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# Epigenetics

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& DNA MODIFICATIONS

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# Epigenetics

For over 45 years, New England Biolabs has been committed to understanding the mechanisms of restriction and methylation of DNA. This expertise in enzymology has led to the development of a suite of validated products for epigenetics research. These unique solutions to study DNA and histone modifications are designed to address some of the challenges of the current methods. NEBNext® Enzymatic Methyl-seq (EM-seq™) and EpiMark® validated reagents simplify epigenetics research and expand the potential for biomarker discovery.

Epigenetics is the study of heritable changes in the phenotype of a cell or organism that are not encoded in the DNA of the genome. The molecular basis of an epigenetic profile arises from covalent modifications of the protein and DNA components of chromatin. The epigenetic profile of a cell often dictates cell memory and cell fate and, thus influences mammalian development.

The epigenetic code is hypothesized to be the combined effects of histone modifications and DNA methylation on gene expression. While the genetic code for an individual is the same in every cell, the epigenetic code is tissue- and cell-specific, and may change over time as a result of aging, disease or environmental stimuli (e.g., nutrition, life style, toxin exposure) (1). Cross-talk between histone modifications, DNA methylation or RNAi pathways are being studied in such areas as cancer, X chromosome inactivation, and imprinting.

## TOOLS & RESOURCES

### Visit NEBNext.com to learn:



- How EM-seq compares to bisulfite sequencing
- How EM-seq minimizes DNA damage and produces high-quality, high-diversity libraries
- What more sensitive detection of 5mC and 5hmC means for your methylome analysis

### Visit www.epimark.com to find:



- An interactive tutorial explaining the phenomenon of epigenetics at the molecular level
- Videos from NEB scientists discussing the concept of epigenetics
- Videos and tutorials from NEB scientists explaining methods for 5hmC and 5mC detection and quantitation

**SODIUM BISULFITE METHOD**

- ✓ Greater DNA damage

**EM-SEQ METHOD**

- ✓ Minimizes DNA damage
- ✓ Superior performance
- More sensitive detection of 5mC and 5hmC

**NEBNext ENZYMATIC METHYL-SEQ WORKFLOW**

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References 1. Tost, J. (2010) *Mol. Biotechnol.* 44, 71–81.

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Visit [www.NEB.com](http://www.NEB.com) for the full list of reagents available for epigenetic studies.

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Find an  
interactive tutorial  
on epigenetics.



\*see back cover for details

# DNA Modifications

DNA can be modified by methylation of cytosine and adenine bases in a wide variety of prokaryotes and eukaryotes (see Table 2). In prokaryotes, DNA methylation is involved in determination of DNA-host specificity, virulence, DNA repair, chromosome replication and segregation, cell cycle regulation and gene expression. In higher eukaryotes, DNA methylation is involved in gene regulation, chromatin structure, differentiation, imprinting, mammalian X chromosome inactivation, carcinogenesis, complex diseases and aging.

## DNA Methylation in Mammals

DNA methylation in mammals primarily occurs on the fifth carbon of the cytosine base (5-methylcytosine, 5mC, see Table 1) of CpG dinucleotides, and approximately 70% to 80% of CpG dinucleotides are methylated in somatic cells. However, 5mC at CpA, CpT and CpC sequences have been found in genomic DNA from mouse embryonic stem cells, and 5mC at CpA sequences are thought to regulate enhancers in mouse brain. Of note, while DNA methylation in mammals primarily occurs at CpG dinucleotides, DNA methylation in plants may occur at CpG, CpHpG and CpHpH sequences, where H is adenine, cytosine, or thymine.

## Methods for Studying DNA Methylation

Study of the DNA methylation patterns on genomic DNA had, until recently, taken one of three approaches – pretreatment with sodium bisulfite, restriction enzymes, or a methylated DNA-binding affinity matrix – with sodium bisulfite treatment and so-called bisulfite sequencing being the gold standard for analysis at the single base level. In 2019, NEB introduced a groundbreaking new method, NEBNext Enzymatic Methyl-seq (EM-seq), which offered myriad advantages over methylome analysis with sodium bisulfite pretreatment. These techniques are compared and contrasted in Table 2 (next page). Both bisulfite treatment and EM-seq can reveal the methylation status of every cytosine residue in the genome, and they are therefore amenable to massively parallel sequencing methods. Methyl-specific differential cleavage of DNA requires restriction enzymes, that are either methylation sensitive or methylation dependent, to fragment genomic DNA for subsequent analysis. This method offers lower resolution data due to the requirement of a range of enzyme recognition sequences and the risk for incomplete digestion. Finally, affinity-based methods use methylated DNA binding proteins or antibodies to enrich the experimental DNA sample for methylated DNA to be analyzed in subsequent steps.

