SANTA CRUZ BIOTECHNOLOGY, INC.

MBD4 siRNA (h): sc-37763

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. and Bird, A. 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: MBD4 (human) mapping to 3q21.3.

PRODUCT

MBD4 siRNA (h) 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 MBD4 shRNA Plasmid (h): sc-37763-SH and MBD4 shRNA (h) Lentiviral Particles: sc-37763-V as alternate gene silencing products.

For independent verification of MBD4 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-37763A, sc-37763B and sc-37763C.

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

MBD4 siRNA (h) is recommended for the inhibition of MBD4 expression in human 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

MBD4 (D-6): sc-271530 is recommended as a control antibody for monitoring of MBD4 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 MBD4 gene expression knockdown using RT-PCR Primer: MBD4 (h)-PR: sc-37763-PR (20 µl, 410 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Dinis, J., et al. 2012. DNA damage response in imatinib resistant chronic myeloid leukemia K562 cells. Leuk. Lymphoma 53: 2004-2014.

RESEARCH USE

For research use only, not for use in diagnostic procedures.