Epigenetics

DNA Methylation
Histone Modification and Chromatin Remodeling
Antibodies for Epigenetics Research
RNAi Tools for Epigenetics Research
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Highlights from this issue:
This issue of Biofiles highlights epigenetics, the study of stable, but potentially reversible alterations in gene expression, that occur without permanent changes in DNA sequence. The significance of epigenetic changes in the development of cancer, autism, and other diseases is being increasingly recognized. Given this developing mechanistic understanding, the field is attracting investigators interested in diverse aspects of chromatin and chromosome biology.

Coming next issue:
The next issue of Biofiles will focus on current research into the area of cancer metabolism. During cellular transformation, many oncogenic cells increasingly rely on aerobic glycolysis for both generating ATP and providing the metabolic intermediates for use in biosynthetic pathways. Much of this metabolic reprogramming may be directly related to the mitogenic mutations that initiate oncogenesis and suggest that targeting these cancer-specific metabolic pathways are viable therapeutic options.

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Cover: Graphically depicts DNA methylation, histone modification, and higher order chromatin structure. The nature of these epigenetic phenomena is now understood to play a pivotal role in development, disease, and general gene expression.
Introduction

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Epigenetic modifications are stable, but potentially reversible alterations in the cell that affect gene expression independent of mutations in the DNA sequence. These modifications can be mitotically transmitted to daughter cells and, in some cases, to the next sexual generation.

The detailed molecular mechanisms underlying epigenetic modifications are still not known; a complex interplay of DNA methylation, chromatin remodeling, and specific forms of RNA-mediated degradation are involved. Epigenetic modifications are thought to occur through two key interconnected processes — DNA methylation and the covalent modification of histones.

Historically, it had been shown DNA is reversibly methylated. In addition, the associated DNA proteins, called histones, are altered by methylation, phosphorylation, acetylation, and other changes. These have been shown to associate with gene regulation. Epigenetic processes are natural and essential to many organism’s functions, but if they occur improperly there can be major adverse health and behavioral effects resulting in diseases like cancers, autism etc.
Introduction to DNA Methylation

Epigenetic regulation starts with DNA wound around a set of completely acetylated histones associated with an activated, fully transcribed gene. Transcriptional repression is initiated by deacetylating multiple lysine residues of histone proteins near the promoter. These lysines can be methylated up to three times per lysine, each time resulting in gene shutdown. Also, the cytosines in the gene can be methylated at their 5’ carbon. Each modification in this chain from acetylation to DNA methylation is associated with a compaction of the gene into dense, untranscribable chromatin.

DNA methylation, histone modifications, and changes to higher-order chromatin structure play a central role in the regulation of mammalian genome organization. The epigenetic signature of any cell provides valuable information about its cellular state, its developmental potential, and its overall health.

DNA methylation is a normally occurring modification in both eukaryotic and prokaryotic organisms. In many plants and animals, it is characterized by the biochemical addition of a methyl group (-CH3) to the cytosine C5 in cytosine-phosphate-guanine (CpG) dinucleotides via a methyltransferase enzyme. In plants, the cytosine can be methylated in the CpG, CpNpG, and CpNpN contexts, where N represents any base except guanine. Bacteria tend to methylate adenosine.

The majority of CpG dinucleotides in the mammalian genome are methylated (5-methylcytosine). Those that are unmethylated typically reside in so-called CpG islands, or G+C-rich regions >300 bp in length where the observed to expected ratio of CpG is >0.6. CpG islands typically reside at the 5’ ends of genes, and the majority of all human genes have CpG islands at their 5’ end. When CpG islands become aberrantly hypermethylated, it is generally associated with decreased expression of the gene. DNA methylation is an epigenetic event that is involved in embryonic development and cell cycle regulation; hence analyzing DNA methylation is necessary for understanding gene expression.
DNA Methylation and Bisulfite Conversion

There are several common ways to determine whether a gene contains methylated DNA. Since mammalian methylation occurs at cytosines, researchers take advantage of the fact that methylated cytosine (meC) is stable to bisulfite treatment but unmethylated cytosine is transformed to uracil under the same conditions. Bisulfite treatment, manifested as Sigma's Imprint® Bisulfite modification kit (MOD50), converts unmethylated cytosine to uracil. These treated samples can then be sequenced to determine the methylation state of the original sample, a process termed bisulfite sequencing. This treatment can also be used to perform methylation-specific PCR, which exploits the C to U change and uses primers that will anneal based on those predicted changes. Bisulfite treatment of DNA is a prerequisite for DNA methylation analysis for many epigenetics-based studies involving methylation profiling and the quantification of methylation status.

Sigma's scientific team systematically investigated the bisulfite treatment of DNA. They studied the chemistries involved in the process and the conversion rates in an effort to limit variability between samples and to improve yield over the conventional methods. They standardized the incubation reaction times, temperature, and pH. The protocol that was developed increased the recovery yields up to 90% of input DNA with >99% C to U conversion. The method has been developed to accommodate purified DNA from biological fluids, cells, or tissue directly as the input material.

Features and Benefits

- One of the fastest bisulfite modification kits available, requires less than two hours
- Small amount of starting material needed for modification, only 50 picograms of DNA or 20 cells
- Greater than 99% unmethylated cytosine deaminated to uracil
- Extremely low degradation, greater than 90% of input DNA is recovered
- Option of convenient one-step protocol
- Consistent and reproducible bisulfite conversion

Best-in-Class Yield and Conversion (MOD50), 100 ng of human genomic DNA was bisulfite treated according to each supplier’s protocol followed by qPCR analysis. The modified and unmodified primer sets within the β-actin gene were then used to determine bisulfite yield. C values were compared to the C value of a 10-ng human genomic standard. Percent conversion was determined after bisulfite modification of 100 ng of human genomic DNA. Comparison of qPCR delta C values between modified and unmodified primers within β-actin demonstrates conversion rates. The graph above highlights the superior yield and conversion rates of the Imprint kit in relation to other suppliers.

Bisulfite sequencing can also be performed after chromatin immunoprecipitation (ChIP-BS), which could be useful for examining DNA methylation status in combination with histone modification on a relatively large scale.

For more information, visit sigma.com/mod50

<table>
<thead>
<tr>
<th>Name</th>
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<tbody>
<tr>
<td>Imprint® DNA Modification Kit</td>
<td>MOD50-1KT</td>
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</tbody>
</table>
Global DNA Methylation Quantification

The overall degree of methylation of a genome can be a useful measure of global regulatory changes. Measurement of this parameter is usually performed after complete digestion to the single base and then analyzed using HPLC or mass spectrophotometry.

Global DNA Modification Kit
The Global DNA Modification Kit (MDQ1), allows the researcher to monitor DNA methylation using a format similar to a sandwich ELISA assay. This is a format more friendly to biological researchers, and is the first of its kind on the market.

Features and Benefits
- ELISA-Based Format
  - Procedure completes in four easy-to-follow steps
  - All reagents supplied, including methylated DNA control
- Quickly Measures Changes in DNA Methylation
  - Detection limit is 5 ng of fully methylated DNA
  - Input DNA may be as low as 10–200 ng
- Complete, Flexible, and Fast
  - Procedure complete in 3.5 hours
  - 96-well format allows option of studying single samples or high-throughput studies
- Extended Range of DNA Sample Sources
  - May be used with DNA from cells, tissues, plasma, and other body fluids

Comparison of two differently methylated lots of DNA with MDQ1.
Methylation status of two lots of Sss I treated DNA was measured by LC-MS. Varying amounts of DNA was also analyzed via the MDQ1 kit. As demonstrated by the graph, the difference in methylation states can easily be detected and the signal generated is proportional to the amount of DNA used in the procedure.

