

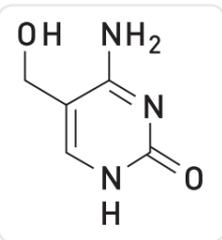
# Exclusive Highly-Specific Kits and Antibodies for DNA Hydroxy-Methylation studies

diagenode

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## Introduction



5-hydroxymethylcytosine (5-hmC) has been recently discovered in mammalian DNA by two US groups (Kriaucionis & Heintz, Science, 2009 and Tahiliani et al., Science, 2009), however, its precise function has not yet been elucidated. This cytosine modification results from the enzymatic conversion of 5-methylcytosine into 5-hydroxymethylcytosine by the TET family of oxygenases. 5-hydroxymethylcytosine may represent a new pathway to demethylate DNA involving a repair mechanism converting hmC to C and, as such open up entirely new perspectives in epigenetic studies. Since its discovery in neuronal Purkinje, granule and ES cells,

studies of this new modified DNA base have been limited by the lack of high quality, validated tools and technologies that discriminate hydroxymethylation from methylation in regulating genome expression. Obtaining a specific assay for 5-hmC is particularly important since standard methods (eg. bisulfite sequencing) cannot distinguish between these two types of methylation.

## Methods

We have developed hMeDIP, MeDIP and dual MeDIP kits for the differential study of the functions of 5-hm, 5-m, and unmodified Cytosines.

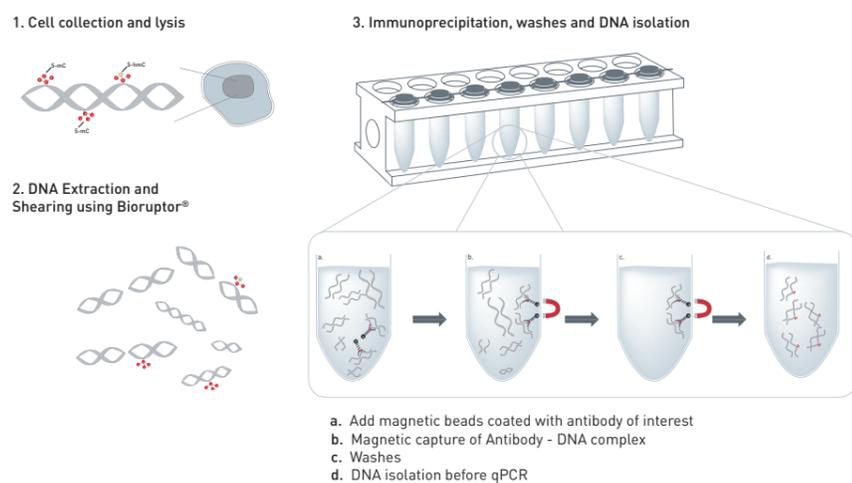


Figure 1. Principle of the MeDIP and hMeDIP assays

Genomic DNA from cultured cells or tissues is prepared, sheared, and then denatured (1. and 2.). Immunoselection and immunoprecipitation can take place using the antibody directed against 5 methylcytosine or 5-hydroxymethylcytosine and antibody binding magnetic beads. After isolation and purification is performed, the IP'd DNA is ready for subsequent analysis such as qPCR, amplification, hybridization on micro-arrays or next generation sequencing.

### Principle of the Dual MeDIP assay

Dual MeDIP is a 2-step immunocapture approach developed by Diagenode to selectively enrich hydroxymethylated or methylated DNA. Genomic DNA is randomly sheared by sonication, then immunoprecipitated sequentially – first with an antibody that specifically recognizes 5-hmC, and then with an antibody

## Results

These affinity-based technologies appear to be the most powerful way to differentially and specifically enrich 5-mC and 5-hmC sequences. Our monoclonal (mouse and rat) and polyclonal (rabbit) antibodies for 5-hmC have been extensively validated using dotblots, immunofluorescence, hMeDIP, as well as hMeDIP followed by next-gen sequencing.

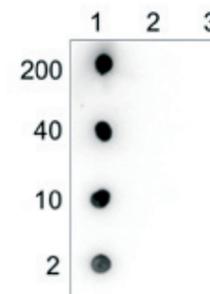


Figure 2. Dot blot analysis of the Diagenode 5-hmC mouse monoclonal antibody with the C, mC and hmC PCR controls.

200 to 2 ng [equivalent of 10 to 0.1 pmol of C-bases] of the hmC (1), mC (2) and C (3) PCR controls from the Diagenode "5-hmC, 5-mC & cytosine DNA Standard Pack" (Cat. No. AF-101-0020) were spotted on a membrane (Amersham Hybond-N+). The membrane was incubated with 2 µg/ml of the mouse 5-hydroxymethylcytosine monoclonal antibody (dilution 1:500). The membranes were exposed for 30 seconds.

