# SANTA CRUZ BIOTECHNOLOGY, INC.

# MBD5 siRNA (h): sc-94756



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, MBD4, MBD5 and MeCP2. MBD5 is a 1,494 amino acid protein containing one MBD domain and one PWWP domain. Localized to the nucleus, MBD5 is expressed in skeletal muscle, kidney, heart, kidney, liver, pancreas and placenta. Mutations in the gene that encodes MBD5 have been found to cause mental retardation autosomal dominant type 1 (MRD1), which is characterized by sub-average general intellectual functioning manifested during the developmental period.

#### REFERENCES

- 1. Boyes, J. and Bird, A. 1991. DNA methylation inhibits transcription indirectly via a methyl-CpG binding protein. Cell 64: 1123-1134.
- Hendrich, B. and Bird, A. 1998. Identification and characterization of a family of mammalian methyl-CpG binding proteins. Mol. Cell. Biol. 18: 6538-6547.
- 3. 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.
- 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.
- Nagase, T., et al. 2000. Prediction of the coding sequences of unidentified human genes. XVII. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. DNA Res. 7: 143-150.
- 6. Roloff, T.C., et al. 2003. Comparative study of methyl-CpG-binding domain proteins. BMC Genomics 4: 1.
- Wagenstaller, J., et al. 2007. Copy-number variations measured by singlenucleotide-polymorphism oligonucleotide arrays in patients with mental retardation. Am. J. Hum. Genet. 81: 768-779.

#### CHROMOSOMAL LOCATION

Genetic locus: MBD5 (human) mapping to 2q23.1.

#### PRODUCT

MBD5 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MBD5 shRNA Plasmid (h): sc-94756-SH and MBD5 shRNA (h) Lentiviral Particles: sc-94756-V as alternate gene silencing products.

For independent verification of MBD5 (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-94756A, sc-94756B and sc-94756C.

#### STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at  $-20^{\circ}$  C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at  $-20^{\circ}$  C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330  $\mu$ l of the RNAse-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNAse-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

# **APPLICATIONS**

MBD5 siRNA (h) is recommended for the inhibition of MBD5 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  $\mu$ M in 66  $\mu$ 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.

#### **RT-PCR REAGENTS**

Semi-quantitative RT-PCR may be performed to monitor MBD5 gene expression knockdown using RT-PCR Primer: MBD5 (h)-PR: sc-94756-PR (20  $\mu$ l). 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.