Effect of Azacytadine on CHO Cell Global Methylation. Azacytadine, a known demethylating agent, was added directly to culture medium. Cells were harvested after 48 hours. DNA was isolated using GenElute™ and global methylation was measured. 40 ng of DNA from treated and non-treated CHO cells was analyzed.
DNA methyltransferases are a family of enzymes that catalyze the transfer of a methyl group to DNA. There are five related DNA cytosine-5-methyltransferases (DNMTs) that transfer a methyl group from S-adenosylmethionine (AdoMet, SAM) to the C-5 position of cytosine. Inhibitors of DNMT activity have been shown to have anti-proliferative activity. These inhibitors can reactivate the expression of genes that have been repressed by DNA methylation.

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<tr>
<th>Nucleoside Analog Inhibitors</th>
<th>Non-Nucleoside Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Name</strong></td>
<td><strong>Cat. No.</strong></td>
</tr>
<tr>
<td>5-Azacytidine</td>
<td>A2385-100MG, A2385-250MG, A2385-1G</td>
</tr>
<tr>
<td>Zebularine</td>
<td>Z4775-5MG, Z4775-25MG</td>
</tr>
<tr>
<td>5-Aza-2'-deoxycytidine</td>
<td>A3656-5MG, A3656-10MG, A3656-50MG</td>
</tr>
</tbody>
</table>

**Nucleoside Analog Inhibitors**

- **5-Azacytidine**: A2385-100MG, A2385-250MG, A2385-1G
- **Zebularine**: Z4775-5MG, Z4775-25MG
- **5-Aza-2'-deoxycytidine**: A3656-5MG, A3656-10MG, A3656-50MG

**Non-Nucleoside Inhibitors**

- **RG108**: P8279-10MG
- **Procanamide hydrochloride**: P9391-25G, P9391-100G
- **Procaine hydrochloride**: P9879-50G, P9879-100G, P9879-250G

**Methyltransferase Proteins and Peptides**

For more information, visit [sigma.com/methyltransferases](https://sigma.com/methyltransferases)

<table>
<thead>
<tr>
<th>Name</th>
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<tbody>
<tr>
<td>DNMT1</td>
<td>Active human</td>
<td>Human DNMT1, (GenBank Accession No. NM_001130823), (amino acids 2-1632), with N-terminal GST tag, MW = 211 kDa, expressed in baculovirus expression system.</td>
<td>1786 DNMT1, human</td>
</tr>
<tr>
<td>DNMT2</td>
<td>Active human</td>
<td>Human DNMT2, (GenBank Accession No. NM_004412), (amino acids 2-292), with N-terminal GST tag, MW = 71 kDa, expressed in baculovirus expression system.</td>
<td>1787 TRDMT1, human</td>
</tr>
<tr>
<td>DNMT3a</td>
<td>Active human</td>
<td>Human DNMT3a, (GenBank Accession No. NM_175629), (amino acids 2-913), with N-terminal GST tag, MW = 128 kDa, expressed in baculovirus expression system.</td>
<td>1788 DNMT3A, human</td>
</tr>
<tr>
<td>Dot1L human</td>
<td>Human DOT1L, (GenBank Accession No. NM_032482), amino acids 2-416 with N-terminal GST tag, MW = 73 kDa, expressed in E. coli expression system.</td>
<td>84444 DOT1L, human</td>
<td>SRP0129-50UG</td>
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<tr>
<td>EZH1/EED/SUZ12 human</td>
<td>Complex of: Human EZH1, GenBank Accession No. NM_001991, amino acids 2-end, with N-terminal His tag, MW = 86 kDa. Human EED, NM_003797, amino acids with N-terminal DDDDK tag (FLAG), MW = 51 kDa. Human SUZ12, NM_015355, amino acids 2-end, with N-terminal His tag.</td>
<td>2145 EZH1, human</td>
<td>SRP0130-50UG</td>
</tr>
<tr>
<td>EZH1/EED/SUZ12/RbAp48/AEBP2 human</td>
<td>Complex of: Human EZH1, GenBank Accession No. NM_001991, amino acids 2-end, with N-terminal His tag, MW = 86 kDa. Human EED, NM_003797, amino acids 2-end, with N-terminal DDDDK tag (FLAG), MW = 51 kDa. Human SUZ12, NM_015355, amino acids 2-end, with N-terminal His tag. Human RbAp48 and AEBP2.</td>
<td>2145 EZH1, human</td>
<td>SRP0131-50UG</td>
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<tr>
<td>EZH2/EED/SUZ12 human</td>
<td>Complex of: Human EZH2, GenBank Accession No. NM_003797, (amino acids 2-end) with N-terminal FLAG tag, MW = 51 kDa. Human SUZ12, NM_015355, amino acids 2-end, with N-terminal His tag.</td>
<td>2146 EZH2, human</td>
<td>SRP0132-50UG</td>
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<tr>
<td>EZH2/EED inactive human</td>
<td>Complex of: Human EZH2, GenBank Accession No. NM_003797, (amino acids 2-end) with N-terminal FLAG tag, MW = 51 kDa, co-expressed in baculovirus expression system.</td>
<td>2146 EZH2, human</td>
<td>SRP0133-20UG</td>
</tr>
<tr>
<td>G9a human</td>
<td>Human G9a (euchromatic histone-lysine N-methyltransferase 2) (GenBank Accession No. NM_006709), (amino acids 785-1210), with N-terminal GST tag, MW = 74.6 kDa, expressed in an E. coli expression system.</td>
<td>10919 EHMT2, human</td>
<td>SRP0135-50UG</td>
</tr>
<tr>
<td>G9a human</td>
<td>Human G9a, (euchromatic histone-lysine N-methyltransferase 2). GenBank Accession No. NM_006709, amino acids 785-1210, with N-terminal GST tag, MW = 74.6 kDa, expressed in a baculovirus infected Sf9 cell expression system.</td>
<td>10919 EHMT2, human</td>
<td>SRP0136-20UG</td>
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Proteins and Peptides for Methyltransferases (continued)

