# SignalSilence<sup>®</sup> DNMT3L siRNA I (Mouse Specific)

 10μM in 300 μl (3 nmol)



Orders 877-616-CELL (2355) orders@cellsignal.com Support 877-678-TECH (8324) info@cellsignal.com Web www.cellsignal.com

rev. 05/16/16

### For Research Use Only. Not For Use In Diagnostic Procedures.

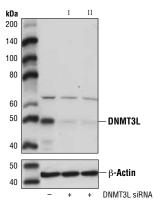
**Species Cross-Reactivity: M** 

**Description:** SignalSilence® DNMT3L siRNA I (Mouse Specific) from Cell Signaling Technology (CST) allows the researcher to specifically inhibit DNMT3L expression using RNA interference, a method whereby gene expression can be selectively silenced through the delivery of double stranded RNA molecules into the cell. All SignalSilence® siRNA products from CST are rigorously tested in-house and have been shown to reduce target protein expression by western analysis.

**Background:** Methylation of DNA at cytosine residues in mammalian cells is a heritable, epigenetic modification that is critical for proper regulation of gene expression, genomic imprinting, and development (1,2). Three families of mammalian DNA methyltransferases have been identified: DNMT1, DNMT2, and DNMT3 (1,2). DNMT1 is constitutively expressed in proliferating cells and functions as a maintenance methyltransferase, transferring proper methylation patterns to newly synthesized DNA during replication. DNMT3A and DNMT3B are strongly expressed in embryonic stem cells with reduced expression in adult somatic tissues. DNMT3A and DNMT3B function as *de novo* methyltransferases that methylate previously unmethylated regions of

DNA. DNMT2 is expressed at low levels in adult somatic tissues and its inactivation affects neither *de novo* nor maintenance DNA methylation.

DNMT3L is a catalytically inactive regulatory factor for the DNMT3A and DNMT3B *de novo* methyltransferases that is expressed at low levels in embryonic stem cells, testis, ovaries, and thymus (1,2). These *de novo* methyltransferases consist of a heterotetrameric complex containing two molecules of DNMT3L, and either two molecules DNMT3A or DNMT3B (3). DNMT3L contains an amino-terminal ATRX-DNMT3-DNMT3L (ADD) domain and a carboxy-terminal methyltransferase-like domain (4-7). The methyltransferase-like domain binds to DNMT3A and DNMT3B to stimulate catalytic activity by increasing the binding of S-adenosylmethionine and DNA (4,5). The ADD domain recruits the methyltransferase complex to transcriptionally inactive regions of the genome by binding to unmethylated histone H3 Lys4 (6,7).



Western blot analysis of extracts from F9 cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® DNMT3L siRNA I (Mouse Specific) (+), or SignalSilence® DNMT3L siRNA II (Mouse Specific) #12308 (+), using DNMT3L Antibody (Mouse Specific) #12309 (upper) or  $\beta$ -Actin (D6A8) Rabbit mAb #8457 (lower). The DNMT3L Antibody (Mouse Specific) confirms silencing of DNMT3L expression, while the  $\beta$ -Actin (D6A8) Rabbit mAb is used as a loading control.

Directions for Use: CST recommends transfection with 100 nM SignalSilence® DNMT3L siRNA I (Mouse Specific) 48 to 72 hours prior to cell lysis. For transfection procedure, follow protocol provided by the transfection reagent manufacturer. Please feel free to contact CST with any questions on use.

Each vial contains the equivalent of 100 transfections, which corresponds to a final siRNA concentration of 100 nM per transfection in a 24-well plate with a total volume of 300  $\mu l$  per well.

Quality Control: Oligonucleotide synthesis is monitored base by base through trityl analysis to ensure appropriate coupling efficiency. The oligo is subsequently purified by affinity-solid phase extraction. The annealed RNA duplex is further analyzed by mass spectrometry to verify the exact composition of the duplex. Each lot is compared to the previous lot by mass spectrometry to ensure maximum lot-to-lot consistency.

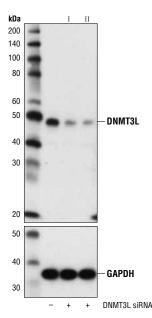
#### Entrez-Gene ID #29947 Swiss-Prot Acc. #Q9UJW3

**Storage:** DNMT3L siRNA I (Mouse Specific) is supplied in RNAse-free water. *Aliquot and store at -20°C*.

## Please visit www.cellsignal.com for a complete listing of recommended companion products.

#### **Background References:**

- (1) Hermann, A. et al. (2004) *Cell. Mol. Life Sci.* 61, 2571–2587.
- (2) Turek-Plewa, J. and Jagodziński, P.P. (2005) *Cell. Mol. Biol. Lett.* 10, 631–647.
- (3) Jia, D. et al. (2007) Nature 449, 248-251.
- (4) Holz-Schietinger, C. and Reich, N.O. (2010) *J. Biol. Chem.* 285, 29091–29100.
- (5) Suetake, I. et al. (2004) J. Biol. Chem. 279, 27816-27823.
- (6) Ooi, S.K. et al. (2007) Nature 448, 714-717.
- (7) Otani, J. et al. (2009) EMBO Rep. 10, 1235-1241.



Western blot analysis of extracts from F9 cells, transfected with 100 nM SignalSilence® Control siRNA (Unconjugated) #6568 (-), SignalSilence® DNMT3L siRNA I (Mouse Specific) (+), or SignalSilence® DNMT3L siRNA II (Mouse Specific) #12308 (+), using DNMT3L (E1Y7Q) Rabbit mAb (Mouse Specific) #13451 (upper) or GAPDH (D16H11) XP® Rabbit mAb #5174 (lower). The DNMT3L (E1Y7Q) Rabbit mAb (Mouse Specific) confirms silencing of DNMT3L expression, while the GAPDH (D16H11) XP® Rabbit mAb is used as a loading control.

Ë

 Applications Key:
 W—Western
 IP—Immunoprecipitation
 IHC—Immunohistochemistry
 ChIP—Chromatin Immunoprecipitation
 IF—Immunofluorescence
 F—Flow cytometry
 E-P—ELISA-Peptide

 Species Cross-Reactivity Key:
 H—human
 M—mouse
 R—rat
 Hm—hamster
 Mk—monkey
 Mi—mink
 C—chicken
 Dm—D. melanogaster
 X—Xenopus
 Z—zebrafish
 B—bovine

 Dg—dog
 Pg—pig
 Sc—S. cerevisiae
 Ce—C. elegans
 Hr—Horse
 AII—all species expected
 Species enclosed in parentheses are predicted to react based on 100% homology.