

Methyl-CpG-binding domain protein 1

UniProt

Function: Transcriptional repressor that binds CpG islands in promoters where the DNA is methylated at position 5 of cytosine within CpG dinucleotides. Binding is abolished by the presence of 7-mG that is produced by DNA damage by methylmethanesulfonate (MMS). Acts as transcriptional repressor and plays a role in gene silencing by recruiting AFT7IP, which in turn recruits factors such as the histone methyltransferase SETDB1. Probably forms a complex with SETDB1 and ATF7IP that represses transcription and couples DNA methylation and histone 'Lys-9' trimethylation. Isoform 1 and isoform 2 can also repress transcription from unmethylated promoters.

Subcellular location: Nucleus speckle. NOTE: Nuclear, in a punctate pattern. Associated with euchromatic regions of the chromosomes, with pericentromeric regions on chromosome 1 and with telomeric regions from several chromosomes.

Tissue specificity: Widely expressed.

Three antibodies: MBD1-1, MBD1-2 and MBD1-3 were tested and 1 antibody was approved for IHC. MBD1-1 was selected for full protein profiling.

MBD1-1 (CAB036003)



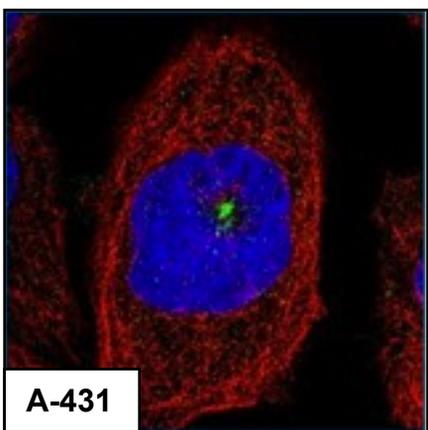
Immunohistochemistry

IHC protocol: HIER pH 6, Dilution 1:10000

IHC test staining: Nuclear and cytoplasmic staining in many tissues. Brush border was stained in intestine and kidney.

IHC Annotators comments

Normal cells generally showed moderate to strong nuclear immunoreactivity which in most cases was combined with weaker cytoplasmic positivity. The gastrointestinal tract, renal tubules and prostate gland among other showed luminal membranous positivity.

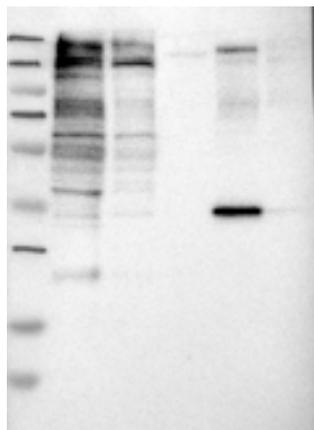


Immunofluorescence

IF Overlay: antibody (green), anti-tubuline (red) and DAPI (blue)

IF Localisation: Staining of centrosomes in all three cell lines.

IF Validation: Subcellular localization not consistent with literature.



Western blot

WB Size markers (kDa): 230, 130, 95, 72, 56, 36, 28, 17, 11

WB Lanes: Marker(1), RT-4(2), U251 MG(3), Plasma(4), Liver(5), Tonsil(6)

WB Target weight (kDa): 72, 67, 65, 61, 60, 59, 55, 52

WB Validation: Not supportive (Only bands not corresponding to the predicted size)