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Gene ID</th>
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<tbody>
<tr>
<td>MLL human</td>
<td>Human MLL, GenBank Accession No. NM_005933, amino acids 3592-3969, with N-terminal His tag, MW = 44.2 kDa, expressed in E. coli expression system.</td>
<td>4297 MLL, human</td>
<td>SRP0137-50UG</td>
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<tr>
<td>NSD2 (782-end)/SUV39H1 full length domain Active human</td>
<td>Human NSD2 (GenBank Accession No. NM_133330), amino acids 782-end with N-terminal DDDDDK (FLAG)-His tag, MW = 66.5 kDa, expressed in baculovirus infected SF9 cell expression system.</td>
<td>7468 WHSC1, human</td>
<td>SRP0138-20UG</td>
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<tr>
<td>PRDM2 (RIZ1) human</td>
<td>Human PRDM2, RIZ1, GenBank Accession No. NM_012231, amino acids 2-200, with N-terminal GST tag, MW = 48.6 kDa, expressed in E.coli expression system.</td>
<td>7799 PRDM2, human</td>
<td>SRP0139-100UG</td>
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<td>PRMT1 human</td>
<td>Human PRMT1, GenBank Accession No. NM_001536, amino acids 2-end, with N-terminal GST tag, MW = 68 kDa, expressed in a baculovirus infected SF9 cell expression system.</td>
<td>3276 PRMT1, human</td>
<td>SRP0140-50UG</td>
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<tr>
<td>PRMT3 human</td>
<td>Human PRMT3, GenBank Accession No. NM_057988, amino acids 2-end, with N-terminal GST tag, MW = 86 kDa, expressed in an E. coli expression system.</td>
<td>10196 PRMT3, human</td>
<td>SRP0142-50UG</td>
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<tr>
<td>PRMT4 Active human</td>
<td>Human PRMT4 (CARM1) (GenBank Accession No. NM_199141), amino acids 2-end, with N-terminal His tag, MW = 66 kDa, expressed in a baculovirus infected SF9 cell expression system.</td>
<td>10498 CARM1, human</td>
<td>SRP0143-20UG</td>
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<tr>
<td>PRMT5 Active human</td>
<td>Human PRMT5 (GenBank Accession No. NM_006109), amino acids 2-end, with N-terminal DDDDDK tag (FLAG), MW = 73 kDa, expressed in a FreeStyle 293-F cells.</td>
<td>10419 PRMT5, human</td>
<td>SRP0145-20UG</td>
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<tr>
<td>PRMT5 (SF9) Active human</td>
<td>Human PRMT5 (GenBank Accession No. NM_006109), amino acids 2-end, with N-terminal DDDDDK tag (FLAG), MW = 73 kDa, expressed in a baculovirus infected SF9 cell expression system.</td>
<td>10419 PRMT5, human</td>
<td>SRP0146-20UG</td>
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<tr>
<td>PRMT6 human</td>
<td>Human PRMT6 (GenBank Accession No. NM_018137), amino acids 2-end, with N-terminal DDDDDK tag (FLAG), MW = 35 kDa, expressed in FreeStyle 293-F cells.</td>
<td>55170 PRMT6, human</td>
<td>SRP0147-20UG</td>
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<tr>
<td>Set2 human</td>
<td>Human Set2, HYP8, GenBank Accession No. NM_014159, amino acids 915-1211, with N-terminal GST tag, MW = 59.7 kDa, expressed in E. coli expression system.</td>
<td>29072 SETD2, human</td>
<td>SRP0148-100UG</td>
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<tr>
<td>Set7/SET9 human</td>
<td>Human recombinant SET7/9, amino acids 52-end, with N-terminal GST tag, GenBank Accession No. NM_030648, expressed in a E. coli expression system, MW = 61 kDa.</td>
<td>80054 SETD7, human</td>
<td>SRP0149-100UG</td>
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<td>SRT8 Active human</td>
<td>Human SRT8 (GenBank Accession No. NM_020382), (amino acids 195-352) with N-terminal GST tag, MW = 44 kDa, expressed in an E. coli expression system.</td>
<td>387893 SETD8, human</td>
<td>SRP0150-50UG</td>
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<tr>
<td>SMYD2 Active human</td>
<td>Human SMYD2 (GenBank Accession No. NM_020197), full length with N-terminal DDDDDK tag (FLAG), MW = 49.7 kDa, expressed in a baculovirus infected SF9 cell expression system.</td>
<td>56950 SMYD2, human</td>
<td>SRP0151-20UG</td>
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<tr>
<td>SMYD3 (35-end) Active human</td>
<td>Human SMYD3 (GenBank Accession No. NM_022743), amino acids 35-end, with N-terminal GST tag, MW=64 kDa, expressed in a baculovirus infected SF9 cell expression system.</td>
<td>64754 SMYD3, human</td>
<td>SRP0152-20UG</td>
</tr>
<tr>
<td>SMYD3 (full length) Active human</td>
<td>Human SMYD3 (GenBank Accession No. BC031010), full length, with N-terminal DDDDDK tag (FLAG), MW = 50 kDa, expressed in a baculovirus infected SF9 cell expression system.</td>
<td>64754 SMYD3, human</td>
<td>SRP0153-20UG</td>
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<tr>
<td>SUV39H1 human</td>
<td>Human SUV39H1, GenBank Accession No. NM_003173, amino acids 82-412, with N-terminal GST tag, MW = 64 kDa, expressed in E.coli expression system.</td>
<td>6839 SUV39H1, human</td>
<td>SRP0154-50UG</td>
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<tr>
<td>SUV39H2 human</td>
<td>Human SUV39H2, GenBank Accession No. NM_024670, amino acids 26-350, with N-terminal GST tag, MW = 63 kDa, expressed in E.coli expression system.</td>
<td>79723 SUV39H2, human</td>
<td>SRP0155-50UG</td>
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<tr>
<td>SUV4-20H1 human</td>
<td>Human SUV4-20H1 (GenBank Accession No. NM_017635), amino acids 2-387, with N-terminal GST tag, MW = 70 kDa, expressed in E.coli expression system.</td>
<td>51111 SUV420H1, human</td>
<td>SRP0156-50UG</td>
</tr>
<tr>
<td>SUV4-20H2 human</td>
<td>Human SUV4-20H2 (GenBank Accession No. NM_032701), amino acids 2-280, with N-terminal GST tag, MW = 58 kDa, expressed in E.coli expression system.</td>
<td>84787 SUV420H2, human</td>
<td>SRP0157-50UG</td>
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<tr>
<td>Metnase (SETMAR) human</td>
<td>Human Metnase (SETMAR, or SET domain and mariner transposase fusion) (GenBank Accession No. NM_006515), amino acids 14-end, with N-terminal FLAG tag, MW = 77 kDa, expressed in FreeStyle 293-F cells.</td>
<td>6419 SETMAR, human</td>
<td>SRP0165-20UG</td>
</tr>
<tr>
<td>DNMT3B/DNMT3L Active human</td>
<td>Complex of: Human DNA methyltransferase 3B (DNMT3B), GenBank Accession No. NM_006892, amino acids 564-853 (end) with N-terminal GST tag, MW = 59 kDa, DNMT3L (GenBank Accession No. NM_013369), amino acids 160-387 (end), with an N-terminal His tag, MW = 27 kDa co-expressed in baculovirus-infected SF9 cell expression system.</td>
<td>1789 DNMT3B, human</td>
<td>SRP0166-10UG</td>
</tr>
<tr>
<td>DNMT1 active from mouse</td>
<td>Murine DNA methyltransferase 3 (DNMT1) (GenBank Accession No. NM_01003961), amino acids 2-859 (end), with N-terminal His tag and C-terminal FLAG tag, MW = 59 kDa, expressed in E. coli via a baculovirus expression system.</td>
<td>13436 DNMT1, mouse</td>
<td>SRP0167-10UG</td>
</tr>
<tr>
<td>NSD2 catalytic domain Active human</td>
<td>Human NSD2 (MMSET) (GenBank Accession No. NM_133330), amino acids 941-1240 with N-terminal GST tag, MW = 62 kDa, expressed in E. coli.</td>
<td>7468 WHSC1, human</td>
<td>SRP0175-50UG</td>
</tr>
<tr>
<td>SUV39H1 full length Active human</td>
<td>Human SUV39H1 (GenBank Accession No. NM_003173), full length (amino acids 2-end), with N-terminal GST tag, MW = 74 kDa, expressed in baculovirus infected SF9 cell expression system.</td>
<td>6839 SUV39H1, human</td>
<td>SRP0176-50UG</td>
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</tbody>
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Proteins and Peptides for Demethylases

