SANTA CRUZ BIOTECHNOLOGY, INC.

NEPH1 siRNA (m): sc-44770

BACKGROUND

Glomerular visceral epithelial cells, also known as podocytes, maintain the selective filtration barrier of the renal glomerulus. NEPH1, a member of the immunoglobulin superfamily, plays a critical role in functional barrier development. Loss of NEPH1 expression, like that of its structural relative nephrin, results in nephrotic syndromes and proteinuria leading to perinatal death. NEPH1 associates with nephrin as well as ZO-1 and localizes with them to the glomerular slit diaphragm. Interaction with nephrin occurs via the extracellular domain of NEPH1 and with ZO-1 in a PDZ binding motif of the cytoplasmic tail. Mutation of a putative threonine phosphorylation site within the cytoplasmic domain abrogates interaction with ZO-1, implying that phosphorylation regulates this interaction, and may effect the recruitment of the appropriate signal transduction components to the complex.

REFERENCES

1. Donoviel, D.B., Freed, D.D., Vogel, H., Potter, D.G., Hawkins, E., Barrish, J.P., Mathur, B.N., Turner, C.A., Geske, R., Montgomery, C.A., Starbuck, M., Brandt, M., Gupta, A., Ramirez-Solis, R., Zambrowicz, B.P. and Powell, D.R. 2001. Proteinuria and perinatal lethality in mice lacking NEPH1, a novel protein with homology to nephrin. Mol. Cell. Biol. 21: 4829-1836.
2. Huber, T.B., Schmidts, M., Gerke, P., Schermer, B., Zahn, A., Hartleben, B., Sellin, L., Walz, G. and Benzing, T. 2003. The carboxyl terminus of NEPH family members binds to the PDZ domain protein zonula occludens-1. J. Biol. Chem. 278: 13417-13421.
3. Barletta, G.M., Kovari, I.A., Verma, R.K., Kerjaschki, D. and Holzman, L.B. 2003. Nephrin and NEPH1 co-localize at the podocyte foot process intercellular junction and form *cis* hetero-oligomers. J. Biol. Chem. 278: 19266-19271.
4. Liu, G., Kaw, B., Kurfis, J., Rahmanuddin, S., Kanwar, Y.S. and Chugh, S.S. 2003. NEPH1 and nephrin interaction in the slit diaphragm is an important determinant of glomerular permeability. J. Clin. Invest. 112: 209-221.

CHROMOSOMAL LOCATION

Genetic locus: Kirrel (mouse) mapping to 3 F1.

PRODUCT

NEPH1 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 NEPH1 shRNA Plasmid (m): sc-44770-SH and NEPH1 shRNA (m) Lentiviral Particles: sc-44770-V as alternate gene silencing products.

For independent verification of NEPH1 (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-44770A, sc-44770B and sc-44770C.

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

NEPH1 siRNA (m) is recommended for the inhibition of NEPH1 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

NEPH1 (F-6): sc-373787 is recommended as a control antibody for monitoring of NEPH1 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 NEPH1 gene expression knockdown using RT-PCR Primer: NEPH1 (m)-PR: sc-44770-PR (20 µl, 514 bp). 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.