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Pitx1 shRNA (h) Lentiviral Particles: sc-44015-V

BACKGROUND

Pitx1 and Pitx2 are highly homologous, bicoid-related transcription factors. Pitx1 is a bicoid-related homeodomain factor that exhibits preferential expression in the hindlimb, as well as expression in the developing anterior pituitary gland and first branchial arch. Deletion of the Pitx1 locus results in decreased distal expression of the hindlimb-specific marker, the T-box factor (TBX4). Pitx1 may modulate morphogenesis, growth and patterning of a specific hindlimb region, and serves as a component of the variables that influence morphological and growth distinctions in forelimb and hindlimb identity. Pitx2 was initially identified as the gene responsible for human Rieger syndrome, an autosomal dominant condition that causes developmental abnormalities. Pitx2 is a transcription factor that regulates cardiac positioning and pituitary and tooth morphogenesis. Pitx2 also regulates lung symmetry by encoding "leftness" of the lung. Pitx2 is asymmetrically expressed in the left lateral-plate mesoderm, and mutant mice with laterality defects show altered patterns of Pitx2 expression that correlate with changes in the visceral symmetry. The genes which encode Pitx1 and Pitx2 map to human chromosomes 5q31.1 and 4q25, respectively.

REFERENCES

1. Crawford, M.J., et al. 1997. Human and murine PTX1/Ptx1 gene maps to the region for Treacher Collins syndrome. *Mamm. Genome* 8: 841-845.
2. Gage, P.J., et al. 1997. Pituitary homeobox 2, a novel member of the bicoid-related family of homeobox genes, is a potential regulator of anterior structure formation. *Hum. Mol. Genet.* 6: 457-464.
3. Hollemann, T., et al. 1999. XPitx-1: a homeobox gene expressed during pituitary and cement gland formation of *Xenopus* embryos. *Mech. Dev.* 88: 249-252.
4. Szeto, D., et al. 1999. Role of the Bicoid-related homeodomain factor Pitx1 in specifying hindlimb morphogenesis and pituitary development. *Genes Dev.* 13: 484-494.

CHROMOSOMAL LOCATION

Genetic locus: PITX1 (human) mapping to 5q31.1.

PRODUCT

Pitx1 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 Pitx1 siRNA (h): sc-44015 and Pitx1 shRNA Plasmid (h): sc-44015-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.

APPLICATIONS

Pitx1 shRNA (h) Lentiviral Particles is recommended for the inhibition of Pitx1 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

Pitx1 (G-4): sc-271435 is recommended as a control antibody for monitoring of Pitx1 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 goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker[™] compatible goat anti-mouse IgG-HRP: sc-2031 (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 goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Pitx1 gene expression knockdown using RT-PCR Primer: Pitx1 (h)-PR: sc-44015-PR (20 μ l). Annealing temperature for the primers should be $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ 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.

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