SANTA CRUZ BIOTECHNOLOGY, INC.

LMX1B siRNA (h): sc-38721

BACKGROUND

Nail-patella syndrome (NPS) is an autosomal dominant disorder characterized by dyplasia of finger nails, skeletal anomalies and, frequently, renal disease. NPS is caused by putative loss-of-function mutations in the transcription factor LMX1B. LMX1B belongs to the LIM-homeodomain family, members of which are known to be important for pattern formation during development. 22 novel mutations may occur in the gene encoding LMX1B and the type and distribution of the mutations support the hypothesis that NPS is the result of haploinsufficiency for LMX1B. LMX1B is also necessary for normal development of the eye and in regulating dopaminergic neurogenesis and may be involved in developmental glaucoma and the aetiology of idiopathic Parkinson's disease. Specifically, LMX1B along with LIM1 control the initial trajectory of motor axons in the developing mammalian limb. In addition, LMX1B directly regulates the coordinated expression of α 3(IV) and α 4(IV) collagen required for normal glomerular basement membrane (GBM) morphogenesis, and the dysregulation of LMX1B in GBM contributes to the renal pathology and nephrosis in NPS.

REFERENCES

1. Kania, A., et al. 2000. Coordinate roles for LIM homeobox genes in directing the dorsoventral trajectory of motor axons in the vertebrate limb. Cell 102: 161-173.
2. Knoers, N.V., et al. 2000. Nail-patella syndrome: identification of mutations in the LMX1B gene in Dutch families. J. Am. Soc. Nephrol. 11: 1762-1766.
3. Hamlington, J.D., et al. 2001. Twenty-two novel LMX1B mutations identified in nail patella syndrome (NPS) patients. Hum. Mutat. 18: 458.
4. Kim, B.S., et al. 2001. Targeted disruption of the myocilin gene (Myoc) suggests that human glaucoma-causing mutations are gain of function. Mol. Cell. Biol. 21: 7707-7713.
5. Morello, R., et al. 2001. Regulation of glomerular basement membrane collagen expression by LMX1B contributes to renal disease in nail patella syndrome. Nat. Genet. 27: 205-208.
6. Ramsden, D.B., et al. 2001. The aetiology of idiopathic Parkinson's disease. Mol. Pathol. 54: 369-380.

CHROMOSOMAL LOCATION

Genetic locus: LMX1B (human) mapping to 9q33.3.

PRODUCT

LMX1B siRNA (h) 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 LMX1B shRNA Plasmid (h): sc-38721-SH and LMX1B shRNA (h) Lentiviral Particles: sc-38721-V as alternate gene silencing products.

For independent verification of LMX1B (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-38721A, sc-38721B and sc-38721C.

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

LMX1B siRNA (h) is recommended for the inhibition of LMX1B 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

LMX1B (1D12): sc-293262 is recommended as a control antibody for monitoring of LMX1B 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 LMX1B gene expression knockdown using RT-PCR Primer: LMX1B (h)-PR: sc-38721-PR (20 µl). 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.