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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) 



Pendrin shRNA (h) Lentiviral Particles: sc-44009-V

BACKGROUND

Pendred syndrome (PDS), an autosomal recessive disorder, is the most common form of syndromic deafness characterized by congenital sensorineural hearing loss and goiter. The gene associated with PDS is mapped to chromosome 7 and encodes a putative transmembrane protein designated Pendrin. Several mutations in the gene have been identified and account for about 10% of hereditary deafness. Pendrin transcripts are expressed at significant levels in the thyroid, inner ear, fetal cochlea and kidney, but expression is drastically reduced in thyroid carcinomas. Pendrin functions as a transporter of chloride and iodide, but not sulfate, in these tissues. Pendrin is an apical anion transporter in intercalated cells of proximal tubule and cortical collecting ducts, which mediate renal bicarbonate secretion and Cl^-/OH^- , $\text{Cl}^-/\text{HCO}_3^-$ and $\text{Cl}^-/\text{formate}$ exchange in kidney. Pendrin is expressed throughout the endolymphatic duct and sac in distinct areas of the utricle and saccule and in the external sulcus region within the cochlea, where it plays a role in the development of ion gradients.

REFERENCES

1. Everett, L.A., et al. 1997. Pendred syndrome is caused by mutations in a putative sulphate transporter gene (PDS). *Nat. Genet.* 17: 411-422.
2. Coyle, B., et al. 1998. Molecular analysis of the PDS gene in Pendred syndrome. *Hum. Mol. Genet.* 7: 1105-1112.
3. Everett, L.A., et al. 1999. Expression pattern of the mouse ortholog of the Pendred's syndrome gene (Pds) suggests a key role for Pendrin in the inner ear. *Proc. Natl. Acad. Sci. USA* 96: 9727-9732.
4. Scott, D.A., et al. 1999. The Pendred syndrome gene encodes a chloride-iodide transport protein. *Nat. Genet.* 21: 440-443.
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CHROMOSOMAL LOCATION

Genetic locus: SLC26A4 (human) mapping to 7q22.3.

PRODUCT

Pendrin 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 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 Pendrin siRNA (h): sc-44009 and Pendrin shRNA Plasmid (h): sc-44009-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

Pendrin shRNA (h) Lentiviral Particles is recommended for the inhibition of Pendrin expression in human 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

Pendrin (E-20): sc-16894 is recommended as a control antibody for monitoring of Pendrin 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 Pendrin gene expression knockdown using RT-PCR Primer: Pendrin (h)-PR: sc-44009-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.

PROTOCOLS

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