diagenode
Innovating Epigenetics Solutions

TECHNICAL DATASHEET

5-methylcytosine (5-mC) monoclonal antibody 33D3 - Premium

Cat. No. C15200081-100

Type: Monoclonal	Specificity: Human, mouse, rat, cow, other (wide range): positive
Size: 100 µg	Isotype: IgG1
Concentration: 1.24 µg/µl	Host: Mouse
Lot No.: RD-004	Purity: Protein A purified monoclonal antibody.
Storage buffer: PBS containing 0.05% azide.	Storage conditions: 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.	

Last Data Sheet Update: October 22, 2018

Description

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

Applications

Applications	Suggested dilution	References
MeDIP/MeDIP-seq *	0.5 - 1 µg/IP	Fig 1, 2
Dot Blotting	1:100	Fig 3
IF	1:500	Fig 4

* Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 0.5-5 µg per IP.

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Validation Data

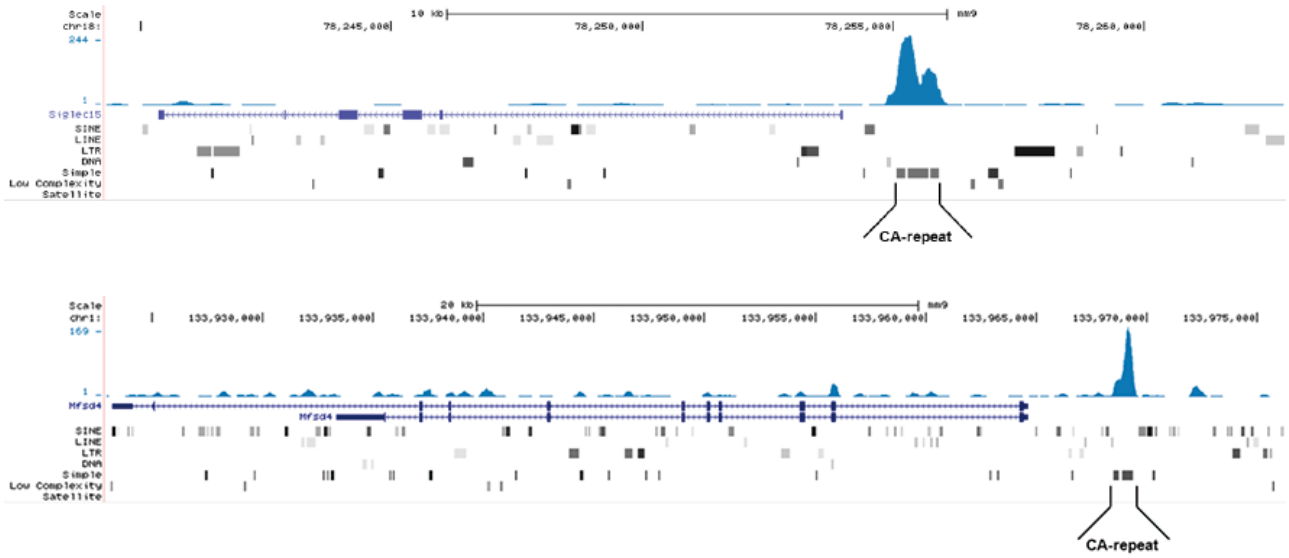

Figure 1. MeDIP-seq with the Diagenode monoclonal antibody directed against 5-mC
Genomic DNA from E14 ES cells was sheared with the Bioruptor® to generate random fragments (size range 300 to 700 bp). One µg of the fragmented DNA was ligated to Illumina adapters and the resulting DNA was used for a standard MeDIP assay, using 2 µg of the Diagenode monoclonal against 5-mC (Cat. No. C15200081). After recovery of the methylated DNA, Illumina sequencing libraries were generated and sequenced on an Illumina Genome Analyzer according to the manufacturer's instructions. Figure 1A and 1B show Genome browser views of CA simple repeat elements with read distributions specific for 5-mC at 2 gene locations (SigleC15 and Mfsd4). Visual inspection of the peak profiles in a genome browser reveals high enrichment of CA simple repeats in affinity-enriched methylated fragments after MeDIP with the Diagenode 5-mC monoclonal antibody.

