SANTA CRUZ BIOTECHNOLOGY, INC.

MBD2 siRNA (m): sc-35866

BACKGROUND

Methylation of DNA contributes to the regulation of gene transcription in both mammalian and invertebrate systems. DNA methylation predominates on cytosine residues that are present in dinucleotide motifs consisting of a 5' cytosine followed by guanosine (CpG), and it requires the enzymatic activity of DNA methyltransferase, which results in transcriptional repression of the methylated gene. Several proteins have been identified that associate with the methyl-CpG sites, and they include methyl-CpG binding protein-1 (MBD1), MBD2, MBD3 and MeCP2. Expression of the MBD proteins is highest in somatic tissues. MBD1 binds in a context-specific manner to methyl-CpG rich domains and, in turn, mediates the transcriptional inhibition that is commonly observed with DNA methylation. Similarly, MBD2 inhibits transcription of methylated genes by associating with histone deacetylase (HDAC1) within the MeCP1 repressor complex. In addition, MBD4, which is also designated MED1, associates with the mismatch repair protein MLH1 and preferentially binds to methylated cytosine residues in mismatched base pairs. MeCP2 binds tightly to chromosomes in a methylation-dependent manner and associates with a corepressor complex containing the transcriptional repressor mSin3A and histone deacetylases.

REFERENCES

1. Boyes, J., et al. 1991. DNA methylation inhibits transcription indirectly via a methyl-CpG binding protein. Cell 64: 1123-1134.
2. Nan, X., et al. 1998. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. Nature 393: 386-389.
3. Hendrich, B., et al. 1998. Identification and characterization of a family of mammalian methyl-CpG binding proteins. Mol. Cell. Biol. 18: 6538-6547.
4. Hendrich, B., et al. 1999. Genomic structure and chromosomal mapping of the murine and human MBD1, MBD2, MBD3 and MBD4 genes. Mamm. Genome 10: 906-912.
5. Ohki, I., et al. 1999. Solution structure of the methyl-CpG-binding domain of the methylation-dependent transcriptional repressor MBD1. EMBO J. 18: 6653-6661.

CHROMOSOMAL LOCATION

Genetic locus: Mbd2 (mouse) mapping to 18 E2.

PRODUCT

MBD2 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 MBD2 shRNA Plasmid (m): sc-35866-SH and MBD2 shRNA (m) Lentiviral Particles: sc-35866-V as alternate gene silencing products.

For independent verification of MBD2 (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-35866A, sc-35866B and sc-35866C.

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

MBD2 siRNA (m) is recommended for the inhibition of MBD2 expression in mouse cells.

GENE EXPRESSION MONITORING

MBD2 (C-11): sc-514062 is recommended as a control antibody for monitoring of MBD2 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 MBD2 gene expression knockdown using RT-PCR Primer: MBD2 (m)-PR: sc-35866-PR (20 µl, 464 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Hwang, C.K., et al. 2007. Evidence of endogenous µ opioid receptor regulation by epigenetic control of the promoters. Mol. Cell. Biol. 27: 4720-4736.
2. Mahmood, N., et al. 2024. Methyl-CpG binding domain protein 2 (Mbd2) drives breast cancer progression through the modulation of epithelial-to-mesenchymal transition. Exp. Mol. Med. 56: 959-974.

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.