A wide variety of analytical and enzymatic methods may be employed downstream of methyl-enrichment steps to characterize genomic DNA. Analytical methods, including high-performance liquid chromatography (HPLC) and matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), are routinely used to quantify modified nucleobases in complex DNA. Though HPLC is quantitative and reproducible, it is poorly suited to high-throughput applications due to a requirement for high input amounts, although recent work has lowered the minimum input to nanogram levels (1). MALDI-TOF MS is both quantitative and amenable to higher throughput applications. Other downstream methylome analysis methods include end-point PCR, real-time PCR, primer extension, single-stranded conformational polymorphism assays, blotting, microarrays, and sequencing. Selecting a method(s) will depend on your sample size and experimental goals (2, see also [www.epimark.com](http://www.epimark.com)).

### TOOLS & RESOURCES



Visit [NEBNext.com](http://NEBNext.com) for more information on NEBNext Enzymatic Methyl-seq, an enzyme-based alternative to bisulfite sequencing

Table 1: Types of DNA Modifications

METHYLATED BASE	ORGANISM	DNA METHYLATION SEQUENCE
C5-methylcytosine	Bacteria	Varies (e.g., CCAGG, CCTGG)
	Some Fungi, Some Insects, Mammals	CpG, CpH*pG, CpH*pH
	Plants	CpG, CpH*pG, CpH*pH
C5-hydroxymethylcytosine	Bacteriophages	Varies (e.g., CCGG, GATC); Some contain only modified cytosines
	Mammals	CpG, CpH*pG, CpH*pH
N4-methylcytosine	Bacteria	Varies (e.g., CTC TTC, CCCGGG)
N6-methyladenine	Bacteria, Bacteriophages, Archaea, Protists, Some Fungi, Plants	Varies (e.g., GATC, GANTC, GAAGAG)

\* = Adenine, Cytosine, or Thymine

### References

- Song, L., et al (2005) Anal. Chem., 77, 504–510.
- Laird, P.W. (2010) Nat. Rev. Genet. 11, 191–203.

