DNA METHYLATION

SOLUTIONS TO STUDY DNA METHYLATION:
TARGETED AND GENOME-WIDE

Optimal for NGS, Sanger sequencing, Pyrosequencing & qPCR
Understanding DNA Methylation

DNA methylation plays key roles in numerous biological processes such as genomic imprinting, control of transcription, embryonic development, stem cell reprogramming and carcinogenesis. In mammals, DNA methylation occurs primarily as 5-methylcytosine (5-mC), which is usually found at CpG dinucleotides.

An additional mark, 5-hydroxymethylcytosine (5-hmC), has been implicated with an alternative role as a regulator of gene expression and cellular differentiation as well as an intermediary in the active demethylation process.

Study 5-mC and 5-hmC

**Sodium bisulfite** converts unmethylated cytosine to uracil while methylated cytosines remain unchanged:
- Single nucleotide resolution
- Gene-specific and genome-wide analyses

**Capture methylated/hydroxymethylated DNA** by choosing one of two techniques:
- Antibodies are used to capture methylated or hydroxymethylated DNA
- Methylated DNA-binding proteins (MBD) are used to capture methylated DNA

In both cases, enriched DNA is analyzed with locus-specific PCR or by NGS:
- Gene-specific and genome-wide analyses
- Compatible with degraded DNA or small fragments

Let Us Do Your DNA Methylation Assays for You

Diagenode offers **DNA Methylation Profiling Services** using our different technologies, and we provide you with ready-to-publish data.
Find your DNA methylation analysis solution

Target

Resolution

Level

I want to study

Methylation

Hydroxymethylation

Gene-specific

Single nucleotide

100-500 bp

Gene-specific

Genome-wide

Gene-specific

Genome-wide

100-500 bp

Innovating Epigenetics Solutions
according to your needs

25x10⁶ CpGs coverage

- **Premium WGBS Kit**: Generate bisulfite-converted libraries ready for single or paired-end sequencing of the whole genome
  - C02030034

4x10⁶ CpGs coverage

- **Premium RRBS Kit**: Generate bisulfite-converted libraries ready for single and paired-end sequencing of CpG-rich parts of the genome
  - C02030032

- **Premium Bisulfite Kit**: Bisulfite convert gDNA ready for region-specific analysis like (q)PCR, Sanger/pyrosequencing [SP]
  - C02030030

**MagMeDIP-seq Package**

- **MagMeDIP-qPCR**: From gDNA extraction to library preparation - prepare ready-to-sequence libraries of immunoprecipitated methylated DNA
  - C02010040

- **MagMeDIP qPCR**: Prepare libraries of captured methylated DNA combining MBD technology with the MicroPlex Library Prep Kit
  - C02020010  C05010014

**MethylCap Kit + MicroPlex**

- **MethylCap Kit**: Precipitate methylated DNA with a specific antibody
  - C02010020

- **MethylCap Kit**: Capture methylated DNA via methyl-binding domain (MBD) of human MeCP2 protein
  - C02020010

- **hMeDIP Kit**: Precipitate hydroxymethylated DNA with a specific antibody
  - C02010031
MagMeDIP qPCR/hMeDIP or MagMeDIP-seq

Perform DNA immunoprecipitation (-sequencing) to estimate the DNA modification status of your sample using the 5-methylcytosine antibody (5-mC) or 5-hydroxymethylcytosine antibody (5-hmC).

**STEP 1**
Lyse cells and extract DNA

**STEP 2**
DNA shearing

**STEP 3**
Prepare library for MeDIP-seq

**STEP 4 & 6**
Immunoprecipitate, wash and isolate DNA

4 qPCR
5 PCR amplification
6 Next-Generation Sequencing
<table>
<thead>
<tr>
<th>AUTO EQUIPMENT NEEDS</th>
<th>WHICH KIT FOR WHICH STEP?</th>
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<tbody>
<tr>
<td>IP-Star® Compact Automated System B03020002</td>
<td>✓</td>
</tr>
<tr>
<td>Bioruptor® B01020001 B01060010</td>
<td>✓</td>
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<tr>
<td>Magnetic rack DiaMag02 B04000001</td>
<td>✓</td>
</tr>
<tr>
<td>XL GenDNA Extraction Module C03030020</td>
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<tr>
<td>iDeal Library Preparation Kit C05010020</td>
<td>✓</td>
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<tr>
<td>DNA isolation with iPure Kit v2 C03010014</td>
<td>✓</td>
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<tr>
<td>hMeDIP Kit C02010031</td>
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<tr>
<td>MagMeDIP-qPCR Kit C02010020</td>
<td>✓</td>
</tr>
<tr>
<td>MagMeDIP-seq Package C02010040</td>
<td>✓</td>
</tr>
</tbody>
</table>

