SANTA CRUZ BIOTECHNOLOGY, INC.

Six3 siRNA (m): sc-38789

BACKGROUND

The Six proteins (sine oculis) are a family of homeodomain transcription factors that share a conserved DNA binding domain. Six3 is required for the specification and proliferation of the eye field in vertebrates and may be involved in some developmental disorders of the brain. Expression of Six3 is detected in human embryos as early as five to seven weeks of gestation; expression is maintained in the eye throughout the entire period of fetal development. At 20 weeks of gestation, expression of Six3 in the human retina has been observed in ganglion cells and in cells of the inner nuclear layer. Six3 maps to human chromosome 2p21, between genetic markers D2S119 and D2S288. The map position of human Six3 overlaps the positions of two dominant disorders (holoprosencephaly type 2 and malattia leventinese) with ocular phenotypes that have been assigned to this chromosomal region.

REFERENCES

1. Granadino, B., et al. 1999. Genomic cloning, structure, expression pattern and chromosomal location of the human Six3 gene. Genomics 55: 100-105.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 603714. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
3. Del Bene, F., et al. 2004. Direct interaction of geminin and Six3 in eye development. Nature 427: 745-749.
4. Hisaoka, M., et al. 2004. Coexpression of Nor1 and Six3 proteins in extraskeletal myxoid chondrosarcomas without detectable NR4A3 fusion genes. Cancer Genet. Cytogenet. 152: 101-107.
5. Aijaz, S., et al. 2005. Expression analysis of Six3 and Six6 in human tissues reveals differences in expression and a novel correlation between the expression of Six3 and the genes encoding isocitrate dehyhrogenase and cadherin 18. Genomics 86: 86-99.
6. Conte, I., et al. 2005. Comparative analysis of Six3 and Six6 distribution in the developing and adult mouse brain. Dev. Dyn. 234: 718-725.
7. Gestri, G., et al. 2005. Six3 functions in anterior neural plate specification by promoting cell proliferation and inhibiting Bmp4 expression. Development 132: 2401-2413.

CHROMOSOMAL LOCATION

Genetic locus: Six3 (mouse) mapping to 17 E4.

PRODUCT

Six3 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 Six3 shRNA Plasmid (m): sc-38789-SH and Six3 shRNA (m) Lentiviral Particles: sc-38789-V as alternate gene silencing products.

For independent verification of Six3 (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-38789A, sc-38789B and sc-38789C.

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

Six3 siRNA (h) is recommended for the inhibition of Six3 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

Six3 (A-1): sc-398797 is recommended as a control antibody for monitoring of Six3 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 Six3 gene expression knockdown using RT-PCR Primer: Six3 (m)-PR: sc-38789-PR (20 µl, 443 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.