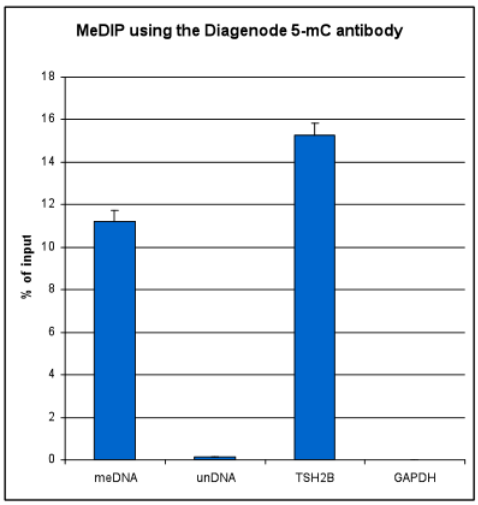

Figure 2. MeDIP results obtained with the Diagenode monoclonal antibody directed against 5-mC
MeDIP (Methylated DNA immunoprecipitation) was performed on 1 µg fragmented human genomic DNA using 0.2 µg of the Diagenode monoclonal antibody against 5-mC (cat. No. C15200081) and the MagMeDIP Kit (cat. No. C02010021). The fragmented DNA was spiked with the internal controls present in the kit (methylated DNA (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 2 shows the recovery expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).

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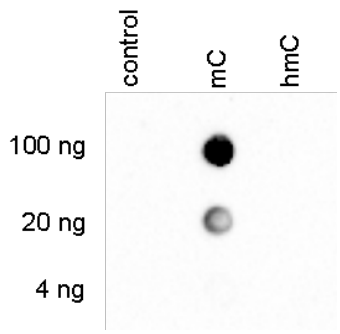

Figure 3. Dot blot analysis using the Diagenode monoclonal antibody directed against 5-mC

To demonstrate the specificity of the Diagenode antibody against 5-mC (cat. No. C15200081), 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. C02040010). One hundred to 4 ng (equivalent of 5 to 0.2 pmol of C-bases) of the controls were spotted on a membrane. Figure 3 shows a high specificity of the antibody for the methylated control.

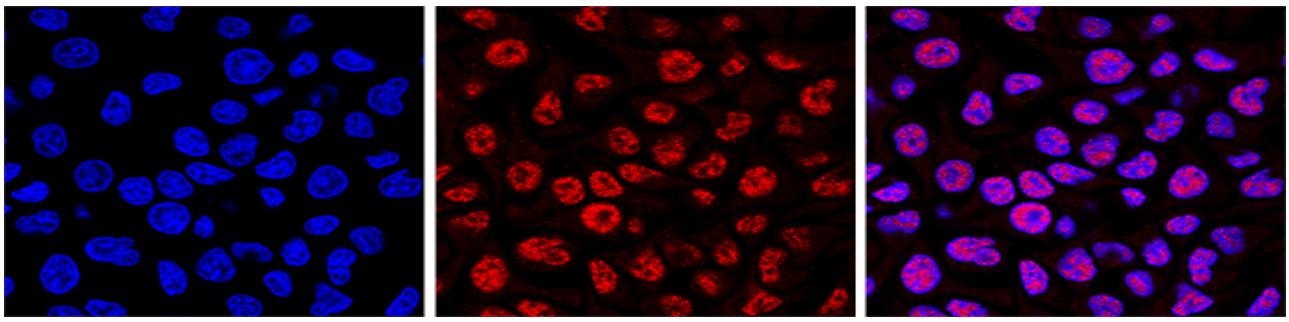

Figure 4. Immunofluorescence using the Diagenode monoclonal antibody directed against 5-mC

HeLa cells were stained with the Diagenode antibody against 5-mC (Cat. No. C15200081) and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/TX-100 containing 1% BSA. The cells were immunofluorescently labelled with the 5-mC antibody (middle) diluted 1:500 in blocking solution followed by an anti-mouse antibody conjugated to Alexa594. The left panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.

