SANTA CRUZ BIOTECHNOLOGY, INC.

Dnmt2 siRNA (m): sc-35206

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 residing in the dinucleotide CpG motif, and this methylation results in transcriptional repression of the target gene. The Dnmt enzymes are encoded by independent genes. Dnmt1 is the most abundant, and it preferentially methylates hemimethylated DNA and coordinates gene expression during development. Additional mammalian Dnmt proteins include Dnmt2 and Dnmt3. Dnmt2 lacks the large N-terminal regulator domain of Dnmt1, is expressed at substantially lower levels in adult tissues, and is likely involved in methylating newly integrated retroviral DNA. Dnmt3a and Dnmt3b are encoded by two distinct genes, but both are abundantly expressed in embryonic stem cells, where they also methylate CpG motifs on DNA.

REFERENCES

1. 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.
2. Okano, M., et al. 1998. Dnmt2 is not required for *de novo* and maintenance methylation of viral DNA in embryonic stem cells. Nucleic Acids Res. 26: 2536-2540.
3. Hsieh, C.L. 1999. *In vivo* activity of murine *de novo* methyltransferases, Dnmt3a and Dnmt3b. Mol. Cell. Biol. 19: 8211-8218.
4. Walsh, C.P. and Bestor, T.H. 1999. Cytosine methylation and mammalian development. Genes Dev. 13: 26-34.
5. Cardoso, M.C. and Leonhardt, H. 1999. DNA methyltransferase is actively retained in the cytoplasm during early development. J. Cell Biol. 147: 25-32.
6. Bigey, P., et al. 2000. Transcriptional regulation of the human DNA methyltransferase (Dnmt1) gene. Gene 242: 407-418.
7. Fuks, F., et al. 2000. DNA methyltransferase Dnmt1 associates with histone deacetylase activity. Nat. Genet. 24: 88-91.

CHROMOSOMAL LOCATION

Genetic locus: Trdmt1 (mouse) mapping to 2 A1.

PRODUCT

Dnmt2 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 Dnmt2 shRNA Plasmid (m): sc-35206-SH and Dnmt2 shRNA (m) Lentiviral Particles: sc-35206-V as alternate gene silencing products.

For independent verification of Dnmt2 (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-35206A, sc-35206B and sc-35206C.

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

Dnmt2 siRNA (m) is recommended for the inhibition of Dnmt2 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

Dnmt2 (D-9): sc-365001 is recommended as a control antibody for monitoring of Dnmt2 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 Dnmt2 gene expression knockdown using RT-PCR Primer: Dnmt2 (m)-PR: sc-35206-PR (20 µl, 621 bp). 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.