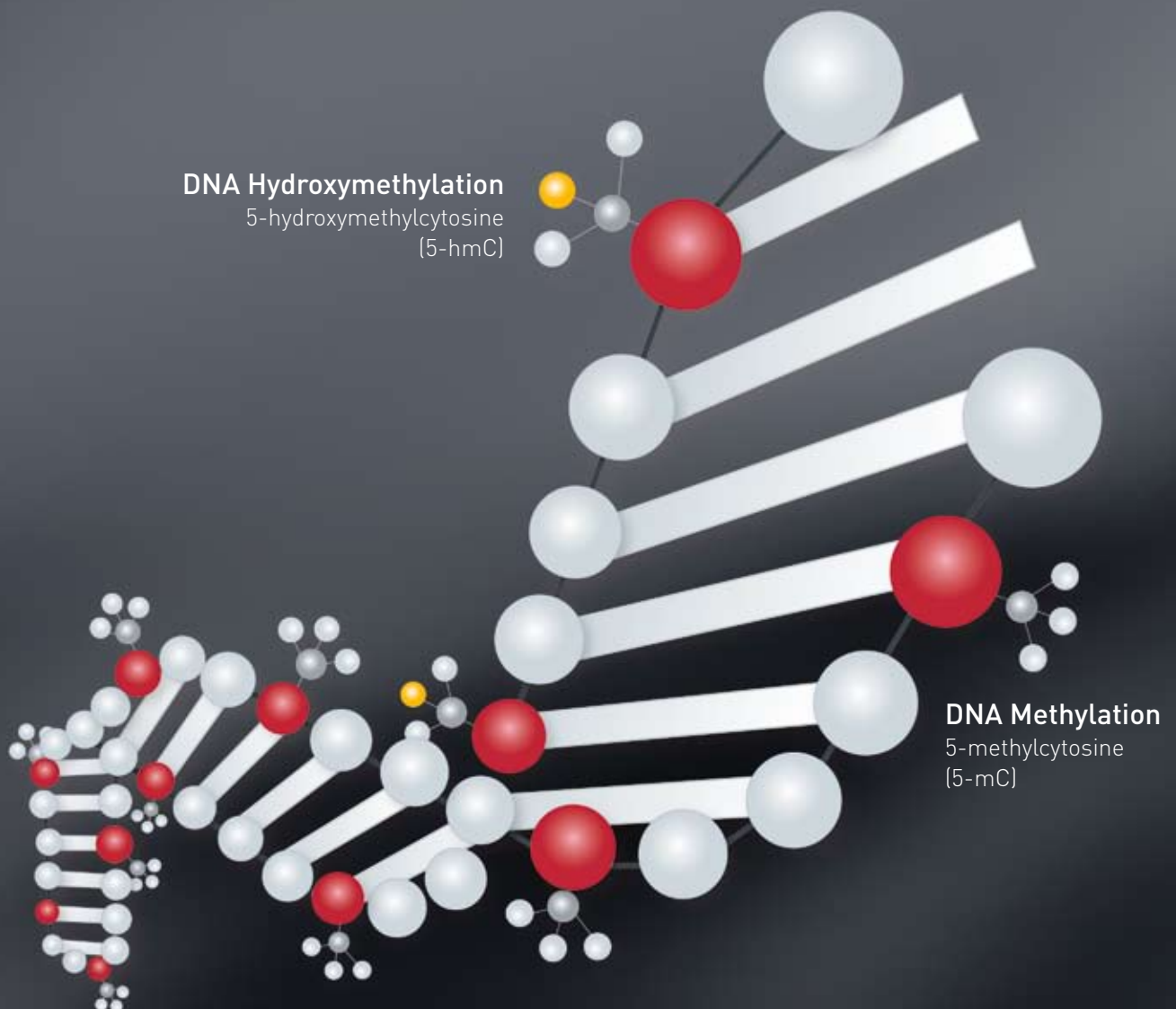


# DNA Methylation Solutions

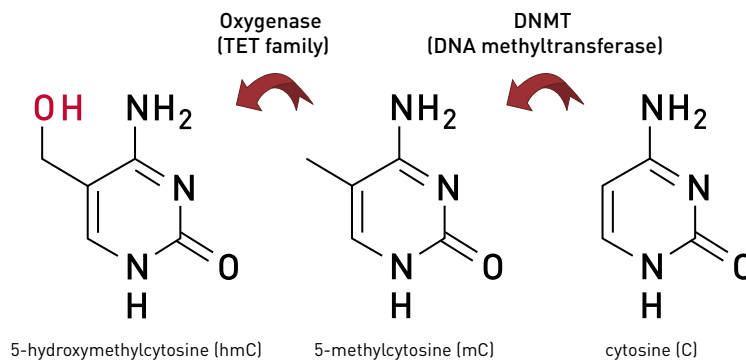
A complete range of quality products for all DNA methylation studies



# Consistent & Straightforward DNA Methylation Analysis

## Choosing your DNA methylation technique

Diagenode offers sonication instruments, reagent kits, high quality antibodies, and high-throughput automation capability to address all of your specific DNA methylation analysis requirements. Our methylated and hydroxymethylated DNA immunoprecipitation (**MeDIP** & **hMeDIP**) product line of full solution kits and high performance antibodies allow cost-effective and reliable genome-wide DNA methylation analysis. Diagenode's **MagMeDIP** and **hMeDIP** kits use a robust, highly sensitive **5-methylcytosine (33D3)** and **5-hydroxymethylcytosine** specific antibodies for enrichment of methylated and hydroxymethylated sites. Alternatively, our **MethylCap**<sup>®</sup> kit, based on the high affinity of methyl binding domain (MBD) proteins towards methylated DNA, can be used. Our **MagBisulfite** kit utilizes bisulfite conversion, a chemical method that converts all unmethylated cytosine to uracil. This method enables comparison of bisulfite-converted DNA to untreated DNA and thus the determination of which cytosines were methylated in the original sample, allowing analysis at the nucleotide level. **MeDIP**, **hMeDIP**, **MethylCap**<sup>®</sup> and **MagBisulfite** kits are available in 'AUTO' formats for the **IP-Star**<sup>®</sup> **Automated System** which facilitates large-scale DNA methylation studies.



