SANTA CRUZ BIOTECHNOLOGY, INC.

NKCC1 siRNA (r): sc-270468

BACKGROUND

Na-K-Cl cotransporters (NKCC) are channel proteins that aid in the transcellular movement of chloride across both secretory and absorptive epithelia. NKCC1 is expressed in muscle cells, neurons, and red blood cells. In the basolateral membrane of secretory epithelia, NKCC1 mediates active chloride secretion. The gene encoding human NKCC1 maps to chromosome 5q23.3. In mice, disruption of the NKCC1 gene leads to deafness and impaired balance. NKCC2 is specifically expressed in the kidney where it mediates active reabsorption of sodium chloride in the thick ascending limb of the loop of Henle. NKCC2 is sensitive to the clinically important diuretics furosemide and bumetanide. The gene encoding human NKCC2 maps to chromosome 15q21.1 and mutations in this gene lead to Bartter's syndrome, an inherited hypokalaemic alkalosis. NCCT is a thiazide-sensitive Na-Cl cotransporter that is primarily expressed in the distal convoluted tubule of the kidney where it accounts for a significant fraction of net renal sodium reabsorption. The gene for human NCCT map to chromosome 16q13. Mutations in the gene encoding NCCT cause Gitelman's syndrome, a subset of Bartter's syndrome.

REFERENCES

1. Xu, J.C., et al. 1994. Molecular cloning and functional expression of the bumetanide-sensitive Na-K-Cl cotransporter. Proc. Natl. Acad. Sci. USA 91: 2201-2205.
2. Payne, J.A., et al. 1995. Primary structure, functional expression, and chromosomal localization of the bumetanide-sensitive Na-K-Cl cotransporter in human colon. J. Biol. Chem. 270: 17977-17985.
3. Quaggin, S.E., et al. 1995. Localization of the renal Na-K-Cl cotransporter gene (Slc12a1) on mouse chromosome 2. Mamm. Genome 6: 557-558.
4. Simon, D.B., et al. 1996. Gitelman's variant of Bartter's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter. Nat. Genet. 12: 24-30.
5. Mastroianni, N., et al. 1996. Molecular cloning, expression pattern, and chromosomal localization of the human Na-Cl thiazide-sensitive cotransporter (SLC12A3). Genomics 35: 486-493.
6. Mastroianni, N., et al. 1996. Novel molecular variants of the Na-Cl cotransporter gene are responsible for Gitelman syndrome. Am. J. Hum. Genet. 59: 1019-1026.

CHROMOSOMAL LOCATION

Genetic locus: Slc12a2 (rat) mapping to 18p12.

PRODUCT

NKCC1 siRNA (r) 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 NKCC1 shRNA Plasmid (r): sc-270468-SH and NKCC1 shRNA (r) Lentiviral Particles: sc-270468-V as alternate gene silencing products.

For independent verification of NKCC1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270468A, sc-270468B and sc-270468C.

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

NKCC1 siRNA (r) is recommended for the inhibition of NKCC1 expression in rat 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

NKCC1 (F-4): sc-514858 is recommended as a control antibody for monitoring of NKCC1 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 NKCC1 gene expression knockdown using RT-PCR Primer: NKCC1 (r)-PR: sc-270468-PR (20 µl, 591 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.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.