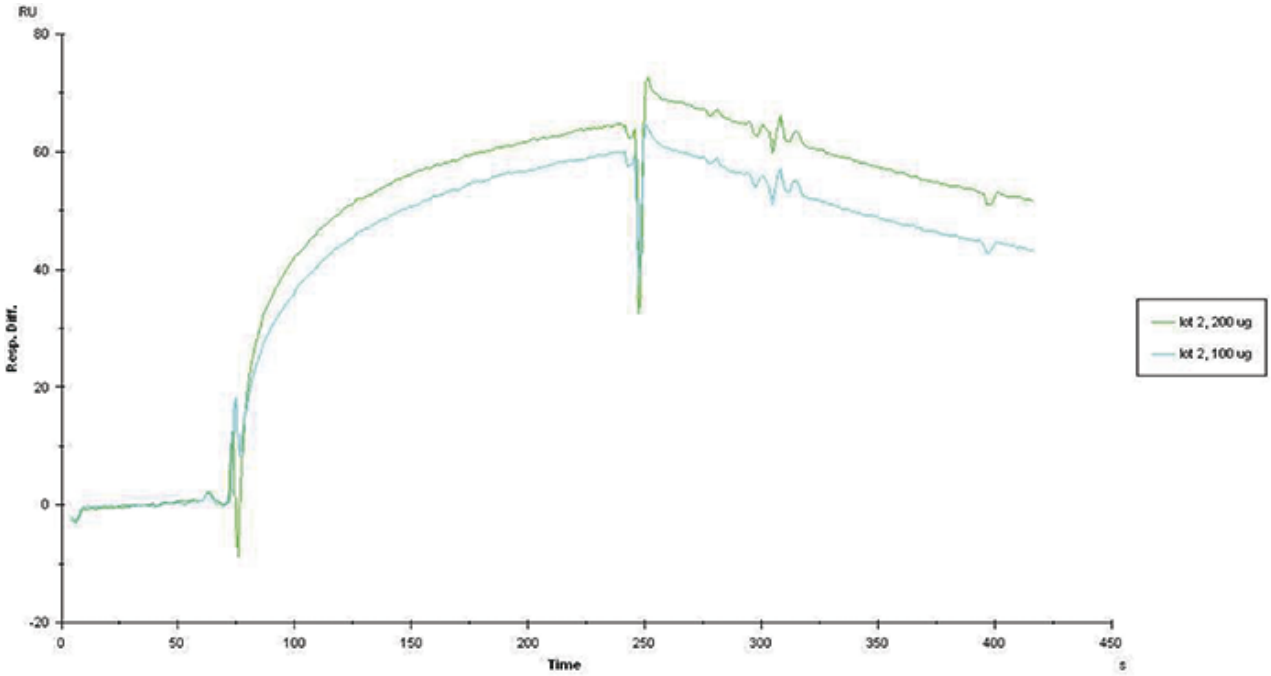

Figure 5. Surface plasmon resonance (SPR) analysis of the the Diagenode monoclonal antibody directed against 5-mC

A synthesized biotin-labeled 5-mC conjugate was immobilized on a CM4 BIAcore sensorchip (GE Healthcare, France). Briefly, two flowcells were prepared by sequential injections of EDC/NHS, streptavidin, and ethanolamine. One of these flowcells served as negative control (biotinylated spacer without 5-mC), while biotinylated 5-mC conjugate was injected in the other one, to get an immobilization level of 55 response units (RU). All SPR experiments were performed, using HBS-N buffer (10 mM HEPES,150 mM NaCl, pH 7.4), at a flow rate of 5 µl/min. Interaction assays involved injections of 2 different dilutions of the Diagenode 5-mC monoclonal antibody (Cat. No. C15200081) over the biotinylated 5-mC conjugate and negative control surfaces, followed by a 3 min washing step with HBS-N buffer to allow dissociation of the complexes formed. At the end of each cycle, the streptavidin surface was regenerated by injection of 0.1M citric acid (pH=3).

The sensorgrams correspond to the biotinylated 5-mC conjugate surface signal subtracted with the negative control. Data from the sensorgrams that reached binding equilibrium were used for Scatchard analysis. The value of the dissociation constant (kd) obtained by global fitting and 1:1 Langmuir model is 65 nM.