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Pax-2/5/8 shRNA (h) Lentiviral Particles: sc-43996-V

BACKGROUND

Pax genes contain paired domains with strong homology to genes in *Drosophila* which are involved in programming early development. The Pax-2 gene, which maps to human chromosome 10q24.31, is expressed in primitive cells of the kidney, ureter, eye, ear, and central nervous system. Mutations in the Pax-2 gene can be responsible for renal hypoplasia. The human Pax-5 gene, which maps to human chromosome 9p13.2, encodes a B cell lineage specific protein called B cell specific activator protein (BSAP), which is expressed in pro-B, pre-B and mature B lymphocytes. BSAP regulates B cell development and influences the balance between immunoglobulin secretion and B cell proliferation. The Pax-8 gene, which maps to human chromosome 2q13, is expressed in the developing and adult thyroid, the developing secretory system and at lower levels, in the adult kidney. Patients who have autosomal dominant mutations of the Pax-8 gene develop thyroid dysgenesis.

REFERENCES

1. Stapleton, P., et al. 1993. Chromosomal localization of seven PAX genes and cloning of a novel family member, PAX-9. *Nat. Genet.* 3: 292-298.
2. Igarashi, P. 1994. Transcription factors and apoptosis in kidney development. *Curr. Opin. Nephrol. Hypertens.* 3: 308-317.
3. Poleev, A., et al. 1995. Distinct functional properties of three human paired-box-protein, PAX8, isoforms generated by alternative splicing in thyroid, kidney and Wilms' tumors. *Eur. J. Biochem.* 228: 899-911.
4. Dorfler, P. et al. 1996. C-terminal activating and inhibitory domains determine the transactivation potential of BSAP (Pax-5), Pax-2 and Pax-8. *EMBO J.* 15: 1971-1982.
5. Mahmoud, M.S., et al. 1996. Altered expression of Pax-5 gene in human myeloma cells. *Blood* 87: 4311-4315.

PRODUCT

Pax-2/5/8 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 Pax-2/5/8 siRNA (h): sc-43996 and Pax-2/5/8 shRNA Plasmid (h): sc-43996-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

Pax-2/5/8 shRNA (h) Lentiviral Particles is recommended for the inhibition of Pax-2, Pax-5 and Pax-8 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

Pax-2/5/8 (G-3): sc-377181 is recommended as a control antibody for monitoring of Pax-2/5/8 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 Pax-2, Pax-5 and Pax-8 gene expression knockdown using RT-PCR Primer: Pax-2/5/8 (h)-PR: sc-43996-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.