C.

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-2.5 siRNA (m) is recommended for the inhibition of Nkx-2.5 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 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-2.5 (A-3): sc-376565 is recommended as a control antibody for monitoring of Nkx-2.5 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-2.5 gene expression knockdown using RT-PCR Primer: Nkx-2.5 (m)-PR: sc-36076-PR (20 µl, 449 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Ning, Y., et al. 2015. Oleanolic acid induces differentiation of neural stem cells to neurons: an involvement of transcription factor Nkx-2.5. Stem Cells Int. 2015: 672312.

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.