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SANTA CRUZ BIOTECHNOLOGY, INC.

Pals1 siRNA (m): sc-44938

BACKGROUND

Two highly conserved complexes are responsible for the assembly of tight junctions, the Crumbs-Pals1-Patj complex and the Cdc42-PAR6-PAR3-aPKC complex. Tight junctions assist in the formation of polarity in the epithelia by establishing a barrier to separate apical and basolateral membranes. Pals1, importantly, mediates interaction between the two complexes, via interaction with PAR6. Loss of Pals1 function results in delayed polarization, decreased transepithelial electrical resistance and an inability to form lumenal cysts. Because tumors exhibit perturbations in epithelial polarity, Pals1 presents a new potential target in the study of carcinogenesis.

REFERENCES

1. Roh, M.H., et al. 2002. The MAGUK protein, Pals1, functions as an adapter, linking mammalian homologues of Crumbs and discs lost. J. Cell Biol. 157: 161-172.
2. Roh, M.H., et al. 2003. The Crumbs3-Pals1 complex participates in the establishment of polarity in mammalian epithelial cells. J. Cell. Sci. 116: 2895-2906.
3. Makarova, O., et al. 2003. Mammalian Crumbs3 is a small transmembrane protein linked to protein associated with LIN-7 (Pals1). Gene 302: 21-29.
4. Penkert, R.R., et al. 2004. Internal recognition through PDZ domain plasticity in the PAR6-Pals1 complex. Nat. Struct. Mol. Biol. 11: 1122-1127.
5. McHugh, E.M., et al. 2004. The GABA transporter GAT-1 and the MAGUK protein Pals1: interaction, uptake modulation, and coexpression in the brain. Mol. Cell. Neurosci. 26: 406-417.
6. Wang, Q., et al. 2004. Tight junction protein PAR6 interacts with an evolutionarily conserved region in the amino terminus of Pals1/stardust. J. Biol. Chem. 279: 30715-30721.
7. Straight, S.W., et al. 2004. Loss of Pals1 expression leads to tight junction and polarity defects. Mol. Biol. Cell 15: 1981-1990.

CHROMOSOMAL LOCATION

Genetic locus: Mpp5 (mouse) mapping to 12 C3.

PRODUCT

Pals1 siRNA (m) 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 Pals1 shRNA Plasmid (m): sc-44938-SH and Pals1 shRNA (m) Lentiviral Particles: sc-44938-V as alternate gene silencing products.

For independent verification of Pals1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44938A, sc-44938B and sc-44938C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

Pals1 siRNA (m) is recommended for the inhibition of Pals1 expression in mouse 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

Pals1 (G-5): sc-365411 is recommended as a control antibody for monitoring of Pals1 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 Pals1 gene expression knockdown using RT-PCR Primer: Pals1 (m)-PR: sc-44938-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.