Our new hMeDIP kit includes a hmc antibody, and positive (ie, fully hydroxy-methylated) and negative (ie, both unmethylated and fullymethylated) DNA controls.

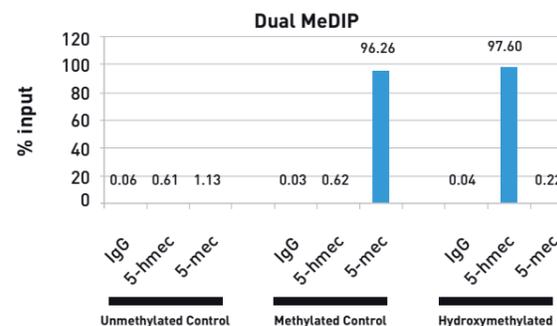


Figure 3. Dual DNA IP results obtained with Diagenode's hMeDIP kit and antibodies against methylated and hydroxymethylated C.

A methylated DNA IP (MagMeDIP) was performed on the unbound fraction of the hydroxymethylated DNA IP (hMeDIP) assays, using the Diagenode antibody directed against 5-hydroxymethylcytosine and 5 methylcytosine. IgG isotype antibody from rat (Cat. No. AF-105-0025) was used as a negative control.

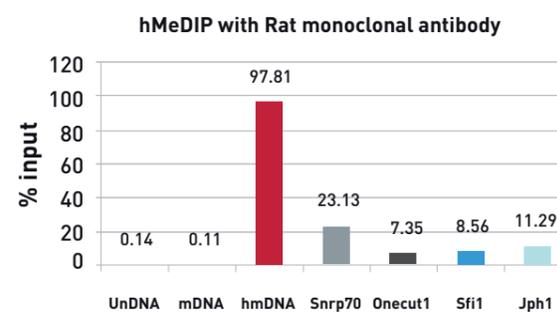


Figure 4. Hydroxymethylated DNA IP results obtained with our hMeDIP kit (Cat. No. AF-104-0016).

Hydroxymethylated DNA IP (hMeDIP) assays were performed using the Diagenode hMeDIP kit. This kit includes: the monoclonal rat antibody against 5-hydroxymethylcytosine, 5-hmC, 5mC & cytosine DNA standards Rat IgG (Cat. No. AF-105-0025). 1 µg of mouse ES cells DNA was spiked with each DNA standard. Snrp70, Onecut1, Sfi1 and Jph1 are hydroxymethylated locus from the mouse DNA sample.

*In situ* cellular staining on interphase ES cells indicates distinct 5-hmC staining, both in overall brightness and in binding patterns, from that obtained with the Diagenode 5-mC antibody clone 33D3.

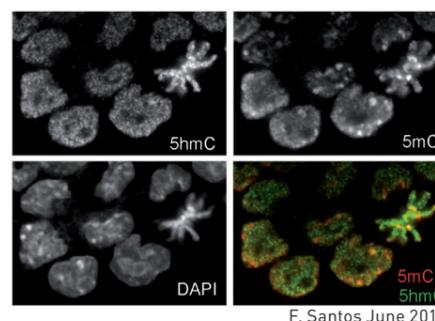


Figure 5. Indirect immunofluorescence results obtained with the antibody directed against 5-methylcytosine and 5-hydroxymethylcytosine from Diagenode.

J1 ES cells were stained with 5-methylcytosine mouse monoclonal antibody (MAB), the monoclonal rat antibody against 5-hydroxymethylcytosine and nuclei were counterstained with DAPI.

Our data are consistent with distinct roles of 5-hmC and 5-mC; our new kits and antibody open the door to novel epigenetics studies, and to clarifying the role of 5-hmC in differentiation, displacing methyl-binding proteins, regulating DNA repair, recruiting chromatin modifiers, and other important functions.

## Conclusions

In conclusion, we present the most complete range of antibodies against 5-hydroxymethylcytosine that have been demonstrated to be highly specific (no cross-reactivity with C- or mC- containing fragments) with our « dual MeDIP » and hMeDIP assays as well as dotblots and that outperform other antibodies on the market. We have developed an efficient test system that can be used to assess the performance of the antibody, and to compare hydroxymethylated and standard methylated DNA distributions. We expect that our assays and antibodies will represent the first tools for evaluating the role of differential methylation in the genome. These tools should be relevant and useful in any cell type.

## Ordering information

Description	Cat. No.	Format
5-hmC monoclonal antibody (rat)	MAB-633HMC-050/100	50 µg or 100 µg
5-hmC monoclonal antibody (mouse)	MAB-31HMC-050/100	50 µg or 100 µg
5-hmC polyclonal antibody (rabbit)	CS-HMC-100	100 µl
5-mC monoclonal antibody clone 33D3 (mouse)	Mab-081-100/500	100 µg or 500 µg
hMeDIP kit x 16 (rat) (for mouse and rabbit antibodies also available)	AF-104-0016	16 rxns