### Understanding DNA Methylation & Hydroxymethylation

One of the fastest growing fields in biology and cancer research is epigenetics. While the underlying genetic code defines which proteins and gene products are synthesized, it is epigenetic control that defines when and where they are expressed. Epigenetic control is often mediated by methylation of cytosine to **5-methylcytosine (5-mC)** in CpG islands and post-translational modification of histones. Methylation of CpGs near promoters is associated with gene silencing, as is deacetylation of histones.

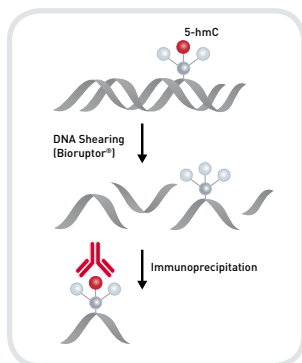
Furthermore, there is substantial interest and speculation in the role of the «sixth DNA base», **5-hydroxymethylcytosine (5-hmC)**, although its precise function has not yet been elucidated. Recent articles demonstrated that the most common approaches (eg. enzymatic approaches, bisulfite sequencing) do not account for 5-hmC (Huang et al., 2010 ; Jin et al., 2010 ; Nestor et al., 2010). Another factor that makes hmC difficult to study is its relatively low abundance. Therefore a full reappraisal of the biological significance of hmC will depend heavily on the tools and methods that allow hmC, 5-mC and C to be distinguished unequivocally and efficiently. The development of the affinity-based technologies (hMeDIP & MeDIP) appear to be the most powerful way and so far the only way to differentially and specifically enrich 5-mC and 5-hmC sequences from complex sources such as genomic DNA.

