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NPT2 shRNA (m) Lentiviral Particles: sc-45203-V

BACKGROUND

Renal tubular reabsorption of phosphate is critical to the maintenance of phosphate homeostasis in mammals. The brush-border membrane Na^+/P_i cotransport systems in proximal tubules play a major role in this process. The renal Na^+/P_i cotransporter NPT2 is expressed in the brush border membrane (BBM) of proximal tubular cells. NPT2 gene expression is crucial for PTH effects on renal P_i handling. However, renal expression of the sodium/phosphate cotransporter gene, NPT2, is not required for regulation of renal 1α -hydroxylase by phosphate. NPT2 is an integral membrane protein expressed in kidney and lung. The gene encoding human NPT1 maps to chromosome 6q21.3-p23, while the gene encoding human NPT2 maps to chromosome 5q35.

REFERENCES

- Chong, S.S., et al. 1993. Molecular cloning of the cDNA encoding a human renal sodium phosphate transport protein and its assignment to chromosome 6p21.3-p23. *Genomics* 18: 355-359.
- Chong, S.S., et al. 1995. Cloning, genetic mapping, and expression analysis of a mouse renal sodium-dependent phosphate cotransporter. *Am. J. Physiol.* 268: F1038-1045.
- Kos, C.H., et al. 1996. Comparative mapping of Na^+ -phosphate cotransporter genes, NPT1 and NPT2, in human and rabbit. *Cytogenet. Cell Genet.* 75: 22-24.
- Hoag, H.M., et al. 1999. Effects of Npt2 gene ablation and low-phosphate diet on renal Na^+ /phosphate cotransport and cotransporter gene expression. *J. Clin. Invest.* 104: 679-686.
- Zhao, N., et al. 2000. Npt2 gene disruption confers resistance to the inhibitory action of parathyroid hormone on renal sodium-phosphate cotransport. *Endocrinology* 141: 2159-2165.

CHROMOSOMAL LOCATION

Genetic locus: Slc34a1 (mouse) mapping to 13 B1.

PRODUCT

NPT2 shRNA (m) 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 μl 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 NPT2 siRNA (m): sc-45203 and NPT2 shRNA Plasmid (m): sc-45203-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

NPT2 shRNA (m) Lentiviral Particles is recommended for the inhibition of NPT2 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μl 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

NPT2 (V-20): sc-33928 is recommended as a control antibody for monitoring of NPT2 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 NPT2 gene expression knockdown using RT-PCR Primer: NPT2 (m)-PR: sc-45203-PR (20 μl). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.