SANTA CRUZ BIOTECHNOLOGY, INC.

nephrocystin-3 siRNA (m): sc-61181

BACKGROUND

The nephrocystin proteins comprise a family of five enzymes that commonly interact with p130Cas (BCAR1), proline-rich tyrosine kinases, calmodulin, and tensin, indicating that these proteins may participate in a common signaling pathway. The NPHP3 gene encodes nephrocystin-3, a protein that interacts with nephrocystin and may play a role in renal tubular function and development. Nephrocystin-3 contains a tubulin-tyrosine ligase (TTL) domain, a coiled-coil (CC) domain, and a tetratrico peptide repeat (TPR) domain. It is expressed widely at a low level, specifically in tissues such as the heart, placenta, liver, skeletal muscle, kidney and pancreas. In the brain and lung, nephrocystin-3 is expressed at a very low level. Mutations in NPHP3 may cause nephronophthisis type 3, a recessive disorder affecting adolescents characterized by sclerosing tubulointerstitial nephropathy, alterations of tubular basement membranes, tubular atrophy and dilatation, and renal cyst development primarily at the corticomedullary junction. These symptoms lead to chronic renal failure in affected individuals.

REFERENCES

1. Omran, H., et al. 2000. Identification of a new gene locus for adolescent nephronophthisis, on chromosome 3q22 in a large Venezuelan pedigree. Am. J. Hum. Genet. 66: 118-127.
2. Omran, H., et al. 2001. Human adolescent nephronophthisis: gene locus synteny disease in pcy mice. J. Am. Soc. Nephrol. 12: 107-113.
3. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 608002. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Olbrich, H., et al. 2003. Mutat degeneration and hepatic fibrosis. Nat. Genet. 34: 455-459.
5. Tanner, J.A. and Tanner, G.A. 2004. Dietary potassium citrate does not harm the pcy mouse. Exp. Biol. Med. 230: 57-60.

CHROMOSOMAL LOCATION

Genetic locus: Nphp3 (mouse) mapping to 9 F1.

PRODUCT

nephrocystin-3 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 nephrocystin-3 shRNA Plasmid (m): sc-61181-SH and nephrocystin-3 shRNA (m) Lentiviral Particles: sc-61181-V as alternate gene silencing products.

For independent verification of nephrocystin-3 (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-61181A, sc-61181B and sc-61181C.

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

nephrocystin-3 siRNA (m) is recommended for the inhibition of nephrocystin-3 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

nephrocystin-3 (3B1): sc-517129 is recommended as a control antibody for monitoring of nephrocystin-3 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 nephrocystin-3 gene expression knockdown using RT-PCR Primer: nephrocystin-3 (m)-PR: sc-61181-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.