#### Customer feedback

I was very happy to discover the Diagenode MeDIP kit. After many months of trying to optimise an in-house protocol that was not giving reliable results, it was a joy to come across a method that includes a positive and negative control in every tube. The kit worked without any problems and the customer service has been stellar. Highly recommended to anyone embarking on MeDIP!

Noémi Roy, University of Oxford, United Kingdom

# DNA Hydroxymethylation Products

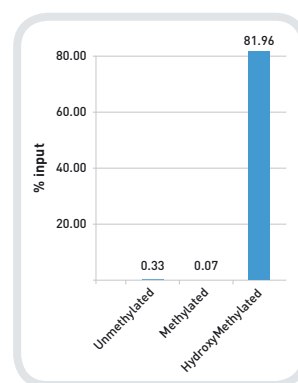


## hMeDIP kits

- Immunoprecipitation with 5-hydroxymethylcytosine (5-hmC) antibody
- Wide range of monoclonal & polyclonal antibodies available
- Optimal for genome-wide screening of hydroxymethylated CpG regions
- Generation of highly consistent results with internal and external controls
- Simple and fast protocol

Figure 1. hMeDIP results

hMeDIP was performed using the hMeDIP kit (mouse monoclonal antibody). The IgG isotype antibody from mouse was used as negative control. 1 µg of HeLa cells DNA was prepared and sonicated with the Bioruptor® to obtain DNA fragments of 300-500 bp. Unmethylated, methylated and hydroxymethylated spike-in controls have been used. Finally qPCR using specific primer pairs for the unmethylated, methylated and hydroxymethylated DNA sequences has been performed.



## Antibodies against 5-hydroxymethylcytosine

- Highly specific
- Extensively validated
- Wide range of monoclonal & polyclonal antibodies available

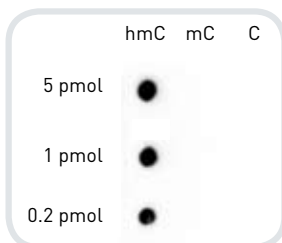


Figure 2. Dot blot analysis using the Diagenode polyclonal antibody directed against 5-hmC

To demonstrate the specificity of the Diagenode antibody against 5-hmC (Cat. No. pAb-HMC-050), 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 1:1,000. Figure 2 shows a high specificity of the antibody for the hydroxymethylated control.

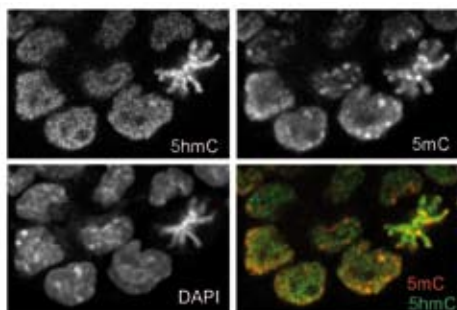


Figure 3. Immunofluorescence results obtained with Diagenode's 5-hmC (monoclonal rat) and 5-mC (monoclonal mouse) antibodies

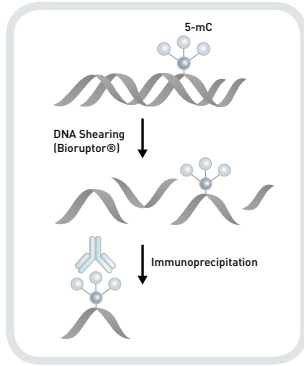
Mouse ES cells and primary embryonic mouse fibroblasts were grown on coverslips. After fixation in PFA and washing in PBS, cells were depurinated and blocked in PBS-Tween-BSA buffer. Cells were then incubated with the Diagenode's 5-mC monoclonal mouse antibody (clone 33D3, MAb-081-100; MAb-081-500) or with the Diagenode's 5-hmC monoclonal rat antibody (MAb-633HMC-100; MAb-633HMC-050). Fluorescent secondary antibodies specific for each mab were used to reveal the specific DNA staining.