For more details, go to sigma.com/demethylases

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<tr>
<td>LSD1 Active human</td>
<td>Human LSD1, AOFA2, amine oxidase, flavin containing, domain 2, GenBank Accession No. NM_015013, amino acids 158-end with N-terminal GST tag, MW = 103 kDa, expressed in E. coli expression system.</td>
<td>23028 KDM1A, human</td>
<td>SRP0122-50UG</td>
</tr>
<tr>
<td>JMID2C Active human</td>
<td>Human JMID2C, GenBank Accession No. BC1435711 (amino acids 2-372) with N-terminal GST tag, MW = 69 kDa, expressed in a baculovirus infected Sf9 cell expression system.</td>
<td>23081 KDM4C, human</td>
<td>SRP0123-20UG</td>
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<tr>
<td>CoREST human</td>
<td>Human recombinant CoREST, GenBank Accession No. NM_015156, amino acids 305-end, with N-terminal His tag, MW = 20 kDa, expressed in E. coli expression system.</td>
<td>23186 RCOR1, human</td>
<td>SRP0124-100UG</td>
</tr>
<tr>
<td>JMID3/KDM6B Active human</td>
<td>Human JMID3/KDM6B histone demethylase (amino acids 1043-end), Genbank Accession No. NM_0001080424, with C-terminal FLAG-tag, MW = 71 kDa, expressed in Sf9 cells via a baculovirus expression system.</td>
<td>23135 KDM6B, human</td>
<td>SRP0162-20UG</td>
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<tr>
<td>JMID2A Active human</td>
<td>Human JMID2A, also known as JHDM3A and KDM4A (GenBank Accession No. NM_014663), amino acids 1-480, with C-terminal FLAG-tag, MW = 56 kDa, expressed in Sf9 cells using a baculovirus expression system.</td>
<td>9682 KDM4A, human</td>
<td>SRP0163-20UG</td>
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<tr>
<td>EED human</td>
<td>Human Embryonic Ectoderm Development (hEED), also known as WAIT-1, GenBank Accession No. NM_003797, amino acids 2-end with N-terminal FLAG-tag, MW = 51 kDa, expressed in Sf9 cells via a baculovirus expression system.</td>
<td>8726 EED, human</td>
<td>SRP0168-50UG</td>
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<tr>
<td>JMID2B Active human</td>
<td>Human JMID2B, also known as JHDM3B and KDM4B (GenBank Accession No. NM_015015), amino acids 1-500, with C-terminal FLAG-tag, MW = 57 kDa, expressed in Sf9 cells using a baculovirus expression system.</td>
<td>23030 KDM4B, human</td>
<td>SRP0169-20UG</td>
</tr>
</tbody>
</table>

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Gene expression is governed by complex mechanisms including transcription factor binding to DNA and coordinated changes in chromatin structure. The primary protein components of chromatin are the histones, which are assembled along with DNA into larger complexes known as nucleosomes. Each nucleosome contains two copies of the core histones, H2A, H2B, H3, and H4, each of which has an accessible amino terminal tail with a high proportion of lysines and arginines. Modifications of histone proteins constitute an important mechanism of gene regulation.

Histone modifications are an early indicator of epigenetic regulation, and one way to study this phenomenon is via chromatin immunoprecipitation (ChIP). ChIP monitors DNA-protein interactions and allows the chromatin structure surrounding a specific DNA sequence to be analyzed. This technique, used with histone modification-specific antibodies, identifies and quantifies those genomic regions containing the targeted histone modifications. The protocol starts with cells, and uses a crosslinking agent to chemically link the DNA and its interacting proteins. The resulting DNA is isolated, sheared, and precipitated using a protein-specific antibody (e.g. acetylated histone). The crosslinks are reversed or broken, and the precipitated DNA, now enriched for sequences that interact with the protein of interest, is examined to determine which genomic regions are present. Detection can be via PCR when looking for a few genes, or can be done using microarrays (ChIP-chip) or parallel (deep) sequencing (ChIP-seq).

**Imprint® Chromatin Immunoprecipitation Kit (CHP1)**

Sigma combines speed and convenience with performance in the Imprint Chromatin Immunoprecipitation Kit (CHP1). The Imprint ChIP Kit provides a complete solution for chromatin immunoprecipitation including columns and reagents for DNA purification. Sigma’s CHP1 Kit is designed for abundant targets, such as histone modifications, and is available in a plate format. The kit affords a high-throughput system, ideal for researchers screening multiple samples for histone modifications.

**Features and Benefits**

- **Fast** – Total protocol time of less than 6 hours, making the Imprint ChIP kit the fastest on the market
- **Sensitive** – As few as 10,000 cells required for each ChIP sample
- **Convenient** – Fewest steps of any available ChIP protocol
- **Flexible** – Protocols for cells or tissue, and convenient strip-well format for high-throughput applications
- **Complete** – Includes columns and reagents for DNA purification as well as an integrated protocol for amplification with the GenomePlex® technology

For more information, visit [sigma.com/chp1](http://sigma.com/chp1)

<table>
<thead>
<tr>
<th>Name</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imprint® Chromatin Immunoprecipitation Kit</td>
<td>CHP1-24RXN, CHP1-96RXN</td>
</tr>
</tbody>
</table>

One day is sufficient to acquire ChIP enriched DNA using Sigma’s Imprint ChIP1 protocol. ChIP with antibodies against mouse IgG, H3K9ac, RNAP, and H3K27me3 was completed on 10,000 and 100,000 SW480 cells per reaction. A fraction (1%) of the sonicated chromatin was set aside as “input” DNA before the antibody affinity manipulations. DNA was purified following cross link reversal. The resulting enriched DNA was probed for GAPDH using specific primers by qPCR. Percent input was calculated by $100 \times 2^{(Ct \text{ adjusted Input} - Ct \text{ Enriched})}$. Input DNA Ct was adjusted from 1% to 100% equivalent by subtracting 6.644 Cts or Log2 100.
Imprint Ultra Chromatin Immunoprecipitation Kit (CHP2)

Sigma’s second-generation Imprint Ultra Chromatin Immunoprecipitation (ChIP) kit was developed for maximum sensitivity and optimum next-generation sequencing results. The DNA-blocked “Staph-Seq” cells are ideally suited for studying recruitment of low-abundance transcription factors (TF) in genome-wide location analysis experiments such as ChIP-chip and ChIP-Seq. The Imprint Ultra ChIP Kit provides a complete solution for Chromatin Immunoprecipitation including columns and reagents for DNA purification. The kit allows researchers to explore the genome-wide binding sites of low abundance TF’s as well as novel histone modifications. The flexible format allows for immunoprecipitation and purification of DNA from mammalian cells or tissue.

