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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

Pitx2 siRNA (m): sc-152281

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 and 4q25-q26, 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. and Camper, S.A. 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. Lin, C., et al. 1999. Pitx2 regulates lung asymmetry, cardiac positioning and pituitary and tooth morphogenesis. *Nature* 401: 279-282.
4. Kioussi, C., et al. 1999. A model for the development of the hypothalamic-pituitary axis: transcribing the hypophysis. *Mech. Dev.* 81: 23-35.
5. Hollemann, T. and Pieler, T. 1999. XPitx1: a homeobox gene expressed during pituitary and cement gland formation of *Xenopus* embryos. *Mech. Dev.* 88: 249-252.
6. 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: Pitx2 (mouse) mapping to 3 G3.

PRODUCT

Pitx2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Pitx2 shRNA Plasmid (m): sc-152281-SH and Pitx2 shRNA (m) Lentiviral Particles: sc-152281-V as alternate gene silencing products.

For independent verification of Pitx2 (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-152281A, sc-152281B and sc-152281C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Pitx2 siRNA (m) is recommended for the inhibition of Pitx2 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 μ M in 66 μ l. 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

Pitx2 (H-1): sc-390457 is recommended as a control antibody for monitoring of Pitx2 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-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Pitx2 gene expression knockdown using RT-PCR Primer: Pitx2 (m)-PR: sc-152281-PR (20 μ l, 504 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.