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

Hydroxymethylation

I want to study

Methylation

Single nucleotide

100-500 bp

100-500 bp

100-500 bp

Gene-specific

Gene-specific

Genome-wide

Genome-wide

Innovating Epigenetics Solutions
according to your needs

25x10^6 CpGs coverage

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

C02030034

4x10^6 CpGs coverage

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

C02030032

MagMeDIP-seq Package
Bisulfite convert gDNA ready for region-specific analysis like (q)PCR, Sanger/pyrosequencing [SP]

C02030030

MagMeDIP qPCR
MethylCap Kit + MicroPlex
From gDNA extraction to library preparation - prepare ready-to-sequence libraries of immunoprecipitated methylated DNA

C02010040

MethylCap Kit
Prepare libraries of captured methylated DNA combining MBD technology with the MicroPlex Library Prep Kit

C02020010 C05010014

MethylCap Kit
Precipitate methylated DNA with a specific antibody

C02010020

hMeDIP Kit
Capture methylated DNA via methyl-binding domain (MBD) of human MeCP2 protein

C02020010

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
### AUTO EQUIPMENT NEEDS

<table>
<thead>
<tr>
<th>Step</th>
<th>Equipment</th>
<th>Kit Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lyse cells and extract DNA</td>
<td>IP-Star® Compact Automated System B03020002</td>
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<tr>
<td></td>
<td>DNA shearing</td>
<td>Bioruptor® B01020001</td>
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<tr>
<td>2</td>
<td>Prepare library for MeDIP-seq</td>
<td>XL GenDNA Extraction Module C03030020</td>
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<tr>
<td>3</td>
<td>Immunoprecipitate, wash and isolate DNA</td>
<td>iDeal Library Preparation Kit C05010020</td>
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<tr>
<td></td>
<td>ImmunoPCR amplification</td>
<td>DNA isolation with IPure Kit v2 C03100014</td>
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<tr>
<td></td>
<td>Next-Generation Sequencing</td>
<td>hMeDIP Kit C02010031</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MagMeDIP qPCR Kit C02010020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MagMeDIP-seq Package C0201040</td>
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### WHICH KIT FOR WHICH STEP?

<table>
<thead>
<tr>
<th>Step</th>
<th>Kit Code</th>
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<tbody>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<tr>
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<td>4</td>
<td>✓ ✓ ✓</td>
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<tr>
<td>5-6</td>
<td>✓ ✓ ✓</td>
</tr>
<tr>
<td>5-6</td>
<td>✓ ✓ ✓</td>
</tr>
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</table>
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

**SPECIES**
- Human
- Animals
- Plants

**SAMPLES**
- Blood
- Stem cells
- Tissue

The possibilities are limitless

**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

**Bisulfite conversion based kits**
- C0203032 - Premium RRBS Kit - 24 rxns
- C02030033 - Premium RRBS Kit - 96 rxns

Superior coverage.

Comparison of CpG coverage between competing technologies.
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>Premium Bisulfite</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>
</tr>
<tr>
<td><strong>Downstream application</strong></td>
<td>Sanger, Pyroseq, (q)PCR</td>
<td>NGS</td>
<td>NGS</td>
</tr>
<tr>
<td><strong>Initial fragment size (bp)</strong></td>
<td>gDNA</td>
<td>gDNA</td>
<td>100-400</td>
</tr>
<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>✓</td>
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<td><strong>Restriction enzyme</strong></td>
<td>na</td>
<td>✓</td>
<td>na</td>
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<tr>
<td><strong>All buffers</strong></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<tr>
<td><strong>Controls</strong></td>
<td>✓</td>
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<td><strong>All library prep reagents</strong></td>
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<td>✓</td>
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<tr>
<td><strong>Indexes</strong></td>
<td>na</td>
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<td>24 included</td>
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<td>(C05010032 for more)</td>
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<td><strong>Purification</strong></td>
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<td><strong>Beads for IP</strong></td>
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<td><strong>Automated Kits</strong></td>
<td>C02030030 (40 rxns)</td>
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<tr>
<td><strong>Manual Kits</strong></td>
<td>C02030031 (50 rxns)</td>
<td>C02030032 (24 rxns)</td>
<td>C02030033 (96 rxns)</td>
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<td><strong>SERVICES</strong></td>
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### KITS

