

MeCP2 siRNA (h2): sc-156056

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

- Boyes, J. and Bird, A. 1991. DNA methylation inhibits transcription indirectly via a methyl-CpG binding protein. *Cell* 64: 1123-1134.
- Nan, X., et al. 1998. Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* 393: 386-389.
- Hendrich, B. and Bird, A. 1998. Identification and characterization of a family of mammalian methyl-CpG binding proteins. *Mol. Cell. Biol.* 18: 6538-6547.

CHROMOSOMAL LOCATION

Genetic locus: MECP2 (human) mapping to Xq28.

PRODUCT

MeCP2 siRNA (h2) 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 MeCP2 shRNA Plasmid (h2): sc-156056-SH and MeCP2 shRNA (h2) Lentiviral Particles: sc-156056-V as alternate gene silencing products.

For independent verification of MeCP2 (h2) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-156056A, sc-156056B and sc-156056C.

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

MeCP2 siRNA (h2) is recommended for the inhibition of MeCP2 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 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

MeCP2 (G-6): sc-137070 is recommended as a control antibody for monitoring of MeCP2 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 MeCP2 gene expression knockdown using RT-PCR Primer: MeCP2 (h2)-PR: sc-156056-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- He, S., et al. 2015. Inhibition of DNA methylation and methyl-CpG-binding protein 2 suppresses RPE transdifferentiation: relevance to proliferative vitreoretinopathy. *Invest. Ophthalmol. Vis. Sci.* 56: 5579-5589.
- Li, X., et al. 2016. The significance of the increased expression of phosphorylated MeCP2 in the membranes from patients with proliferative diabetic retinopathy. *Sci. Rep.* 6: 32850.

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.