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SANTA CRUZ BIOTECHNOLOGY, INC.

PCLN-1 siRNA (h): sc-42588

BACKGROUND

Tight junctions mediate the regulation of the paracellular pathway between epithelial and endothelial cells. They form close connections to eliminate the extracellular space and regulate the flow of solutes between cells. The human gene PCLN-1 (paracellin-1) is related to the claudin family of integral membrane proteins, which localize to tight junctions. PCLN-1 contains four transmembrane domains and intracellular amino- and carboxy-termini, characteristic of the other claudin family members, and is detected only at the tight junctions of kidney tissue. PCLN-1 forms an intercellular pore and controls the resorption of magnesium and calcium in the thick ascending limb of Henle (TAL). Mutations in PCLN-1 cause renal magnesium wasting, which may contribute to a rare autosomal recessive disease, renal hypomagnesemia with hypercalciuria and nephrocalcinosis.

REFERENCES

1. de Rouffignac, C., et al. 1994. Renal magnesium handling and its hormonal control. Physiol. Rev. 72: 305-322.
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3. Kelepouris, E., et al. 1998. Hypomagnesemia: renal magnesium handling. Semin. Nephrol. 18: 56-73.
4. Madara, J.L. 1998. Regulation of the movement of solutes across tight junctions. Annu. Rev. Physiol. 60: 143-159.
5. Wong, V., et al. 1999. Paracellular Channels! Science 285: 62.
6. Furuse, M., et al. 1999. Manner of interaction of heterogeneous claudin species within and between tight junction strands. J. Cell Biol. 147: 891-903.
7. Kubota, K., et al. 1999. Ca^{2+}-independent cell-adhesion activity of claudins, a family of integral membrane proteins localized at tight junctions. Curr. Biol. 9: 1035-1038.
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CHROMOSOMAL LOCATION

Genetic locus: CLDN16 (human) mapping to 3q28.

PRODUCT

PCLN-1 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 PCLN-1 shRNA Plasmid (h): sc-42588-SH and PCLN-1 shRNA (h) Lentiviral Particles: sc-42588-V as alternate gene silencing products.

For independent verification of PCLN-1 (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-42588A, sc-42588B and sc-42588C.

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

PCLN-1 siRNA (h) is recommended for the inhibition of PCLN-1 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

PCLN-1 (7A2): sc-130561 is recommended as a control antibody for monitoring of PCLN-1 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 PCLN-1 gene expression knockdown using RT-PCR Primer: PCLN-1 (h)-PR: sc-42588-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.