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SHOX2 shRNA (h) Lentiviral Particles: sc-44100-V

BACKGROUND

Homeodomain proteins (HP) are transcriptional regulators that coordinate the expression of genes involved in development, differentiation and cellular transformation. HPs are characterized by a conserved domain of 60 amino acid residues that recognize and bind a site in the regulatory region of the target gene. SHOX2, also designated SHOT, is a human paired-related homeobox gene with two known isoforms, SHOX2A and SHOX2B, which are products of alternative splicing. The SHOX2A and SHOX2B isoforms differ in N-terminal residues and an alternatively-spliced C-terminal exon. Both isoforms contain a C-terminal OAR domain, a motif characteristic of cranio-facially-expressed homeodomain proteins. Transcripts of *Og12X*, the mouse ortholog of SHOX2, have been isolated in the aorta, female genitalia, diecephalon, mesencephalon, myelencephalon, nasal capsula, palate, eyelid and limbs of developing mouse embryo. *Og12x* localization and expression patterns suggest that SHOX2 may play a role in the pathology of Cornelia de Lange syndrome, a multisystem disorder that is characterized by somatic and cognitive retardation, characteristic facial features and limb abnormalities. The human SHOX2 gene maps to chromosome 3q25.32 and encodes a 331 amino acid protein.

REFERENCES

1. Blaschke, R.J., et al. 1998. SHOT, a SHOX-related homeobox gene, is implicated in craniofacial, brain, heart and limb development. *Proc. Natl. Acad. Sci. USA* 95: 2406-2411.
2. Semina, E.V., et al. 1998. A new human homeobox gene *Og12x* is a member of the most conserved homeobox gene family and is expressed during heart development in mouse. *Hum. Mol. Genet.* 7: 415-422.
3. Chariot, A., et al. 1999. The homeodomain-containing proteins: an update on their interacting partners. *Biochem. Pharmacol.* 58: 1851-1857.

CHROMOSOMAL LOCATION

Genetic locus: SHOX2 (human) mapping to 3q25.32.

PRODUCT

SHOX2 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see SHOX2 siRNA (h): sc-44100 and SHOX2 shRNA Plasmid (h): sc-44100-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

SHOX2 shRNA (h) Lentiviral Particles is recommended for the inhibition of SHOX2 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

SHOX2 (N-15): sc-21898 is recommended as a control antibody for monitoring of SHOX2 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor SHOX2 gene expression knockdown using RT-PCR Primer: SHOX2 (h)-PR: sc-44100-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.