NEW

### hMeDIP-seq reference paper

Hume Stroud, Suhua Feng, Shannon Morey Kinney, Sriharsa Pradhan & Steven E. Jacobsen. 5-hydroxymethylcytosine is associated with enhancers and gene bodies in human embryonic stem cells. *Genome Biology*, 12:R54, 2011.

# DNA Methylation Products

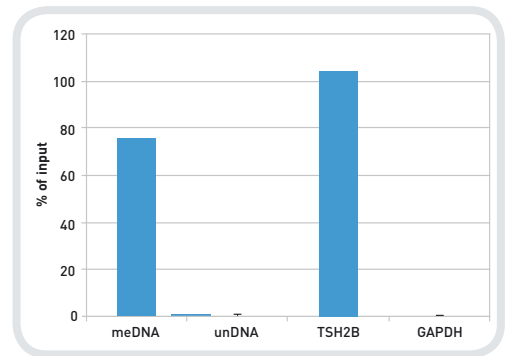


## MeDIP kits

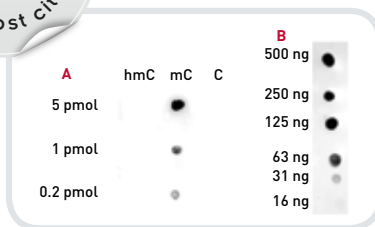
- Immunoprecipitation with the most sensitive & published anti-5-mC antibody – **clone 33D3**
- Optimal for genome-wide screening of methylated CpGs
- Generation of highly consistent results with internal and external controls in 24h
- Minimal error with all reagents in 1 tube

**Figure 4. MeDIP results obtained with Diagenode's 5-mC antibody (33D3)**

MeDIP (Methylated DNA immunoprecipitation) was performed on fragmented genomic DNA from U2OS cells using the Diagenode monoclonal antibody against 5-mC (clone 33D3, Cat. No. MAb-081-100/500) and the MagMeDIP Kit (Cat. No. mc-magme-048). The fragmented DNA was spiked with internal controls prior to performing the IP. QPCR was performed using 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.



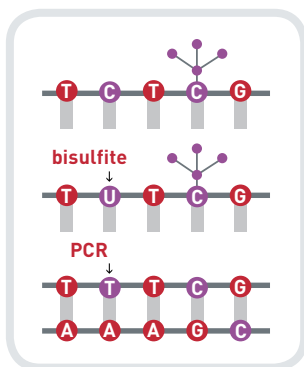
## Antibody against 5-methylcytosine (clone 33D3)



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

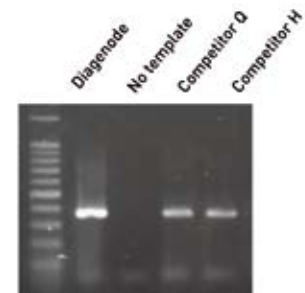
**Figure 5A:** Dot blot analysis was performed using "5-hmC, 5-mC & cytosine DNA Standard Pack" (Cat. No. AF-101-0002). One hundred to 4 ng (equivalent to 5 to 0.2 pmol of C-bases) of the controls were spotted on a membrane (Amersham Hybond-N+). The antibody was used at a dilution of 1:250. Figure 5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

**Figure 5B:** Dot blot was performed as described above on decreasing amounts of DNA isolated from phage XP12.



## Bisulfite conversion kits

- Complete conversion in **3.5 hours**
- Magnetic bead - based purification provides **maximum yield** of converted DNA
- High sensitivity - Down to 1 ng
- Conversion efficiency > 99%

