

Dnmt3L siRNA (m): sc-37762

BACKGROUND

Methylation at the 5'-position of cytosine is the only known naturally occurring covalent modification of the mammalian genome. DNA methylation requires the enzymatic activity of DNA 5-cytosine methyltransferase (Dnmt) proteins, which catalyze the transfer of a methyl group from S-adenosyl methionine to the 5'-position of cytosines, thereby repressing expression of the target gene. Dnmt3L (DNA (cytosine-5)-methyltransferase 3-like) is a 387 amino acid protein that contains one ADD-type zinc finger and is a member of the Dnmt family. Localized to the nucleus and expressed at low levels in thymus, testis and ovary, Dnmt3L does not exhibit DNA methyltransferase activity, but is able to stimulate *de novo* methylation by Dnmt3 and is thought to play a key role in the establishment of genomic imprints. Additionally, Dnmt3L interacts with histone deacetylase 1 (HDAC1) and, through this interaction, mediates transcriptional repression. Multiple isoforms of Dnmt3L exist due to alternative splicing events.

REFERENCES

- Yoder, J.A., et al. 1997. DNA (cytosine-5)-methyltransferases in mouse cells and tissues. Studies with a mechanism-based probe. *J. Mol. Biol.* 270: 385-395.
- Walsh, C.P., et al. 1999. Cytosine methylation and mammalian development. *Genes Dev.* 13: 26-34.
- Hsieh, C.L. 1999. *In vivo* activity of murine *de novo* methyltransferases, Dnmt3a and Dnmt3b. *Mol. Cell. Biol.* 19: 8211-8218.
- Aapola, U., et al. 2000. Isolation and initial characterization of a novel zinc finger gene, DNMT3L, on 21q22.3, related to the cytosine-5-methyltransferase 3 gene family. *Genomics* 65: 293-298.
- Fuks, F., et al. 2000. DNA methyltransferase Dnmt1 associates with histone deacetylase activity. *Nat. Genet.* 24: 88-91.
- Suetake, I., et al. 2006. Stimulation effect of Dnmt3L on the DNA methylation activity of Dnmt3a2. *J. Biochem.* 140: 553-559.
- Ooi, S.K., et al. 2007. DNMT3L connects unmethylated lysine 4 of histone H3 to *de novo* methylation of DNA. *Nature* 448: 714-717.
- Jia, D., et al. 2007. Structure of Dnmt3a bound to Dnmt3L suggests a model for *de novo* DNA methylation. *Nature* 449: 248-251.

CHROMOSOMAL LOCATION

Genetic locus: Dnmt3L (mouse) mapping to 10 C1.

PRODUCT

Dnmt3L 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 Dnmt3L shRNA Plasmid (m): sc-37762-SH and Dnmt3L shRNA (m) Lentiviral Particles: sc-37762-V as alternate gene silencing products.

For independent verification of Dnmt3L (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-37762A, sc-37762B and sc-37762C.

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

Dnmt3L siRNA (m) is recommended for the inhibition of Dnmt3L 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.

GENE EXPRESSION MONITORING

Dnmt3L (A-4): sc-393603 is recommended as a control antibody for monitoring of Dnmt3L 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 Dnmt3L gene expression knockdown using RT-PCR Primer: Dnmt3L (m)-PR: sc-37762-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.

PROTOCOLS

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