

5-mC antibody (33D3)

Cat. No. C15200081

Lot:	RD-007
Size:	10 µg / 100 µg / 500 µg
Type:	Monoclonal
Isotype:	IgG1
Source:	Mouse
Concentration:	1.2 µg/µl

Specificity:	Human, mouse, rat, cow, other (wide range): positive
Purity:	Protein A purified monoclonal antibody
Storage buffer:	PBS containing 0.05% azide

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

Applications

Applications	Suggested dilution	References
MeDIP/MeDIP-seq*	0.1 – 2 µg per IP	Fig 1, 2
Dot blotting	1:300	Fig 3
Immunofluorescence	1:500	Fig 4

*Please note that the optimal antibody amount per IP should be determined by the end-user. We recommend testing 0.1-5 µg per IP.

**Dot blot was only performed to demonstrate the specificity. This antibody is not recommended for dot blot on biological samples

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Results

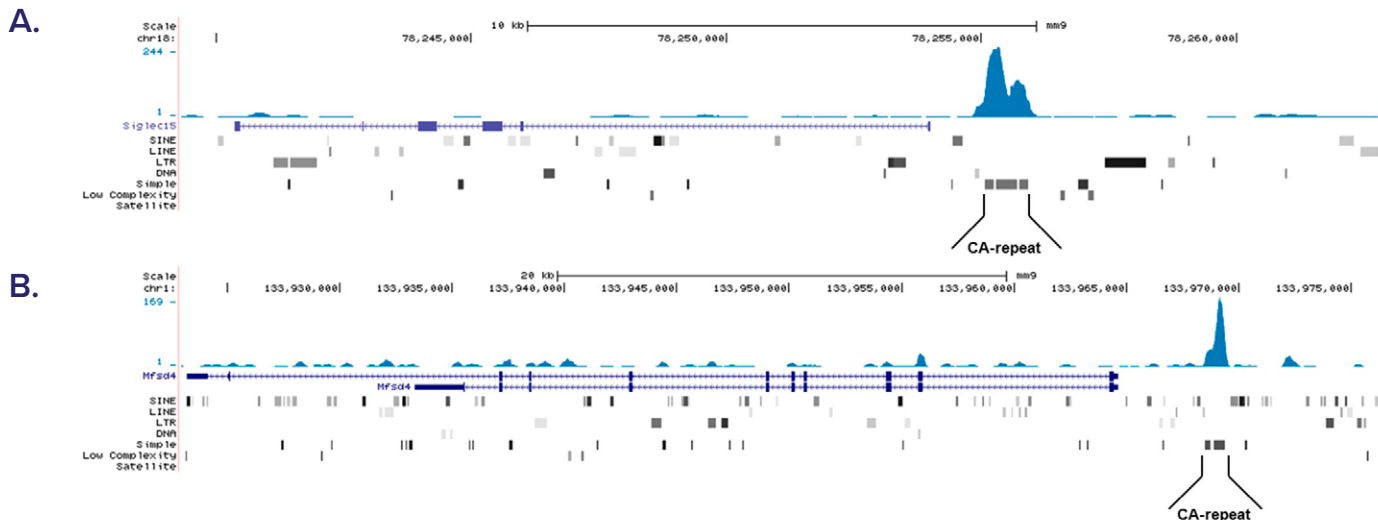


Figure 1: MeDIP-seq with the Hologic Diagenode monoclonal antibody directed against 5-mC

MeDIP was performed on 1 µg sheared genomic DNA using the Hologic Diagenode MagMeDIP kit (cat. No. C02010021) and 0.2 µg of the Hologic Diagenode monoclonal against 5-mC (Cat. No. C15200081). The libraries were subsequently analysed on an Illumina NovaSeq sequencer (2x75 paired-end reads) according to the manufacturer's instructions. The tags were aligned to the human genome (hg19) using the BWA algorithm. 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 Hologic Diagenode 5-mC monoclonal antibody.

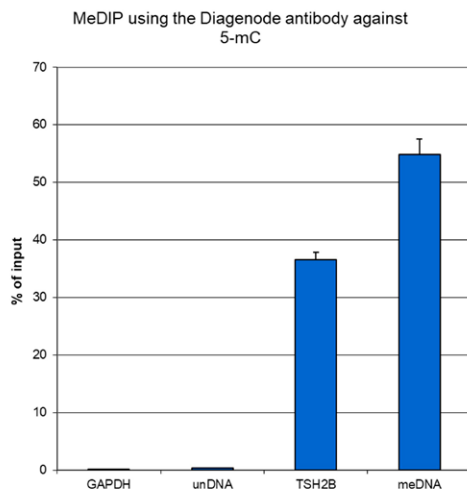


Figure 2: MeDIP results obtained with the Hologic Diagenode monoclonal antibody directed against 5-mC

MeDIP (Methylated DNA immunoprecipitation) was performed on 1 µg fragmented human genomic DNA using 0.2 µg of the Hologic 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).

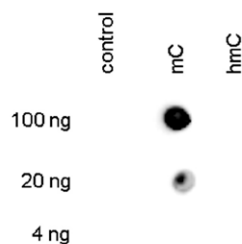


Figure 3: Dot blot analysis using the Hologic Diagenode monoclonal antibody directed against 5-mC

To demonstrate the specificity of the Hologic Diagenode antibody against 5-mC (cat. No. C15200081), a Dot blot analysis was performed using the hmC, mC and C controls from the Hologic 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. The antibody was used at a dilution of 1:300. Figure 3 shows a high specificity of the antibody for the methylated control.

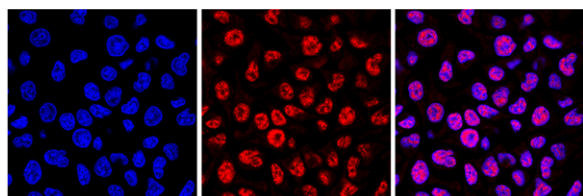


Figure 4: Immunofluorescence using the Hologic Diagenode monoclonal antibody directed against 5-mC

HeLa cells were stained with the Hologic 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.