- **Sensitivity**—CHP2 out performs all the kits in the market designed for detection of low abundance TF’s, requires as little as 2 x 10^6 cells
- **Greater Capacity**—compatible with a wide range of cell numbers ranging from 2-10^8

CHIs were performed with 2 μL of EZH2 Ab (Diagenode, pAb39) and 1 μL of Rabbit IgG (Sigma, I5006) with chromatin from DU145 cells as indicated (10 million cells on left panel and 2 million on right panel). 2 μL of ChIP’ed DNA (out of 30 μL eluate) was analyzed by qPCR using primers for the sonic hedgehog (SHH) gene promoter, a target for EZH2 containing polycomb repression complex, and a non-target ZNF333 gene (ZNF333-3’).
### Imprint® ChIP Validated Antibodies

<table>
<thead>
<tr>
<th>Name</th>
<th>Cat. No.</th>
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<tbody>
<tr>
<td>Monoclonal Anti-5-methylcytosine (33D3) antibody produced in mouse</td>
<td>SAB4800001-100UG</td>
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<tr>
<td>Monoclonal Anti-5-hydroxymethylcytosine antibody produced in rat</td>
<td>SAB4800002-100UG</td>
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<tr>
<td>Anti-H3K4me3, (N-terminal) antibody produced in rabbit</td>
<td>SAB4800003-50UG</td>
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<tr>
<td>Anti-H3K9me3 antibody produced in rabbit</td>
<td>SAB4800004-50UG</td>
</tr>
<tr>
<td>Anti-H3K36me3 antibody produced in rabbit</td>
<td>SAB4800005-50UG</td>
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<tr>
<td>Anti-H3K9/14ac antibody produced in rabbit</td>
<td>SAB4800006-50UG</td>
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<tr>
<td>Anti-H3K4me2, (N-terminal) antibody produced in rabbit</td>
<td>SAB4800007-50UG</td>
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<td>Anti-H3Pan antibody produced in rabbit</td>
<td>SAB4800008-50UG</td>
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<tr>
<td>Anti-H3K27me3 antibody produced in rabbit</td>
<td>SAB4800009-100UL</td>
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<tr>
<td>Anti-MeCP2 antibody produced in rabbit</td>
<td>SAB4800010-100UL</td>
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<tr>
<td>Anti-HDAC1, (C-terminal) antibody produced in rabbit</td>
<td>SAB4800011-100UL</td>
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<td>Monoclonal Anti-H3K9me3 antibody produced in mouse</td>
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<td>Anti-H3K79me2 antibody produced in rabbit</td>
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<td>Anti-H3K36me3 antibody produced in rabbit</td>
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<td>Anti-H4K20me3 antibody produced in rabbit</td>
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<td>SAB4800017-50UG</td>
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<tr>
<td>Monoclonal Antibody-HDAC1, (C-terminal) antibody produced in mouse</td>
<td>SAB4800018-50UG</td>
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<tr>
<td>Monoclonal Antibody-HDAC1, (C-terminal) antibody produced in mouse</td>
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<tr>
<td>Monoclonal Antibody-H4K20me1 antibody produced in mouse</td>
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<td>Anti-H3K36me1 antibody produced in rabbit</td>
<td>SAB4800021-50UG</td>
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<td>Anti-H3S10p antibody produced in rabbit</td>
<td>SAB4800023-50UG</td>
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<tr>
<td>Anti-H3K36me2 antibody produced in rabbit</td>
<td>SAB4800024-50UG</td>
</tr>
</tbody>
</table>

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Whole Genome Amplification Technology (WGA)
The GenomePlex® Whole Genome Amplification (WGA) family of products provide a robust and accurate method of amplifying nanogram quantities of starting material into microgram yields with minimal allele drop out. It utilizes a proprietary amplification technology based upon random fragmentation of genomic DNA and conversion of the resulting small fragments to PCR-amplifiable OmniPlex® library molecules flanked by universal priming sites. The OmniPlex library is then PCR amplified using universal oligonucleotide primers and a limited number of cycles.

The GenomePlex Whole Genome Amplification kits have been very successfully applied to ChIP DNA amplification and is the method of choice for generating more copies of a fragmented DNA sample. WGA has been used in a variety of applications including amplifying DNA from formalin-fixed paraffin-embedded (FFPE) tumor tissue, genotyping single-nucleotide polymorphisms (SNPs), and amplifying single copies of chromosomes. It is suitable for use with purified genomic DNA from a variety of sources. GenomePlex WGA uses nanogram quantities of starting genomic DNA, which after PCR yields 5–10 μg of WGA product. After purification, the WGA product can be analyzed in a manner similar to any genomic or FFPE tissues.

For details on whole genome amplification technology, visit sigma.com/wga

<table>
<thead>
<tr>
<th>Name</th>
<th>Cat. No.</th>
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<tbody>
<tr>
<td>GenomePlex Whole Genome Amplification (WGA) Kit</td>
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<tr>
<td>GenomePlex Complete Whole Genome Amplification (WGA) Kit</td>
<td>WGA2-10RXN, WGA2-50RXN, WGA2-500RXN</td>
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<td>GenomePlex WGA Reamplification Kit</td>
<td>WGA3-50RXN</td>
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<td>GenomePlex Single Cell Whole Genome Amplification Kit</td>
<td>WGA4-10RXN, WGA4-50RXN</td>
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<tr>
<td>GenomePlex Tissue Whole Genome Amplification Kit</td>
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</table>

Whole Genome Amplification Sequencing Technology (SEQX)
The SeqPlex DNA Amplification Kit for whole genome amplification (WGA) is designed to facilitate next-generation sequencing (NGS) from extremely small quantities or from degraded/highly fragmented DNA. The yields from chromatin immunoprecipitation (ChIP) or formalin-fixed paraffin-embedded tissue samples (FFPE) are often less than required for successful NGS library preparation. The SeqPlex kit allows the user to pre-amplify these and other small quantity/highly fragmented DNA samples for input into a NGS workflow. This kit is an extension of the WGA product line and has been developed to integrate into the Illumina, Solid, or 454 sequencing workflows.

<table>
<thead>
<tr>
<th>Name</th>
<th>Cat. No.</th>
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<tbody>
<tr>
<td>SeqPlex DNA Amplification Kit</td>
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<td>SeqPlex DNA Amplification Kit</td>
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<td>SeqPlex DNA Amplification Kit</td>
<td>SEQX-500RX</td>
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</tbody>
</table>

Whole Transcriptome Amplification Technology (WTA)
The TransPlex® Whole Transcriptome Amplification (WTA1 and WTA2) kits provide an accurate, fast, and simple method of amplifying total RNA from a variety of sources including blood, fixed and frozen tissue, cell culture, FACS-sorted cells, plants, and microorganisms. The TransPlex WTA technology accurately amplifies total RNA by the utilization of a unique blend of quasi-random primers to ensure accurate transcriptome coverage and rapid amplification of total RNA. The resulting cDNA is suitable for qPCR, microarray, and traditional cloning.

For more information on whole transcriptome amplification, visit sigma.com/wta

<table>
<thead>
<tr>
<th>Name</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TransPlex® Whole Transcriptome Amplification Kit</td>
<td>WTA1-500RXN</td>
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<tr>
<td>TransPlex Complete Whole Transcriptome Amplification Kit</td>
<td>WTA2-10RXN, WTA2-50RXN</td>
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</table>

Histone Modifications
Eukaryotic DNA is highly organized and packaged into the nucleus as chromatin. The primary protein components of chromatin are the core histones H2A, H2B, H3, and H4, which together with DNA form chromatin. In eukaryotes, the packaging of DNA in chromatin regulates DNA metabolic processes such as transcription, replication, and DNA repair. Chromatin structure and function can be affected by various post-translational modifications of the amino-terminal tails of nucleosomal histones. Acetylation results in the loosening of chromatin and lends itself to replication and transcription. The level of acetylation is related to transcription activity. Acetylation induces an open chromatin confirmation that allows the transcription machinery access to promoters. Histone deacetylases (HDACs) and histone acetyltransferases (HATs) are enzymes that influence transcription by selectively deacetylating or acetylating the ε-amino groups of lysines located near the amino termini of core histone proteins. HDACs are also involved in the reversible acetylation of non-histone proteins. HATs and HDACs have major roles in the control of cell fate and their misregulation is involved in diseases like cancer and chronic asthma.