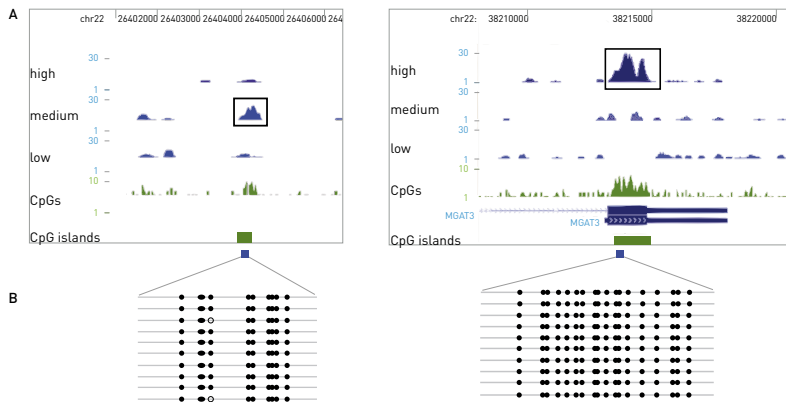


**Figure 6. Highest recovery obtained with the MagBisulfite kit**

Comparison of DNA recovery using the MagBisulfite kit and kits from competitor Q and H. CTS is a highly methylated region in MCF cells.

## MethylCap<sup>®</sup> kit

- Affinity purification of methylated DNA with Methylbinding domain (MBD) protein
- Allows for fractionation of methylated DNA by CpG density
- Compatible with next generation sequencing
- Magnetic beads allow for fast and sensitive capture of methylated DNA
- Includes control primer pairs for the assessment of the capture efficiency



**Figure 7.** Using MethylCap-seq, two methylated regions were detected in different elution fractions according to their methylated CpG density (A). Low, Medium and High refer to the sequenced DNA from different elution fractions with increasing salt concentration. Methylated patterns of these two different methylated regions were validated by bisulfite conversion assay (B).

Data provided by Henk Stunnenberg (Nijmegen Center for Molecular Life Sciences - The Netherlands)

### Reference papers

Arie B. Brinkman, Femke Simmer, Kelong Ma, Anita Kaan, Jingde Zhu, Hendrik G. Stunnenberg. Whole-genome DNA methylation profiling using MethylCap-seq. *Methods* 52, 232–236, 2010.

Christoph Bock, Eleni M Tomazou, Arie B Brinkman, Fabian Müller, Femke Simmer, Hongcang Gu, Natalie Jäger, Andreas Gnirke, Hendrik G Stunnenberg & Alexander Meissner. Quantitative comparison of genome-wide DNA methylation mapping technologies. *Nature Biotechnology*, 28, 1106–1114, 2010.

Joost H.A. Martens, Arie B. Brinkman, Femke Simmer, Kees-Jan Francoijs, Angela Nebbioso, Felicetto Ferrara, Lucia Altucci, and Hendrik G. Stunnenberg. PML-RARA/RXR Alters the Epigenetic Landscape in Acute Promyelocytic Leukemia. *Cancer Cell* 17, 173–185, February 17, 2010.



## SX-8G IP-Star<sup>®</sup> Compact Automated System

- Automation of MeDIP, hMeDIP, MethylCap & MagBisulfite assays
- Unparalleled reproducibility & productivity
- Huge time savings
- Simple operating procedure

All Diagenode DNA methylation assays have been validated on the IP-Star<sup>®</sup> Automated System

Antibodies	Cat. No.	Format
5-hmC monoclonal antibody (rat)	MAB-633HMC-050	50 µg/32 µl
5-hmC monoclonal antibody (rat)	MAB-633HMC-100	100 µg/64 µl
5-hmC monoclonal antibody (mouse)	MAB-31HMC-020	20 µg/20 µl
5-hmC monoclonal antibody (mouse)	MAB-31HMC-050	50 µg/50 µl
5-hmC monoclonal antibody (mouse)	MAB-31HMC-100	100 µg/100 µl
5-hmC polyclonal antibody (rabbit)	pAb-HMC-020	20 µg/20 µl
5-hmC polyclonal antibody (rabbit)	pAb-HMC-050	50 µg/50 µl
5-hmC polyclonal antibody (rabbit)	CS-HMC-020	20 µl
5-hmC polyclonal antibody (rabbit)	CS-HMC-100	100 µl
5-mC monoclonal antibody 33D3	MAB-081-100	100 µg/100 µl
5-mC monoclonal antibody 33D3	MAB-081-500	500 µg/500 µl
5-mC monoclonal antibody cl. b	MAB-006-100	100 µg/100 µl
5-mC monoclonal antibody cl. b	MAB-006-500	500 µg/500 µl

