SANTA CRUZ BIOTECHNOLOGY, INC.

DCTD siRNA (m): sc-142907

BACKGROUND

DCTD (deoxycytidylate deaminase), also known as dCMP deaminase, is a 178 amino acid allosteric enzyme that exists as a homohexamer and belongs to the cytidine and deoxycytidylate deaminase protein family. Using zinc as a cofactor, DCTD catalyzes the deamination of dCMP to dUMP, thereby producing the nucleotide substrate (dUMP) that is used by thymidylate synthase (TS). TS uses 5,10-methyl-enetetrahydrofolate (methylene-THF) and dUMP in the synthesis of 2'-deoxythymidine-5'-monophosphate (dTMP), an essential precursor for DNA biosynthesis. Due to its role in the synthesis of dUMP, DCTC plays an important role in the creation of DNA. The activity of DCTD is regulated by the presence of dCTP and dTTP, two end products in the DCTD metabolic pathway. Multiple isoforms of DCTD are expressed due to alternative splicing events.

REFERENCES

1. Tyrsted, G., et al. 1987. Deoxycytidylate deaminase activity in non-stimulated and phytohemagglutinin-stimulated human lymphocytes, and in leukemic cells. Mol. Cell. Biochem. 76: 27-34.
2. Weiner, K.X., et al. 1993. Primary structure of human deoxycytidylate deaminase and overexpression of its functional protein in *Escherichia coli*. J. Biol. Chem. 268: 12983-12989.
3. Weiner, K.X., et al. 1995. Chromosomal location and structural organization of the human deoxycytidylate deaminase gene. J. Biol. Chem. 270: 18727-18729.
4. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 607638. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
5. Rush, J., et al. 2005. Immunoaffinity profiling of tyrosine phosphorylation in cancer cells. Nat. Biotechnol. 23: 94-101.
6. Gilbert, J.A., et al. 2006. Gemcitabine pharmacogenomics: cytidine deaminase and deoxycytidylate deaminase gene resequencing and functional genomics. Clin. Cancer Res. 12: 1794-1803.
7. Liskay, R.M., et al. 2007. Involvement of deoxycytidylate deaminase in the response to S_n1-type methylation DNA damage in budding yeast. Curr. Biol. 17: R755-R757.

CHROMOSOMAL LOCATION

Genetic locus: Dctd (mouse) mapping to 8 B1.2.

PRODUCT

DCTD 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 DCTD shRNA Plasmid (m): sc-142907-SH and DCTD shRNA (m) Lentiviral Particles: sc-142907-V as alternate gene silencing products.

For independent verification of DCTD (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-142907A, sc-142907B and sc-142907C.

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

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

DCTD (F-9): sc-376659 is recommended as a control antibody for monitoring of DCTD 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 DCTD gene expression knockdown using RT-PCR Primer: DCTD (m)-PR: sc-142907-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.

PROTOCOLS

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