diagenode
A Hologic Company

TECHNICAL DATASHEET

5-methylcytosine (5-mC) antibody - clone 33D3

Cat. No. C15200081

Type: Monoclonal, **MeDIP/MeDIP-seq grade**

Isotype: IgG1

Source: Mouse

Lot: RD-006

Size: 10 µg
50 µg
500 µg

Concentration: 1.1 µg/µl

Specificity: Human, mouse, rat, cow, other (wide range): positive.

Purity: Protein A purified monoclonal antibody.

Storage: Store at -20°C; for long storage, store at -80°C. Avoid multiple freeze-thaw cycles.

Storage buffer: PBS containing 0.05% azide.

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.

Diagenode sa. BELGIUM | EUROPE
LIEGE SCIENCE PARK
Rue du Bois Saint-Jean, 3
4102 Seraing - Belgium
Tel: +32 4 364 20 50
Fax: +32 4 364 20 51
orders@diagenode.com
info@diagenode.com

Diagenode LLC. USA | NORTH AMERICA
400 Morris Avenue, Suite 101
Denville, NJ 07834 - USA
Tel: +1 862 209-4680
Fax: +1 862 209-4681
orders.na@diagenode.com
info.na@diagenode.com

Last update: May, 2023

Results

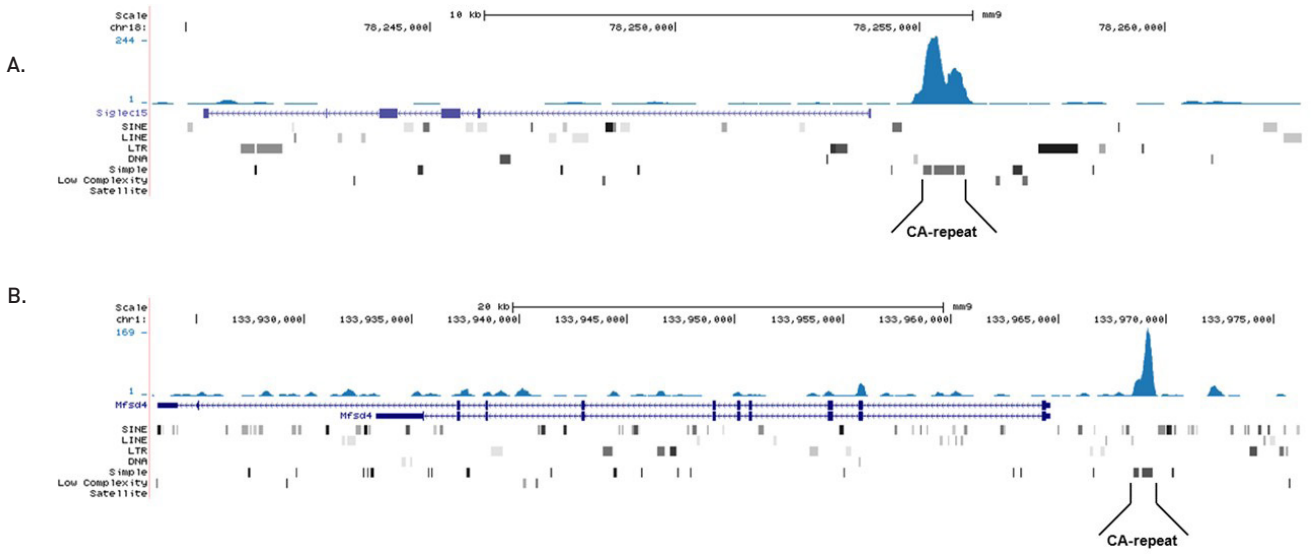

Figure 1. MeDIP-seq with the Diagenode monoclonal antibody directed against 5-mC

MeDIP was performed on 1 µg sheared genomic DNA using the Diagenode MagMeDIP kit (cat. No. C02010021) and 0.2 µg of the 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 Diagenode 5-mC monoclonal antibody.

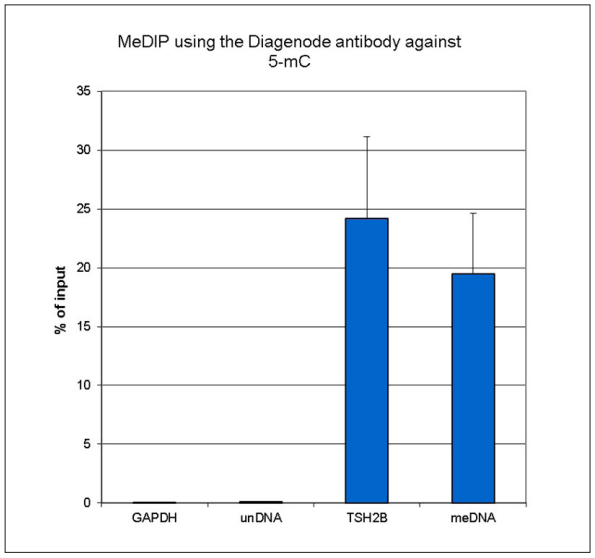

Figure 2. MeDIP results obtained with the Diagenode monoclonal antibody directed against 5-mC

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).

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. The antibody was used at a dilution of 1:300. Figure 3 shows a high specificity of the antibody for the methylated control.

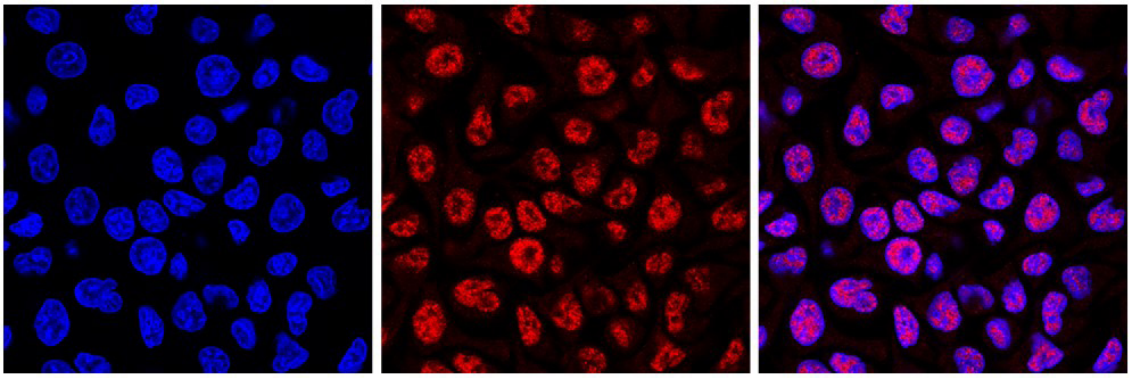

Figure 4. Immunofluorescence using the Diagenode monoclonal antibody directed against 5-mC

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.