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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

NCCT siRNA (m): sc-42516

BACKGROUND

NCCT (Na-Cl cotransporter), also known as NCC, TSC (thiazide-sensitive sodium-chloride cotransporter) or SLC12A3 (solute carrier family 12 (sodium/chloride transporters), member 3), is a 1,021 amino acid cell membrane protein that is predominately expressed in kidney. Belonging to the SLC12A transporter family, NCCT plays an important role in renal sodium reabsorption, functioning as a renal thiazide-sensitive sodium-chloride cotransporter. Mutations to the NCCT gene are the cause of Gitelman syndrome, an autosomal recessive disorder characterized by hypokalemic alkalosis combined with hypomagnesemia, low urinary calcium, and increased renin activity associated with normal blood pressure.

REFERENCES

1. 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.
2. 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.
3. Takeuchi, K., et al. 1996. Association of a mutation in thiazide-sensitive Na-Cl cotransporter with familial Gitelman's syndrome. *J. Clin. Endocrinol. Metab.* 81: 4496-4499.
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. Lemmink, H.H., et al. 1998. Novel mutations in the thiazide-sensitive NaCl cotransporter gene in patients with Gitelman syndrome with predominant localization to the C-terminal domain. *Kidney Int.* 54: 720-730.
6. Melander, O., et al. 2000. Genetic variants of thiazide-sensitive NaCl-cotransporter in Gitelman's syndrome and primary hypertension. *Hypertension* 36: 389-394.

CHROMOSOMAL LOCATION

Genetic locus: Slc12a3 (mouse) mapping to 8 C5.

PRODUCT

NCCT 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 NCCT shRNA Plasmid (m): sc-42516-SH and NCCT shRNA (m) Lentiviral Particles: sc-42516-V as alternate gene silencing products.

For independent verification of NCCT (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-42516A, sc-42516B and sc-42516C.

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

NCCT siRNA (m) is recommended for the inhibition of NCCT 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NCCT gene expression knockdown using RT-PCR Primer: NCCT (m)-PR: sc-42516-PR (20 μ l). 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.