

## dNT-2 siRNA (h): sc-38997

### 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 two 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., Johansson, M., Gallinaro, L., Ferraro, P., Hellman, U., Karlsson, A., Reichard, P. and Bianchi, V. 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., Gallinaro, L., Milanesi, E., Frigimelica, E., Reichard, P. and Bianchi, V. 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., Ferraro, P., Moras, M., Reichard, P., and Bianchi, V. 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: NT5M (human) mapping to 17p11.2.

### PRODUCT

dNT-2 siRNA (h) 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see dNT-2 shRNA Plasmid (h): sc-38997-SH and dNT-2 shRNA (h) Lentiviral Particles: sc-38997-V as alternate gene silencing products.

For independent verification of dNT-2 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-38997A, sc-38997B and sc-38997C.

### 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

dNT-2 siRNA (h) is recommended for the inhibition of dNT-2 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  $\mu$ M in 66  $\mu$ 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 dNT-2 gene expression knockdown using RT-PCR Primer: dNT-2 (h)-PR: sc-38997-PR (20  $\mu$ 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.

### PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.