**STEP 1**
Lyse cells and extract DNA

**STEP 2**
DNA shearing

**STEP 3**
Prepare library for MeDIP-seq

**STEP 4**
Immunoprecipitate, wash and isolate DNA

**STEP 5 & 6**
120 130 50 100 150 200 250 300 350 400 450
12 34 56 78 91 01 11 21 31 41 51 61 71 81 92 02 12 22 32 42 52 62 72 82 93 03 13 23 33 43 53 63 73 83 94 04 14 24 34 44 5
5-6 4 4 5-6
Your Full MeDIP-seq Solution

The MagMeDIP-seq Package, our complete solution, includes MagMeDIP qPCR, IPure, iDeal Library prep and an optimized MeDIP-seq protocol to offer the highest accuracy, reliability and performance.

- Start-to-finish: Complete kits include DNA extraction module, highly validated antibody 33D3, spike-in controls and qPCR primer pairs for control regions
- Easy to use with user-friendly magnetic beads and racks
- Validated protocols provide reproducible and consistent data - using as low as 100 ng input DNA
- Highest quality purification with IPure
- Validated shearing protocol with the Bioruptor
- Suitable for all species

Species and sample possibilities - no limit

The possibilities are limitless

- SPECIES: Human, Animals, Plants
- SAMPLES: Blood, Stem cells, Tissue

Immunoprecipitation kit
C02010040 - MagMeDIP-seq Package - 10 rxns
Validated MagMeDIP assures results

High specificity of the MagMeDIP-seq Package.
The coverage profiles on the control regions of one human blood sample show excellent specificity of results.

(A) No methylation detected in the negative control region.

(B) High methylation detected in the positive control region.

Excellent coverage across inputs.
MagMeDIP-seq Package shows consistent coverage across a range of DNA inputs, 100 ng, 250 ng, and 1 µg.

The perfect combination for best-in-class results:
magnetic IP technology with ultra-validated antibodies

Diagenode emphasizes strict quality standards and actual validation for all of our antibodies. Our partnerships with the leading epigenetics experts and epigenetics consortiums have allowed us to validate our antibodies to the highest degree, guaranteeing specificity, reproducibility, and success for each and every immunoprecipitation assay.

- Rigorous QC with stringent validation - every lot tested and compared to prior lot
- Accessible and transparent validation data from current lots
- Comprehensive selection
Bisulfite conversion

Bisulfite conversion reagent chemically modifies non-methylated cytosines (C) into uracil (U) while methylated cytosines (5-mC) remain unchanged. For single locus analysis, the region of interest is amplified with PCR which can be followed by Sanger sequencing or pyrosequencing.

- **Rapid** bisulfite conversion of DNA - only 1 hour reaction time
- **Simple** workflow, 3 steps
- **High-yields** of converted DNA for methylation analysis

**Bisulfite conversion based kit**
C02030030 - Premium Bisulfite Kit - 50 rxns
Whole Genome Bisulfite Sequencing (WGBS)

Whole Genome Bisulfite Sequencing (WGBS) is a **single nucleotide resolution** technique that allows the user to study DNA methylation sites and their role in **gene regulation**. Diagenode’s Premium WGBS Kit is designed to prepare **single and paired-end** bisulfite converted DNA libraries for sequencing using Illumina® platforms.

Diagenode’s Premium WGBS Kit contains specially designed enzymes and buffers needed for genome-wide bisulfite sequencing.

- Wide range of DNA inputs: **5 ng - 1 µg**.
- Adaptor sets (6-12-24) available separately for multiplexing
- ChIP-bisulfite-sequencing compatible

Visualization of the alignment bam files on Integrative Genome Viewer (IGV) shows excellent coverage (A) of the whole genome using WGBS and (B) of the CpGs areas using RRBS.

(A) WGBS was performed using the Premium Whole Genome Bisulfite Sequencing (WGBS) kit.
(B) RRBS was performed using the Premium Reduced Representation Bisulfite Sequencing (RRBS) kit. For both, after sequencing, reads were aligned on the mm10 reference genome.
Reduced Representation Bisulfite Sequencing (RRBS)

Methylation typically targets cytosine in a CpG context in vertebrates. In RRBS, by using the restriction enzyme MspI (CCGG target sites) followed by size selection, DNA is enriched to represent CpG-rich regions (including CpG islands). Thus, RRBS is a powerful and cost-effective method to efficiently analyze DNA methylation at the single nucleotide level at approximately 20x lower cost compared to whole genome bisulfite sequencing (WGBS).

