SANTA CRUZ BIOTECHNOLOGY, INC.

NEPH3 siRNA (h): sc-75899

BACKGROUND

NEPH3 (nephrin-like protein 3), also referred to as NLG1, FILTRIN or KIRREL2 (kin of IRRE-like 2), is a 708 amino acid protein that is a member of the immunoglobulin superfamily of cell adhesion molecules. NEPH3 consists of five extracellular Ig-like repeats, a transmembrane domain, several glycosylation sites and a cytoplasmic domain that has a stretch of nine conserved residues. NEPH3 localizes to the interpodocyte filtration slit in the kidney and to the Langerhans islet β cells in the pancreas. NEPH3 is thought to be involved in the maintenance of the glomerular filtration barrier in the kidney and in pancreas development. Downregulation of NEPH3 is implicated in several proteinuric diseases. Five isoforms exists due to alternative splicing events.

REFERENCES

1. Ihalmo, P., et al. 2003. Filtrin is a novel member of nephrin-like proteins. Biochem. Biophys. Res. Commun. 300: 364-370.
2. Sun, C., et al. 2003. KIRREL2 a novel immunoglobulin superfamily gene expressed primarily in β cells of the pancreatic islets. Genomics 82: 130-142.
3. Online Mendelian Inheritance in Man, OMIM™. 2003. Johns Hopkins University, Baltimore, MD. MIM Number: 607762. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Minaki, Y., et al. 2005. Migrating postmitotic neural precursor cells in the ventricular zone extend apical processes and form adherens junctions near the ventricle in the developing spinal cord. Neurosci. Res. 52: 250-262.
5. Serizawa, S., et al. 2006. A neuronal identity code for the odorant receptor-specific and activity-dependent axon sorting. Cell 127: 1057-1069.
6. Rinta-Valkama, J., et al. 2007. Podocyte-associated proteins FAT, α-actinin-4 and filtrin are expressed in Langerhans islets of the pancreas. Mol. Cell. Biochem. 294: 117-125.
7. Ihalmo, P., et al. 2007. Expression of filtrin in human glomerular diseases. Nephrol. Dial. Transplant. 22: 1903-1909.

CHROMOSOMAL LOCATION

Genetic locus: KIRREL2 (human) mapping to 19q13.12.

PRODUCT

NEPH3 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 NEPH3 shRNA Plasmid (h): sc-75899-SH and NEPH3 shRNA (h) Lentiviral Particles: sc-75899-V as alternate gene silencing products.

For independent verification of NEPH3 (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-75899A, sc-75899B and sc-75899C.

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

NEPH3 siRNA (h) is recommended for the inhibition of NEPH3 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

NEPH3 (G-12): sc-515104 is recommended as a control antibody for monitoring of NEPH3 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 NEPH3 gene expression knockdown using RT-PCR Primer: NEPH3 (h)-PR: sc-75899-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.