Histone Deacetylases
Histone deacetylases (HDACs) are enzymes that remove acetyl groups from lysines of histones and a number of other regulatory and structural proteins. They play critical roles in chromatin remodeling and are involved in transcription regulation, cell-cycle progression, cell survival, and differentiation. Eighteen HDACs have been identified and divided into three distinct enzyme classes.

For more information on histone deacetylase inhibitors visit: sigma.com/deacetylaseinhib
Class I and II HDACs
Class I and II HDACs are zinc-dependent enzymes. Class I, homologous to yeast Rpd3, includes HDAC1, 2, 3, and 8. Class II (HDAC4, 5, 6, 7, 9, 10) resembles yeast Hda1. Class I and II HDACs are associated with malignant transformations and are targets for cancer drugs.

Inhibitors of Class I and II HDACs are largely classified into four groups on the basis of their chemical structures.

**Hydroxamic acid inhibitors**—The most widely used HDAC inhibitors are hydroxamic acid-based. Most are nonspecific for HDAC subclass.

<table>
<thead>
<tr>
<th>Name</th>
<th>Cat. No.</th>
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<tbody>
<tr>
<td>N-Hydroxy-4-(1-naphthalenyl)-benzamide</td>
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<td>1-Naphthohydroxamic Acid</td>
<td>SML0078-5MG</td>
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<td>SML0078-25MG</td>
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<tr>
<td>Diphenylacetohydroxamic acid</td>
<td>D6071-10MG</td>
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<tr>
<td>Tubastatin A hydrochloride</td>
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<td>Tubacin hydrate</td>
<td>SML0065-500UG</td>
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<td>VAHA</td>
<td>SML0011-5MG</td>
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<td>SML0011-25MG</td>
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<td>Butyrylhydroxamic acid</td>
<td>SML0027-5MG</td>
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<td>4-Phenybutyryl hydroxamic acid</td>
<td>SML0026-5MG</td>
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<td>Droxinostat</td>
<td>D6321-5MG</td>
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<td>SAHA</td>
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<td>MC1568</td>
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<td>Scriptaid</td>
<td>S7817-1MG</td>
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<tr>
<td>Oxamflatin</td>
<td>O3139-1MG</td>
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<tr>
<td>Trichostatin A</td>
<td>TB552-1MG</td>
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</table>

**Cyclic tetrapeptide and natural product inhibitors**—Most show selectivity for Class I HDACs.

<table>
<thead>
<tr>
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<th>Cat. No.</th>
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<tbody>
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<td>Trapsin A</td>
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<tr>
<td>(−)-Depudecin</td>
<td>D5816-1MG</td>
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<tr>
<td>Apicidin</td>
<td>A8851-1MG</td>
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**Short-chain fatty acid inhibitors**

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<tbody>
<tr>
<td>AN-9</td>
<td>A8236-5MG</td>
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<tr>
<td></td>
<td>A8236-25MG</td>
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<tr>
<td>Sodium butyrate</td>
<td>BS887-250MG</td>
</tr>
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<td></td>
<td>BS887-1G</td>
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<tr>
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<td>BS887-5G</td>
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<tr>
<td>Valproic acid sodium salt</td>
<td>P4543-10G</td>
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<td>P4543-25G</td>
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**Benzamide and Other Inhibitors**

<table>
<thead>
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<tbody>
<tr>
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<td>CO494-5MG</td>
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<tr>
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<td>CO494-25MG</td>
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<tr>
<td>Spltomicin</td>
<td>S4068-5MG</td>
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<tr>
<td>M344</td>
<td>MS820-1MG</td>
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<td>MS820-5MG</td>
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<tr>
<td>PTACH</td>
<td>PS874-2MG</td>
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<tr>
<td>BATCP</td>
<td>B4061-2MG</td>
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<tr>
<td>MOCPAC</td>
<td>M2195-2MG</td>
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<tr>
<td>APHA Compound 8</td>
<td>A2478-1MG</td>
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<tr>
<td>BML-210</td>
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<td>BB063-25MG</td>
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<tr>
<td>Cl−-994</td>
<td>C0621-5MG</td>
</tr>
<tr>
<td></td>
<td>C0621-25MG</td>
</tr>
</tbody>
</table>

**Class III HDACs — Sirtuins**

Class III deacetylases, the human sirtuins related to yeast Sir2, do not contain a zinc-binding site and are nicotinamide adenine dinucleotide (NAD⁺)-dependent. The sirtuin proteins have been implicated in the regulation of aging and age-associated diseases, such as type-II diabetes, obesity, and neurodegenerative disorders.

<table>
<thead>
<tr>
<th>Name</th>
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<tbody>
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<td>Resveratrol</td>
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**Sirtuin Activators**

<table>
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<td>AGK2</td>
<td>A8231-5MG</td>
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<td>Camlinin</td>
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<td>CO494-25MG</td>
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<td>CHIC-35</td>
<td>C8742-1MG</td>
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<td>EX-527</td>
<td>E7034-5MG</td>
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<td>HR-73</td>
<td>H3416-5MG</td>
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<td>H3416-25MG</td>
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<td>JFD00244</td>
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<td>Nicotinamide</td>
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<td>Ro 31-8220 methanesulfonate salt</td>
<td>R136-1MG</td>
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<td>Sirtinol</td>
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<td>Sirtuinol</td>
<td>S8825-5MG</td>
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<td>S8825-25MG</td>
</tr>
</tbody>
</table>

**HDAC Cell-based Assay Kit**

The Histone Deacetylase Assay Kit provides a simple method for the detection of HDAC activity based on a two-step enzymatic reaction.

For more information on the HDAC kit, visit sigma.com/hdackit
Deacetylases Proteins
For more details, go to sigma.com/deacetylases

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Gene ID</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDAC-1 human</td>
<td>Human HDAC1, GenBank Accession No. NM_004964, full length with C-terminal HIS-DDDDK tag (FLAG*) and C-terminal His tag, MW = 56 kDa, expressed in baculovirus expression system.</td>
<td>3065</td>
<td>SRP0100-50UG</td>
</tr>
<tr>
<td>HDAC 1 from Plasmodium falciparum</td>
<td>Plasmodium falciparum HDAC1, GenBank Accession No. XM_001352091, full length with C-terminal Hist6-DDDDK (FLAG) tags, MW = 53 kDa, expressed in a baculovirus infected S9 cell expression system.</td>
<td>813532</td>
<td>SRP0101-20UG</td>
</tr>
<tr>
<td>HDAC-4 human</td>
<td>Human HDAC4, GenBank Accession No. NM_006037, amino acids 627-1085 with N-terminal GST tag, MW = 75.2 kDa, expressed in baculovirus expression system.</td>
<td>9759</td>
<td>SRP0105-2UG</td>
</tr>
<tr>
<td>HDAC-5 human</td>
<td>Human HDAC5, catalytic domain, GenBank Accession No. NM_001015053, amino acids 657-1123 with C-terminal His tag, MW = 51 kDa, expressed in baculovirus expression system.</td>
<td>10014</td>
<td>SRP0106-5UG</td>
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<tr>
<td>HDAC-6 human</td>
<td>Human HDAC6, GenBank Accession No. BC069243, full length with N-terminal GST tag, MW = 159 kDa, expressed in baculovirus expression system.</td>
<td>10013</td>
<td>SRP0108-50UG</td>
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<tr>
<td>HDAC-7 human</td>
<td>Human HDAC7, GenBank Accession No. AV302468, amino acids 518-end, with N-terminal GST tag, MW = 78 kDa, expressed in baculovirus expression system.</td>
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<td>SRP0109-2UG</td>
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<tr>
<td>HDAC-8 human</td>
<td>Human HDAC8, GenBank Accession No. NM_018486, full length with C-terminal His tag, MW = 42.4 kDa, expressed in a baculovirus expression system.</td>
<td>55869</td>
<td>SRP0110-50UG</td>
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<tr>
<td>HDAC-9 human</td>
<td>Human HDAC9, GenBank Accession No. NM_178423, amino acids 604-1066 with C-terminal His tag, MW = 50.7 kDa, expressed in baculovirus expression system.</td>
<td>9734</td>
<td>SRP0111-5UG</td>
</tr>
</tbody>
</table>

Histone Acetyltransferases (HAT)
Histone acetyltransferases (HAT) are enzymes that acetylate conserved lysine amino acids on histone proteins by transferring an acetyl group from acetyl-CoA to form ε-N-acetyl lysine.

