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NKCC shRNA (h) Lentiviral Particles: sc-43972-V

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 15q15-q21 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

- 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.
- 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.
- 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.
- 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.

CHROMOSOMAL LOCATION

Genetic locus: SLC12A1 (human) mapping to 15q21.1, SLC12A2 (human) mapping to 5q23.3.

PRODUCT

NKCC shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 4 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see NKCC siRNA (h): sc-43972 and NKCC shRNA Plasmid (h): sc-43972-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

NKCC shRNA (h) Lentiviral Particles is recommended for the inhibition of NKCC1 and NKCC2 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

NKCC (H-110): sc-20980 is recommended as a control antibody for monitoring of NKCC1 and NKCC2 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NKCC1 and NKCC2 gene expression knockdown using RT-PCR Primer: NKCC (h)-PR: sc-43972-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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