diagenode
A Hologic Company

TECHNICAL DATASHEET

MBD1 polyclonal antibody

Other names: CXXC3, PCM1, RFT

Cat. No. C15410078
Type: Polyclonal **ChIP-grade**
Source: Rabbit
Lot #: A17-0042
Size: 50 µg/ 72 µl
Concentration: 0.7 µg/µl

Specificity: Human, mouse: positive
Other species: not tested.
Purity: Affinity purified polyclonal antibody in PBS containing 0.05% azide and 0.05% ProClin 300.
Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.
Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Description: Polyclonal antibody raised in rabbit against human MBD1 (Methyl-CpG-binding domain protein 1), using a KLH-conjugated synthetic peptide containing a sequence from the N-terminal part of the protein.

Applications

	Suggested dilution	References
ChIP *	1.5 µg/ChIP	Fig 1
ELISA	1:1,000	Fig 2
Western blotting	1:500	Fig 3

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 1-5 µg per IP.

Target description

MBD1 (UniProt/Swiss-Prot entry Q9UIS9) is a transcriptional repressor that specifically binds to methylated CpG dinucleotides in promoter sequences. MBD1 acts by recruiting a variety of histone deacetylases (HDAC's) and chromatin remodelling factors. MBD1-dependent transcriptional repression is mediated by ATF7IP through the recruitment of factors such as the histone methyltransferase SETDB1. MBD1 probably forms a complex with SETDB1 and ATF7IP which couples DNA methylation to H3K9 trimethylation and represses transcription.

Results

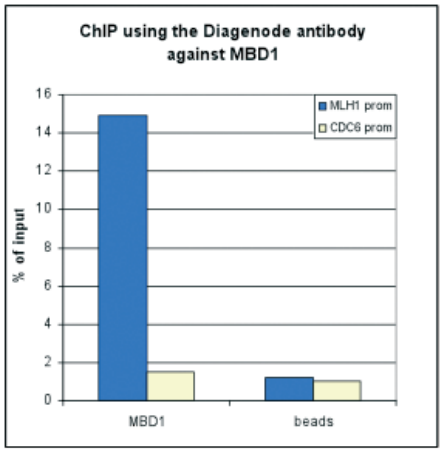

Figure 1. ChIP results obtained with the Diagenode antibody directed against MBD1

ChIP assays were performed using human osteosarcoma (U2OS) cells, the Diagenode antibody against MBD1 (Cat. No. C15410078) and optimized PCR primer sets. Sheared chromatin from 1x10e6 cells and 1.5 µg of antibody were used per ChIP experiment. Beads only were used as a negative IP control. Quantitative PCR was performed with primers for the promoters of the MLH1 gene (used as a positive control) and CDC6 gene (used as a negative control). Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

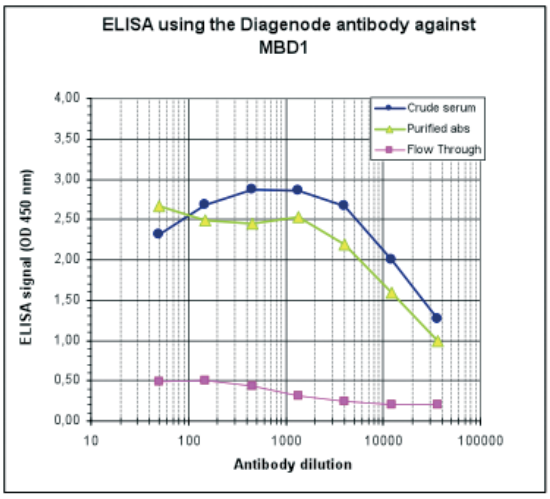

Figure 2. Determination of the antibody titer

To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against human MBD1 (Cat. No. C15410078), crude serum and Flow Through. The plates were coated with the peptide used for immunization of the rabbit. By plotting the absorbance against the antibody dilution (Figure 2), the titer of the purified antibody was estimated to be 1:20,000.

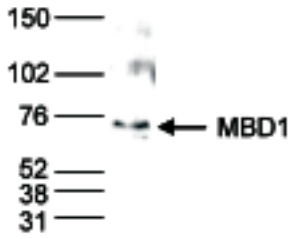

Figure 3. Western blot analysis using the Diagenode antibody directed against MBD1

Nuclear extracts of HeLa cells (40 µg) were analysed by Western blot using the Diagenode antibody against MBD1 (Cat. No. C15410078) diluted 1:500 in TBS-Tween containing 5% skimmed milk. The position of the protein of interest is indicated on the right, the marker (in kDa) is shown on the left.

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