**Hydroxy-/Methylated DNA Immunoprecipitation**

<table>
<thead>
<tr>
<th>MagMeDIP qPCR</th>
<th>MagMeDIP-seq Package</th>
<th>hMeDIP</th>
<th>MethylCap</th>
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<tbody>
<tr>
<td>100-500 bp</td>
<td>100-500 bp</td>
<td>100-500 bp</td>
<td>100-500 bp</td>
</tr>
<tr>
<td>qPCR, NGS</td>
<td>NGS</td>
<td>qPCR, NGS</td>
<td>qPCR, NGS</td>
</tr>
<tr>
<td>100-600 (200)</td>
<td>200</td>
<td>100-600 (200)</td>
<td>100-600 (200)</td>
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<tr>
<td>100-1000</td>
<td>100-1000</td>
<td>&gt;1000</td>
<td>1000</td>
</tr>
<tr>
<td>2-3 days</td>
<td>4</td>
<td>2-3 days</td>
<td>1-2 days</td>
</tr>
<tr>
<td>na</td>
<td>na</td>
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</tbody>
</table>

- **Separately in iDeal Library prep C05010020**
- Separate in iDeal Library prep C05010020
- Separate in MicroPlex Library prep C05010013, C05010014

- Separately in IPure v2 C03010015 or MicroChIP Diapure C03040001

<table>
<thead>
<tr>
<th>C02010013</th>
<th>C02010014 (48 rxns)</th>
<th>C02010020</th>
<th>C02010021</th>
<th>C02010020 (10 rxns)</th>
<th>C02010021 (48 rxns)</th>
<th>C02010040</th>
<th>C02010040 (10 rxns)</th>
<th>C02020010 (48 rxns)</th>
</tr>
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<tbody>
<tr>
<td>na</td>
<td>mAb rat: C02010033</td>
<td>mAb rat: C02010030</td>
<td>mAb rat: C02010031</td>
<td>mAb rat: C02010032</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>mAb mouse: C02010034</td>
<td>mAb mouse: C02010031</td>
<td>mAb mouse: C02010032</td>
<td>mAb mouse: C02010033</td>
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<tr>
<td></td>
<td>polyAb rabbit: C02010035</td>
<td>polyAb rabbit: C02010032</td>
<td>polyAb rabbit: C02010031</td>
<td>polyAb rabbit: C02010030</td>
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<tr>
<td></td>
<td>C02020011 (48 rxns)</td>
<td>C02020010 (48 rxns)</td>
<td>C02020010 (48 rxns)</td>
<td>C02020010 (48 rxns)</td>
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</tbody>
</table>

**MBD based**

<table>
<thead>
<tr>
<th>MagMeDIP-seq</th>
<th>hMeDIP-seq</th>
</tr>
</thead>
<tbody>
<tr>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>

**Features**

- Premium Bisulfite RRBS WGBS MagMeDIP qPCR MagMeDIP-seq Package hMeDIP MethylCap

- **Resolution**
  - Single nt
  - 100-500 bp
  - 100-600 (200)
  - >1000
  - 1000

- **Downstream application**
  - Sanger, Pyroseq, (q)PCR
  - NGS
  - qPCR, NGS

- **Initial fragment size (bp)**
  - gDNA
  - 100-400
  - 100-600 (200)
  - 100-600 (200)

- **Input DNA (ng)**
  - 0.1-2000
  - 100-1000
  - >1000
  - 1000

- **Turnaround time**
  - 1.5-2h
  - 4-5 days
  - 2-3 days
  - 2-3 days

**Kit Content**

- BS conv. reagent
- Restriction enzyme
- All buffers
- Controls
- All library prep reagents
- Purification

- na for manual kits
- na for automated kits

- Separately in IPure v2 C03010015 or MicroChIP Diapure C03040001

- na: not applicable

na: not applicable
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www.diagenode.com