- **Excellent coverage** – 4 million CpGs
- **Confidence in results** – Positive and negative spike-in controls to check conversion efficiency
- **Cost-efficient** – Multiplex up to 8 human/mouse samples per sequencing lane on HiSeq 3000
- **High efficiency and minimal bias** – 99.5% bisulfite conversion rate and reduced amplification

**EXPERTS RECOMMEND IT**

"Our lab has used Diagenode’s Premium RRBS kit on rat brain samples. The protocol is understandable, logical, well-written and is easy to follow. I found it fantastic that I could ask questions from the company, and their answers were really useful. We were able to construct a library, which we ran on BioAnalyzer, and the results looked very nice and ready to be sequenced. I would definitely recommend my colleagues to use the Premium RRBS kit from Diagenode."

**Borbála Veto, Institute of Enzymology, Budapest, Hungary**

**Superior coverage.**

Comparison of CpG coverage between competing technologies.

**Bisulfite conversion based kits**

- C02030032 - Premium RRBS Kit - 24 rxns
- C02030033 - Premium RRBS Kit - 96 rxns
Diagenode RRBS SIP – Software for Intelligent Pooling

Pooling techniques allow you to manipulate fewer tubes, providing an easier-to-handle and cost-effective protocol.

Diagenode’s new online intelligent pooling aid provides the optimal pool design for RRBS to meet your specific sample and analysis needs:

- **Time-saving** – Avoid complex calculations
- **Highest pooling efficiency** based on qPCR quantification – Bring the power of pooling to the highest
- **Powerful** – Incorporates advanced aspects such as number of samples per pool required, the separation between projects, and more
- **Accurate** – Identify outliers
**A quick glance: DNA methylation kit features**

<table>
<thead>
<tr>
<th>Features</th>
<th>Resolution</th>
<th>Bisulfite conversion</th>
<th>RRBS</th>
<th>WGBS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Resolution</strong></td>
<td>Single nt</td>
<td>Single nt</td>
<td>Single nt</td>
<td>Single nt</td>
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<tr>
<td><strong>Downstream application</strong></td>
<td>Sanger, Pyroseq, [q]PCR</td>
<td>NGS</td>
<td>NGS</td>
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<tr>
<td><strong>Initial fragment size (bp)</strong></td>
<td>gDNA</td>
<td>gDNA</td>
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<tr>
<td><strong>Input DNA (ng)</strong></td>
<td>0.1-2000</td>
<td>100</td>
<td>5-1000</td>
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<tr>
<td><strong>Turnaround time</strong></td>
<td>1.5-2h</td>
<td>4-5 days</td>
<td>2-3 days</td>
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<td><strong>BS conv. reagent</strong></td>
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<td><strong>All buffers</strong></td>
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<td><strong>Controls</strong></td>
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<td><strong>All library prep reagents</strong></td>
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<td><strong>Indexes</strong></td>
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<td>24</td>
<td>24 included (C05010032 for more)</td>
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<td><strong>Purification</strong></td>
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<td><strong>Automated Kits</strong></td>
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<td><strong>Manual Kits</strong></td>
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<td>C02030032 (24 rxns)</td>
<td>C02030033 (96 rxns)</td>
<td>C02030034 (8 rxns)</td>
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<tr>
<td><strong>SERVICES</strong></td>
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Innovating Epigenetics Solutions
### KITS

<table>
<thead>
<tr>
<th>Hydroxy-/Methylated DNA Immunoprecipitation</th>
<th>MBD based</th>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MagMeDIP qPCR</strong></td>
<td><strong>MagMeDIP-seq Package</strong></td>
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<tr>
<td></td>
<td></td>
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<tr>
<td>100-500 bp</td>
<td>100-500 bp</td>
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<td>qPCR, NGS</td>
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<td>na</td>
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</table>

Separately in iDeal Library prep C05010020

24 included (C05010032 for more)

Separately in iDeal Library prep C05010020

Separately in MicroPlex Library prep C05010013, C05010014

Separately in IPure v2 C03010015 or MicroChIP Diapure C03040001

|   |   |   |   |
| C02010013 [10 rxns] | C02010014 [48 rxns] | na | C02020011 [48 rxns] |
|                       |                       | mAb rat: C02010033 | polyAb rabbit: C02010035 |
|                       |                       | mAb mouse: C02010034 |

|                    |                       | 16 rxns |                   |
|                    |                       | mAb rat: C02010030 |                   |
|                    |                       | mAb mouse: C02010031 |                   |
|                    |                       | polyAb rabbit: C02010032 |                   |

MagMeDIP-seq | hMeDIP-seq

na: not applicable
Shop online in our EpiStore at
www.diagenode.com