

PRODUCT NAME		
5-mC monoclonal antibody 33D3		
Cat. No. MAb-081-100 MAb-081-500	Type: Monoclonal MeDIP-grade IgG1	Size: 100 µg/ 100 µl 500 µg/ 500 µl
Lot #: GF-001	Source: Mouse	Concentration: 1 µg/µl

Product description: Monoclonal antibody raised in mouse against 5-mC (5-methylcytosine) conjugated to ovalbumine (clone 33D3).

Specificity: Human, mouse: positive
Other species: not tested

Applications	Suggested dilution	References
MeDIP*	1-2 µg per IP	Fig 1
Dot blotting	1:250	Fig 2
Immunofluorescence	1:500	Fig 3
Southern blot	1:200	Fig 4

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

Purity: Monoclonal antibody purified by gel filtration, in PBS containing 0.05% azide.

Storage: Store in small aliquots at -80°C. Avoid multiple freeze-thaw cycles.

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

This antibody has been described in:

(1) Lewis ZA, Honda S, Khlafallah TK, Jeffress JK, Freitag M, Mohn F, Schübeler D, and Selker EU (2009) Relics of repeat-induced point mutation direct heterochromatin formation in *Neurospora crassa* Genome Res 19: 427-437.

Last data sheet update: June 24, 2011

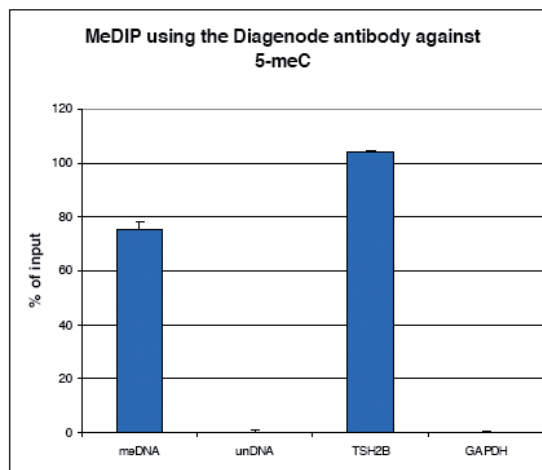


Figure 1

MeDIP results obtained with the Diagenode monoclonal antibody directed against 5-mC

MeDIP (Methylated DNA immunoprecipitation) was performed on fragmented genomic DNA from U2OS cells using the Diagenode monoclonal antibody against 5-mC (Cat. No. MAb-081-100/500) and the MagMeDIP Kit (Cat. No. mc-magme-048). The DNA was prepared with the GenDNA module of the MagMeDIP kit (also available separately, Cat. No. mc-magme-003). The fragmented DNA was spiked with the internal controls present in the kit (methylated (meDNA) as a positive and unmethylated DNA (unDNA) as a negative control) prior to performing the IP. QPCR was performed with optimized primer sets, included in the kit, specific for the methylated and unmethylated DNA controls, and for a known methylated (TSH2B) and unmethylated (GAPDH) genomic region. Figure 1 shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

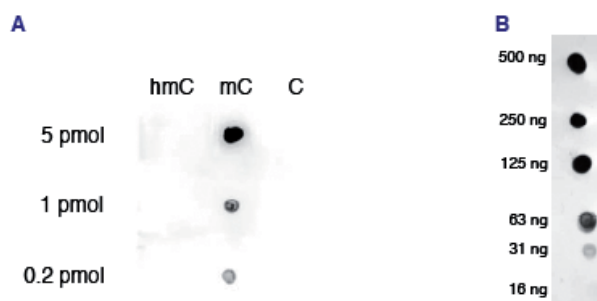


Figure 2

Dot blot analysis using the Diagenode monoclonal antibody directed against 5-mC

Figure 2A: To demonstrate the specificity of the Diagenode antibody against 5-mC (Cat. No. MAb-081-100/500), a Dot blot analysis was performed using the hmC, mC and C controls from the Diagenode "5-hmC, 5-mC & cytosine DNA Standard Pack" (Cat. No. AF-101-0002). One hundred to 4 ng [equivalent of 5 to 0.2 pmol of C-bases] of the controls were spotted on a membrane (Amersham Hybond-N+). The antibody was used at a dilution of 1:250. Figure 2 shows a high specificity of the antibody for the hydroxymethylated control.

Figure 2B: Dot blot was performed as described above on decreasing amounts of DNA isolated from phage XP12.

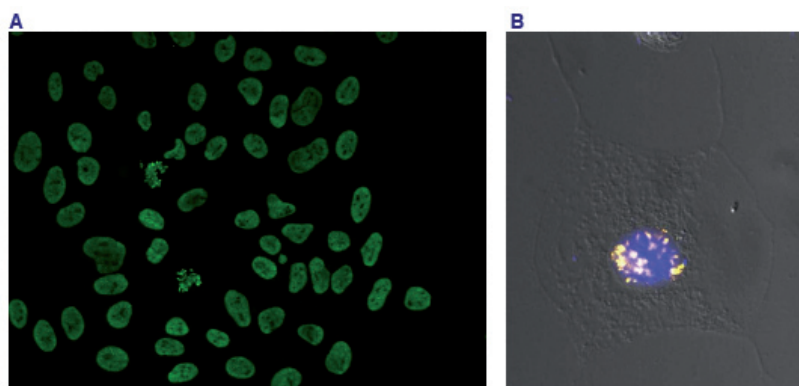


Figure 3

Immunofluorescence results obtained with the Diagenode monoclonal antibody directed against 5-mC

Figure 3A: Human osteosarcoma (U2OS) cells were stained with the Diagenode monoclonal antibody against 5-mC (Cat. No. MAb-081-100/500). Cells were fixed with 2.5% PFA in PBS for 30', permeabilised with 0.5% Triton X-100 for 1 hour and treated with 2N HCl for 1 hour followed by 2 x 5 minutes with 0.1 M borate buffer to depurinate the DNA. After blocking with PBS containing 0.1% TritonX-100 and 1% BSA, the cells were immunofluorescently labeled with the 5-mC antibody diluted 1:500 in blocking solution followed by an anti-mouse antibody conjugated to Alexa488.

Figure 3B: Immunofluorescent staining of an interphase HeLa cell with the Diagenode 5-mC antibody followed by an anti-mouse antibody conjugated to FITC (yellow) and with DAPI (blue).

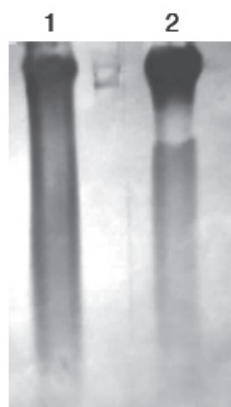


Figure 4

Southern blot analysis using the Diagenode monoclonal antibody directed against 5-mC

Genomic DNA from lymphocytes (2 µg) was digested with the methylation sensitive or methylation sensitive restriction enzymes MspI (lane 1) and HpaII (lane 2), respectively and analysed by Southern blot using the Diagenode monoclonal antibody against 5-mC (Cat. No. MAb-081-100/500).