SANTA CRUZ BIOTECHNOLOGY, INC.

CNT1 siRNA (m): sc-60422

BACKGROUND

Nucleosides play a role in signaling in several physiologic systems, and synthetic analogs of natural nucleosides are often used to treat neoplastic and viral diseases. Plasma membrane transport of nucleosides is mediated by equilibrative and concentrative nucleoside transporters, which may have specificity for purines or pyrimidines. The deduced human 650 amino acid concentrative nucleoside transporter 1 (CNT1) protein is 83% identical to the rat protein and is expressed in the intestine, kidney and liver. CNT1, also designated solute carrier family 28 (sodium-coupled nucleoside transporter), member 1 (SLC28A1), expedites sodium-dependent fluxes of uridine, azidodeoxythymidine (AZT) and adenosine, but not of guanosine or deoxyadenosine, which undergo net renal secretion. CNT1 activity may serve as a putative mechanism for renal reabsorption of physiologic nucleosides and synthetic nucleoside drugs.

REFERENCES

1. Cano-Soldado, P., et al. 2004. Interaction of nucleoside inhibitors of HIV-1 reverse transcriptase with the concentrative nucleoside transporter-1 (SLC28A1). Antivir. Ther. 9: 993-1002.
2. Gray, J.H., et al. 2004. Functional and genetic diversity in the concentrative nucleoside transporter, CNT1, in human populations. Mol. Pharmacol. 65: 512-519.
3. Aymerich, I., et al. 2005. The concentrative nucleoside transporter family (SLC28): new roles beyond salvage? Biochem. Soc. Trans. 33: 216-219.
4. Lai, Y., et al. 2005. Conserved residues F316 and G476 in the concentrative nucleoside transporter 1 (hCNT1) affect guanosine sensitivity and membrane expression, respectively. Am. J. Physiol., Cell Physiol. 288: C39-C45.
5. Rodriguez-Mulero, S., et al. 2005. Expression of concentrative nucleoside transporters SLC28 (CNT1, CNT2 and CNT3) along the rat nephron: effect of diabetes. Kidney Int. 68: 665-672.

CHROMOSOMAL LOCATION

Genetic locus: Slc28a1 (mouse) mapping to 7 D3.

PRODUCT

CNT1 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 CNT1 shRNA Plasmid (m): sc-60422-SH and CNT1 shRNA (m) Lentiviral Particles: sc-60422-V as alternate gene silencing products.

For independent verification of CNT1 (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-60422A, sc-60422B and sc-60422C.

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

CNT1 siRNA (m) is recommended for the inhibition of CNT1 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

CNT1 (G-7): sc-515874 is recommended as a control antibody for monitoring of CNT1 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 CNT1 gene expression knockdown using RT-PCR Primer: CNT1 (m)-PR: sc-60422-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.