MeDIP & hMeDIP kits	Cat. No.	Format
Auto MeDIP kit x16	AF-Auto01-0016	16 rxns
Auto MeDIP kit x100	AF-Auto01-0100	100 rxns
Auto hMeDIP kit	AF-Auto02-0016	16 rxns
MagMeDIP kit x10	mc-magme-A10	10 rxns
MagMeDIP kit x48	mc-magme-048	48 rxns
MeDIP kit	mc-green-003	10 rxns
hMeDIP kit x16 (monoclonal rat ab.)	AF-104-0016	16 rxns
hMeDIP kit x16 (monoclonal mouse ab.)	AF-110-0016	16 rxns
hMeDIP kit x16 (polyclonal rabbit ab.)	AF-111-0016	16 rxns

DNA Methylation Controls & Primer Pairs	Cat. No.	Format
DNA Methylation control package	EF-100-0040	40 rxns
5-hmC, 5-mC & cytosine DNA standard pack	AF-101-0002	2 µg
5-hmC, 5-mC & cytosine DNA standard pack for hMeDIP	AF-107-0040	40 rxns
Human GAPDH promoter Primer Pairs	pp-1044-050/500	50/500 µl
Mouse GAPDH promoter Primer Pairs	pp-1045-050/500	50/500 µl
Rat GAPDH promoter Primer Pairs	pp-1046-050/500	50/500 µl
Human TSH2B Primer Pairs	pp-1041-050/500	50/500 µl
Mouse TSH2B Primer Pairs	pp-1042-050/500	50/500 µl
Rat TSH2B Primer Pairs	pp-1043-050/500	50/500 µl

DNA purification for epigenetic applications	Cat. No.	Format
Auto IPure kit	AL-Auto01-0100	100 rxns
IPure kit x100	AL-100-0100	100 rxns

MBD (Methyl Binding Domain)	Cat. No.	Format
Auto MethylCap kit x48	AF-Auto01-0048	48 rxns
MethylCap kit x48	AF-100-0048	48 rxns
MethylCap protein	mbd-001-100	50 rxns

Bisulfite conversion kits	Cat. No.	Format
MagBisulfite kit	AF-106-0024	24 rxns
MethylEasy™ kit 25 rxns	MEA-BISLPH-025	100 µg/100 µl
MethylEasy™ kit Xceed 40 rxns	MEA-BISXCE-040	40 rxns

DiaMag magnetic beads	Cat. No.	Format
DiaMag anti-mouse IgG-coated magnetic beads	kch-821-220	220 µl
DiaMag protein A-coated magnetic beads	kch-802-220	220 µl
DiaMag protein G-coated magnetic beads	kch-818-220	220 µl
<b>DiaMag1.5 - magnetic rack &amp; disc stand</b>	kch-716-015	1 unit
DiaMag02 - magnetic rack	kch-816-001	1 unit
DiaMag Rotator	VL-100-0001	1 unit

Bioruptor	Cat No	Format
Bioruptor® Standard	UCD-200 TM	1 unit
Bioruptor® Plus	UCD-300 TM	1 unit
Bioruptor® Twin	UCD-400 TM	1 unit
Bioruptor® XL	UCD-500 TM	1 unit
Bioruptor® NGS	UCD-600 TS	1 unit

IP-Star Automated System	Cat No	Format
SX-8G IP-Star® Automated System	UH-001-0001	1 unit
SX-8G IP-Star® Compact Automated System	UH-002-0001	1 unit

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