SANTA CRUZ BIOTECHNOLOGY, INC.

nephrin siRNA (h): sc-36030

BACKGROUND

Nephrin is a member of the immunoglobulin family of cell adhesion molecules that localizes to opposing sites of the secondary foot processes formed by podocytes, a specialized epithelial cell that ensures size- and charge-selective ultrafiltration. The human nephrin gene maps to chromosome 19q13.12 and encodes a 1,241 amino acid protein that contains a transmembrane domain, eight Ig-like modules, and one fibronectin III-like module. Nephrin is expressed in embryonic and adult kidneys and localizes to glomerular podocytes and the glomerular slit diaphragm. Nephrin stimulates mitogen-activated protein kinases and is enhanced by podocin, which binds to the cytoplasmic tail of nephrin. A293 cells treated with Phorbol-12-myristate-13-acetate can up-regulate nephrin, suggesting that protein kinase C is part of an intracellular signalling system, which regulates nephrin.

REFERENCES

1. Holzman, L.B., et al. 1999. Nephrin localizes to the slit pore of the glomerular epithelial cell. Kidney Int. 56: 1481-1491.
2. Huber, T.B., et al. 2001. Interaction with podocin facilitates nephrin signaling. J. Biol. Chem. 276: 41543-41546.
3. Liu, L., et al. 2001. Nephrin is an important component of the barrier system in the testis. Acta Med. Okayama 55: 161-165.
4. Simons, M., et al. 2001. Involvement of lipid rafts in nephrin phosphorylation and organization of the glomerular slit diaphragm. Am. J. Pathol. 159: 1069-1077.
5. Wang, S.X., et al. 2001. Nephrin mRNA regulation by protein kinase C. J. Nephrol. 14: 98-103.
6. Online Mendelian Inheritance in Man, OMIM™. 2001. Johns Hopkins University, Baltimore, MD. MIM Number: 602716: World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/

CHROMOSOMAL LOCATION

Genetic locus: NPHS1 (human) mapping to 19q13.12.

PRODUCT

nephrin 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 nephrin shRNA Plasmid (h): sc-36030-SH and nephrin shRNA (h) Lentiviral Particles: sc-36030-V as alternate gene silencing products.

For independent verification of nephrin (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-36030A, sc-36030B and sc-36030C.

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

nephrin siRNA (h) is recommended for the inhibition of nephrin 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

nephrin (G-8): sc-376522 is recommended as a control antibody for monitoring of nephrin 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 nephrin gene expression knockdown using RT-PCR Primer: nephrin (h)-PR: sc-36030-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kumar, V., et al. 2019. Disruption of APOL1-miR193a axis induces disorganization of podocyte Actin cytoskeleton. Sci. Rep. 9: 3582.

RESEARCH USE

For research use only, not for use in diagnostic procedures.