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IRX2 shRNA (h) Lentiviral Particles: sc-43868-V

BACKGROUND

The Iroquois homeobox gene family of transcription factors regulate aspects of embryonic development including anterior/posterior and dorsal/ventral axis patterning in the central nervous system. The Iroquois family are clustered on two loci, IRXA and IRXB, which map to chromosomes 8 and 13 in mice. The IRXA group includes IRX1, IRX2 and IRX4; the IRXB group comprises IRX3, IRX5 and IRX6. IRX1 and IRX2 are both widely expressed during development in the lung epithelium and also in the ventricular septum. IRX1 and IRX2 also play a role in digit formation (E11.5–E14.5). The IRX gene family members are each expressed in a distinct pattern during mouse heart development. Specifically, IRX1 and IRX2 are expressed in the ventricular septum and IRX3 is expressed in the ventricular trabeculated myocardium. In addition, IRX4 is expressed in the linear heart tube and the AV canal; IRX5 is expressed in the endocardium lining the ventricular and atrial myocardium. Furthermore, the IRX4 gene may modulate cardiac development and function. Although the heart of IRX4⁻ mice appears to develop normally, adult IRX4⁻ mice exhibit cardiomyopathy, including cardiac hypertrophy and decreased contractility.

REFERENCES

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CHROMOSOMAL LOCATION

Genetic locus: IRX2 (human) mapping to 5p15.33.

PRODUCT

IRX2 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 IRX2 siRNA (h): sc-43868 and IRX2 shRNA Plasmid (h): sc-43868-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

IRX2 shRNA (h) Lentiviral Particles is recommended for the inhibition of IRX2 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

IRX2 (N-14): sc-323980 is recommended as a control antibody for monitoring of IRX2 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 IRX2 gene expression knockdown using RT-PCR Primer: IRX2 (h)-PR: sc-43868-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.

PROTOCOLS

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