Histone acetyltransferases (HATs) generally act as transcriptional coactivators.

For a complete list of small molecules for research, visit sigma.com/epism

HAT Activators
CTB was the first known small molecular activator of histone acetyltransferase p300.

<table>
<thead>
<tr>
<th>Name</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPV-106</td>
<td>SML0154-5MG SML0154-25MG</td>
</tr>
<tr>
<td>CTB</td>
<td>C6499-5MG C6499-25MG</td>
</tr>
</tbody>
</table>

Histone Methylation
In the cell nucleus, DNA is wound around groups of histone proteins. Changes in the methylation patterns of these histone proteins impacts gene expression levels. Methylated histones bind DNA more tightly, which inhibits transcription while unmethylated histones lead to increased gene expression. Histone methylation can be broadly grouped into two families, lysine methylation and arginine methylation.

Histone Lysine Methyltransferase (HKMT) Inhibitors

<table>
<thead>
<tr>
<th>Name</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNC0638 hydrate</td>
<td>U4885-5MG U4885-25MG</td>
</tr>
<tr>
<td>BIX-01338 hydrate</td>
<td>B5313-5MG B5313-25MG</td>
</tr>
<tr>
<td>BIX 01294 trihydrochloride hydrate</td>
<td>B9311-5MG B9311-25MG</td>
</tr>
<tr>
<td>Chaetocin from Chaetomium minutum</td>
<td>C9492-1MG</td>
</tr>
</tbody>
</table>

Histone Lysine Demethylase Inhibitors
Lysine specific demethylase I (LSD1, KIA0601, BHC10) has close homology to monoamine oxidases (MAOs), and hence the classic MAO inhibitors pargyline and tranylcypromine have been found to inhibit LSD1, although not with high potency (>100 μM).

<table>
<thead>
<tr>
<th>Name</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Carboxy-8-hydroxyquinoline</td>
<td>SML0067-25MG SML0067-5MG</td>
</tr>
<tr>
<td>N-Oxalylglycine</td>
<td>O9390-10MG O9390-50MG</td>
</tr>
<tr>
<td>trans-2-Phenylcyclopropylamine hydrochloride</td>
<td>PBS11-25MG PBS11-1G PBS11-5G</td>
</tr>
<tr>
<td>Pargyline hydrochloride</td>
<td>PBS103-500MG PBS103-1G PBS103-5G</td>
</tr>
</tbody>
</table>
Protein Arginine Methyltransferase (PRMT) modulators
Most of the PRMT inhibitors mentioned in literature are nonselective and may also inhibit DNMTs and HKMTs.

<table>
<thead>
<tr>
<th>Name</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosin Y</td>
<td>119830-100G</td>
</tr>
<tr>
<td>5'-Deoxy-5'-methylthioadenosine</td>
<td>D5011-25MG, D5011-100MG, D5011-250MG, D5011-1G</td>
</tr>
<tr>
<td>S-5'-Adenosyl-L-homocysteine</td>
<td>A9384-10MG, A9384-25MG, A9384-50MG, A9384-100MG</td>
</tr>
<tr>
<td>AMI-1 sodium salt hydrate</td>
<td>A9232-5MG, A9232-25MG</td>
</tr>
</tbody>
</table>

Histones
For more details, visit sigma.com/histones

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Gene ID</th>
<th>Cat. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSD1 substrate (Di-methylated K4 H3)</td>
<td>Histone H3 peptide methylated at Lysine 4, substrate for LSD1</td>
<td>8353 HIST1H3E, human</td>
<td>SRP0125-500UL</td>
</tr>
<tr>
<td>Histone H3 (2-58) human</td>
<td>Human Histone 3 (GenBank Accession No. NM_003532), (amino acid 2-58) with N-terminal GST tag, MW = 32 kDa, expressed in an E. coli expression system.</td>
<td>8353 HIST1H3E, human</td>
<td>SRP0158-500UG</td>
</tr>
<tr>
<td>Histone H3 peptide (1-21) human</td>
<td>A peptide substrate for Histone 3, K4,K9 methyltransferases. The peptide corresponds to residues 1-21 of histone H3.</td>
<td>8353 HIST1H3E, human</td>
<td>SRP0159-160NMOL</td>
</tr>
<tr>
<td>Histone H3 peptide (21-44) human</td>
<td>A peptide substrate for Histone 3 K27 methyltransferases.</td>
<td>8353 HIST1H3E, human</td>
<td>SRP0160-160NMOL</td>
</tr>
<tr>
<td>Histone H3 full length human</td>
<td>Human Histone 3 (HIST1H3E), GenBank Accession No. NM_003532, amino acids 2-137 (end) with N-terminal His-tag, MW = 15.4 kDa, expressed in an E. coli expression system.</td>
<td>8353 HIST1H3E, human</td>
<td>SRP0177-1MG</td>
</tr>
<tr>
<td>Histone H4 peptide (1-21) human</td>
<td>A peptide substrate for Histone 4 R3 methyltransferases. The peptide corresponds to amino acid residues 1-21 of histone H4.</td>
<td>8370 HIST2H4A, human</td>
<td>SRP0161-160NMOL</td>
</tr>
<tr>
<td>Histone H4 (2-58) human</td>
<td>Human Histone 4 (GenBank Accession No. NM_003548), (2-58) with N-terminal GST-tag, MW = 32 kDa, expressed in an E. coli expression system.</td>
<td>8370 HIST2H4A, human</td>
<td>SRP0164-500UG</td>
</tr>
<tr>
<td>Histone H4 full length human</td>
<td>Human Histone 4 (HIST2H4A) (GenBank Accession No. NM_003548), amino acids 2-104 (end) with N-terminal His-tag, MW = 12.1 kDa, expressed in an E. coli expression system.</td>
<td>8370 HIST2H4A, human</td>
<td>SRP0178-1MG</td>
</tr>
</tbody>
</table>
Antibodies for Epigenetics Research

With a current portfolio of over 50,000 antibodies, Sigma is a leader in providing high-quality antibodies for DNA methylation, chromatin biology, transcriptional regulation, and cellular biology. All backed by our industry leading Bioguarantee program, Sigma’s antibodies help researchers in their quest to elucidate the function, regulation, and interactions of the genes, their encoded proteins, and the epigenetic mechanisms involved.

