SANTA CRUZ BIOTECHNOLOGY, INC.

dNT-1 siRNA (h): sc-38995

BACKGROUND

Deoxyribonucleotidases are catabolic proteins that regulate intracellular deoxyribonucleoside triphosphate pools through substrate cycles. The various substrate specificities of deoxyribonucleotidases suggests that these enzymes have different functions in nucleotide metabolism. For example, dNT-2 is a mitochondrial specific enzyme that regulates a thymidine/dTMP substrate cycle by catalyzing the dephosphorylation of 5'- and 2'(3')-phosphates of uracil and thymine, thereby regulating the size of the intramitochondrial dTTP pool. Human dNT-1 is a cytosolic enzyme that regulates pyrimidine nucleotide pools. Human dNT-2 contains a mitochondrial leader peptide, providing the structural basis for two-step processing during import into the mitochondrial matrix. Mitochondrial dNT-2 is 52% identical to cytosolic deoxyribonucleotidase (dNT-1) and the 2 enzymes share many catalytic properties, however dNT-2 shows a more narrow substrate specificity. The human dNT-2 gene maps to chromosome 17p11.2, which is also a critical region for the Smith-Magenis syndrome, suggesting that dNT-2 may be involved in the etiology of this hereditary disease.

REFERENCES

1. Rampazzo, C., et al. 2000. Mammalian 5'(3')-deoxyribonucleotidase, cDNA cloning, and overexpression of the enzyme in *Escherichia coli* and mammalian cells. J. Biol. Chem. 275: 5409-5415.
2. Rampazzo, C., et al. 2000. A deoxyribonucleotidase in mitochondria: involvement in regulation of dNTP pools and possible link to genetic disease. Proc. Natl. Acad. Sci. USA 97: 8239-8244.
3. Gazziola, C., et al. 2001. Cytosolic high K_m 5'-nucleotidase and 5'(3')-deoxyribonucleotidase in substrate cycles involved in nucleotide metabolism. J. Biol. Chem. 276: 6185-6190.
4. Online Mendelian Inheritance in Man, OMIM™. 2001. Johns Hopkins University, Baltimore, MD. MIM Number: 605292. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. LocusLink Report (LocusID: 56953). http://www.ncbi.nlm.nih.gov/LocusLink/

CHROMOSOMAL LOCATION

Genetic locus: NT5C (human) mapping to 17q25.1.

PRODUCT

dNT-1 siRNA (h) is a target-specific 19-25 nt siRNA 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 dNT-1 shRNA Plasmid (h): sc-38995-SH and dNT-1 shRNA (h) Lentiviral Particles: sc-38995-V as alternate gene silencing products.

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.

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

dNT-1 siRNA (h) is recommended for the inhibition of dNT-1 expression in human 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

dNT-1 (C-10): sc-390041 is recommended as a control antibody for monitoring of dNT-1 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 dNT-1 gene expression knockdown using RT-PCR Primer: dNT-1 (h)-PR: sc-38995-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.