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NEPH2 siRNA (m): sc-149911

BACKGROUND

NEPH2 (nephrin-like protein 2), also known as KIRRE, KIRREL3 (kin of IRRE like 3) or MRD4, is a 778 amino acid single-pass type I membrane protein that belongs to the nephrin-like protein family and immunoglobulin superfamily. Expressed in both fetal and adult brain, as well as podocytes of kidney glomeruli, NEPH2 contains five Ig-like C2-type (immunoglobulin-like) domains and is thought to play a role in the hematopoietic supportive capacity of stroma cells. NEPH2 undergoes alternative splicing to produce two isoforms and contains a C-terminal cytoplasmic domain which it uses to interact with Podocin, a podocyte protein involved in ultrafiltration. Defects in the gene encoding NEPH2 are associated with mental retardation autosomal dominant type 4 (MRD4).

REFERENCES

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2. Sellin, L., et al. 2003. NEPH1 defines a novel family of podocin interacting proteins. FASEB J. 17: 115-117.
3. Ueno, H., et al. 2003. A stromal cell-derived membrane protein that supports hematopoietic stem cells. Nat. Immunol. 4: 457-463.
4. Musiat, K. and Zwolska, D. 2005. Structure and function of the glomerular filtration barrier. Pol. Merk. Lekarski. 18: 317-320.
5. Bhalla, K., et al. 2008. Alterations in CDH15 and KIRREL3 in patients with mild to severe intellectual disability. Am. J. Hum. Genet. 83: 703-713.
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CHROMOSOMAL LOCATION

Genetic locus: Kirrel3 (mouse) mapping to 9 A4.

PRODUCT

NEPH2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NEPH2 shRNA Plasmid (m): sc-149911-SH and NEPH2 shRNA (m) Lentiviral Particles: sc-149911-V as alternate gene silencing products.

For independent verification of NEPH2 (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-149911A, sc-149911B and sc-149911C.

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.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NEPH2 siRNA (m) is recommended for the inhibition of NEPH2 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 μ M in 66 μ l. 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

NEPH2 (G-8): sc-514471 is recommended as a control antibody for monitoring of NEPH2 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 NEPH2 gene expression knockdown using RT-PCR Primer: NEPH2 (m)-PR: sc-149911-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.