Sigma offers an extensive line of antibodies to histones and biologically relevant histone modifications. The various categories of antibodies are:
- Chromatin
- Chromatin remodeling
- Epigenetic regulators
- Histone
- Histone modifications
- Modifying enzyme arginine methylation
- RNA binding protein
- Transcription factors
- Frequently studied epigenetics-related genes

For a complete list of antibodies by category, visit sigma.com/epiantibodies

RNAi Tools for Epigenetics Research

RNA interference (RNAi) is a relatively new technology that is revolutionizing the way researchers study gene expression. RNAi is a highly conserved gene silencing mechanism that occurs in response to the presence of double-stranded RNA. It’s a pathway by which mRNAs can be down-regulated, aberrant RNAs degraded, and alien elements such as viral material, transgenes, and transposable elements can be suppressed.

RNAi works at the post-transcriptional, pre-translational level by targeting endogenous mRNA, with subsequent cleavage of targeted mRNA resulting in the effective knockdown of a given gene’s activity. It is a powerful technique to study gene function in a rapid timeframe. Key to the RNAi processes are small interfering strands (siRNA), which have complementary nucleotide sequences to a targeted RNA strand. The siRNA “guides” proteins within the RNAi pathway to the targeted messenger RNA (mRNA) and “cleaves” them, breaking them down into smaller portions that can no longer be translated into protein and are eventually degraded.

Using this technology, researchers can specifically target/introduce double-stranded RNAs (dsRNAs) that are complimentary to known mRNAs into the cell to specifically destroy that particular mRNA allowing the study of molecular mechanisms.

Although all of the cellular functions and components of the RNAi pathway are not yet completely understood, the recent identification of a class of small non-coding RNAs called microRNAs in different organisms suggests the extent of RNAi-mediated gene regulation may be much greater than previously thought. MicroRNAs (miRNA) are single-stranded RNA molecules of 21–23 nucleotides in length, which regulate gene expression. miRNAs are encoded by genes that are transcribed but not translated into protein (non-coding RNA). Instead, they are processed from primary transcripts known as pri-miRNA to short stem-loop structures called pre-miRNA and finally to functional miRNA. They typically differ from siRNA
because they are processed from single-stranded RNA precursors. Mature miRNA molecules are partially complementary to one or more messenger RNA (mRNA) molecules, and their main function is to down-regulate gene expression. Animal miRNAs are usually complementary to a site in the 3’UTR, whereas, plant miRNAs are usually complementary to coding regions of mRNAs.

The selective and robust effect of RNAi on gene expression makes it a valuable research tool, both in cell culture and in living organisms, because synthetic dsRNA introduced into cells can induce suppression of specific genes of interest. RNAi may also be used for large-scale screens that systematically shut down each gene in the cell, which can help identify the components necessary for a particular cellular process or an event such as cell division. Exploitation of the pathway is also a promising tool in biotechnology and medicine.

**shRNA and siRNA Targets for Epigenetics**

Sigma comprehensive shRNAi and siRNA product offerings consists of over 150,000 pre-cloned shRNA constructs targeting more than 15,000 human and 15,000 mouse genes. The library is available as gene family sets and gene collections related to epigenetics, stem cells, and cancer.

For a detailed list of all the shRNA and siRNA products for Epigenetics genes in Sigma's portfolio, visit the following link: [sigma.com/epigenes](http://sigma.com/epigenes)

**shRNA Gene Sets for Epigenetic Regulators**

For more information visit the following link: [sigma.com/epiregulators](http://sigma.com/epiregulators)

<table>
<thead>
<tr>
<th>Name</th>
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</thead>
<tbody>
<tr>
<td>MISSION® shRNA Human Gene Family Set, Lentiviral Particles</td>
<td>SHB301</td>
</tr>
<tr>
<td>MISSION shRNA Human Gene Family Set, DNA</td>
<td>SHB302</td>
</tr>
<tr>
<td>MISSION shRNA Human Gene Family Set, Bacterial Glycerol Stock</td>
<td>SHB3011</td>
</tr>
</tbody>
</table>

**MISSION Human miRNA Mimics**

MISSION miRNA mimics are small, double-stranded RNA molecules designed to mimic endogenous mature miRNA molecules when transfected into cells. miRNA are known to regulate gene expression in a variety of manners, including translational repression, mRNA cleavage, and deadenylation. MISSION miRNA mimics utilize a proprietary design to minimize potential sense strand off-target effects.

For a detailed list of the available miRNA mimics and additional technical information, visit [sigma.com/mimic](http://sigma.com/mimic)

<table>
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<th>Name</th>
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</tr>
</thead>
<tbody>
<tr>
<td>MISSION microRNA Mimic</td>
<td>HMI1001-SNMOL</td>
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<tr>
<td>MISSION microRNA Mimic</td>
<td>MI00100-15ET</td>
</tr>
</tbody>
</table>

**MISSION Target ID Library**

The MISSION Target ID Library enables bench-top transcriptome-wide human miRNA and ncRNA gene target identification. It has an innovative dual selection system, which makes rapid whole transcriptome miRNA and ncRNA gene target screens accessible to any researcher with minimal reagent, time, or capital equipment expense.

For additional information on the MISSION Target ID Library, visit [sigma.com/targetid](http://sigma.com/targetid)

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</tr>
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<tbody>
<tr>
<td>MISSION Target ID Library</td>
<td>MREH01-15ET</td>
</tr>
</tbody>
</table>
Zinc finger nucleases (ZFNs) are a class of engineered DNA-binding proteins that facilitate targeted editing of the genome by creating double-strand breaks in DNA at user-specified locations.

Double-strand breaks are important for site-specific mutagenesis in that they stimulate the cell’s natural DNA repair processes, namely homologous recombination and Non-Homologous End Joining (NHEJ). By implementing established, field-proven methods, these processes are harnessed to generate precisely targeted genomic edits, resulting in cell lines with targeted gene deletions, integrations, or modifications.

CompoZr ZFNs are used to create modified cell lines with targeted gene deletions, gene insertions, or gene corrections that can be used in studying epigenetics.

**Benefits**
- Rapid disruption of, or integration into, any genomic locus
- Mutations made are permanent and heritable
- Works in a variety of mammalian somatic cell types
- Edits induced through a single transfection experiment
- Knockout or knockin cell lines in as little as two months
- Single or biallelic edits occur in 1–20% of clone population
- No antibiotic selection required for screening

For more information on ZFN technology visit [sigma.com/zfns](http://sigma.com/zfns)

Sigma has off-the-shelf Zinc finger kits for the following genes for Epigenetics and Cancer in its portfolio and the number is increasing.

For more information please go to [sigma.com/epizfns](http://sigma.com/epizfns)

### Zinc Finger Nuclease Kits for Epigenetics

<table>
<thead>
<tr>
<th>Description</th>
<th>Gene ID</th>
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</tr>
</thead>
<tbody>
<tr>
<td>CompoZr Knockout ZFN Kit</td>
<td>CDH1</td>
<td>CKOZFN1009-1KT</td>
</tr>
<tr>
<td>CompoZr Knockout ZFN Kit</td>
<td>DKK1</td>
<td>CKOZFN1145-1KT</td>
</tr>
<tr>
<td>CompoZr Knockout ZFN Kit</td>
<td>DKK1</td>
<td>CKOZFN1144-1KT</td>
</tr>
<tr>
<td>CompoZr Knockout ZFN Kit</td>
<td>HDAC2</td>
<td>CKOZFN1025-1KT</td>
</tr>
<tr>
<td>CompoZr Knockout ZFN Kit</td>
<td>PRMT1</td>
<td>CKOZFN1058-1KT</td>
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For more information on these products, visit [sigma.com/epigenetics](http://sigma.com/epigenetics) or contact your local sales representative.