Table 2: Approaches for Studying DNA Methylation

METHOD	DESCRIPTION	ADVANTAGES	DISADVANTAGES	APPLICATION
<b>NEBNext Enzymatic Methyl-seq (EM-seq)</b>	EM-seq is a new method for detection of 5mC and 5hmC at single-base resolution. In a two-step conversion process, TET2 and an oxidation enhancer protect modified cytosines from downstream deamination. TET2 enzymatically oxidizes 5mC and 5hmC through a cascade reaction into 5-carboxycytosine [5-methylcytosine (5mC) → 5-hydroxymethylcytosine (5hmC) → 5-formylcytosine (5fC) → 5-carboxycytosine (5caC)]. This protects 5mC and 5hmC from deamination. 5hmC can also be protected from deamination by glucosylation to form 5ghmC using the oxidation enhancer. Then, APOBEC deaminates cytosines but does not affect 5caC and 5ghmC. Comparison of sequence information between the reference genome and EM-seq DNA can provide single-nucleotide resolution information about cytosine methylation patterns.	<ul style="list-style-type: none"> <li>• Superior sensitivity of detection of 5mC and 5hmC</li> <li>• High mapping efficiency with uniform GC coverage</li> <li>• Gentle enzymatic process/minimal DNA damage</li> <li>• More CpG data with fewer sequencing runs than WGBS</li> <li>• Works with damaged DNA (e.g., FFPE)</li> <li>• Faster workflow than WGBS</li> <li>• Resolution at the nucleotide level</li> <li>• Automated analysis</li> <li>• Gives % mC at a specific site</li> </ul>	<ul style="list-style-type: none"> <li>• Cannot distinguish between 5mC and 5hmC</li> <li>• Intensive downstream analysis (same as WGBS)</li> </ul>	<ul style="list-style-type: none"> <li>• Whole genome (or single locus) methylation analysis</li> </ul>
<b>Sodium Bisulfite Conversion</b>	Treatment of denatured DNA (i.e., single-stranded DNA) with sodium bisulfite leads to deamination of unmethylated cytosine residues to uracil, leaving 5mC intact. The uracils are amplified as thymines, and 5mC residues are amplified as cytosines in PCR. Comparison of sequence information between the reference genome and bisulfite-treated DNA can provide single-nucleotide resolution information about cytosine methylation patterns.	<ul style="list-style-type: none"> <li>• Resolution at the nucleotide level</li> <li>• Works on 5mC-containing DNA</li> <li>• Automated analysis</li> <li>• Gives % mC at a specific site</li> </ul>	<ul style="list-style-type: none"> <li>• Requires micrograms of DNA input, depending on downstream processes</li> <li>• DNA is often damaged</li> <li>• Multi-step protocol</li> <li>• Potentially incomplete conversion of DNA</li> <li>• Intensive downstream analysis</li> <li>• Cannot distinguish 5mC and 5hmC</li> </ul>	<ul style="list-style-type: none"> <li>• Whole genome or a single DNA locus methylation analysis</li> </ul>
<b>Sequence-Specific Enzyme Digestion</b>	Restriction enzymes are used to generate DNA fragments for methylation analysis. Some restriction enzymes are methylation-sensitive (i.e., digestion is impaired or blocked by methylated DNA). When used in conjunction with an isoschizomer that has the same recognition site, but is methylation insensitive, information about methylation status can be obtained. Additionally, the use of methylation-dependent restriction enzymes (i.e., requires methylated DNA for cleavage to occur) can be used to fragment DNA for sequencing analysis.	<ul style="list-style-type: none"> <li>• High enzyme turnover</li> <li>• Well-studied</li> <li>• Easy-to-use</li> <li>• Availability of recombinant enzymes</li> </ul>	<ul style="list-style-type: none"> <li>• Determination of methylation status is limited by the enzyme recognition site</li> <li>• Overnight protocols</li> <li>• Lower throughput</li> </ul>	<ul style="list-style-type: none"> <li>• Southern blots using MspI/HpaII</li> </ul>
<b>Methylated DNA Immunoprecipitation</b>	Fragmented genomic DNA (restriction enzyme digestion or sonication) is denatured and immunoprecipitated with antibodies specific for 5mC. The enriched DNA fragments can be analyzed by PCR for locus-specific studies or by microarrays (MeDIP-chip) and massively parallel sequencing (MeDIP-seq) for whole genome studies.	<ul style="list-style-type: none"> <li>• Relatively fast</li> <li>• Compatible with array-based analysis</li> <li>• Applicable for high throughput sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• Dependent on antibody specificity</li> <li>• May require more than one 5mC for antibody binding</li> <li>• Requires DNA denaturation</li> <li>• Resolution depends on the size of the immunoprecipitated DNA and for microarray experiments; depends on probe design</li> <li>• Data from repeat sequences may be overrepresented</li> </ul>	<ul style="list-style-type: none"> <li>• Immuno affinity capture</li> </ul>
<b>Methylated DNA-Binding Proteins</b>	Instead of relying on antibodies for DNA enrichment, affinity-based assays use proteins that specifically bind methylated or unmethylated CpG sites in fragmented genomic DNA (restriction enzyme digestion or sonication). The enriched DNA fragments can be analyzed by PCR for locus-specific studies or by microarrays and massively parallel sequencing for whole genome studies.	<ul style="list-style-type: none"> <li>• Well-studied</li> <li>• Does not require denaturation</li> <li>• Compatible with array-based analysis</li> <li>• Applicable for high throughput sequencing</li> </ul>	<ul style="list-style-type: none"> <li>• May require high DNA input</li> <li>• May require a long protocol</li> <li>• Requires salt elutions</li> <li>• Does not give single base methylation resolution data</li> </ul>	<ul style="list-style-type: none"> <li>• Capture of methylated DNA</li> </ul>



# Methylome Analysis (5mC & 5hmC)

The methylome comprises the total of methyl marks attached to the cytosine bases within a genome. Analyzing the complete methylome requires tools that enable the reliable quantitation of methylated cytosines, in most cases requiring the conversion of methylated cytosines into other structures before deamination and sequence comparison.

## NEBNext Enzymatic Methyl-seq (EM-seq) Kit

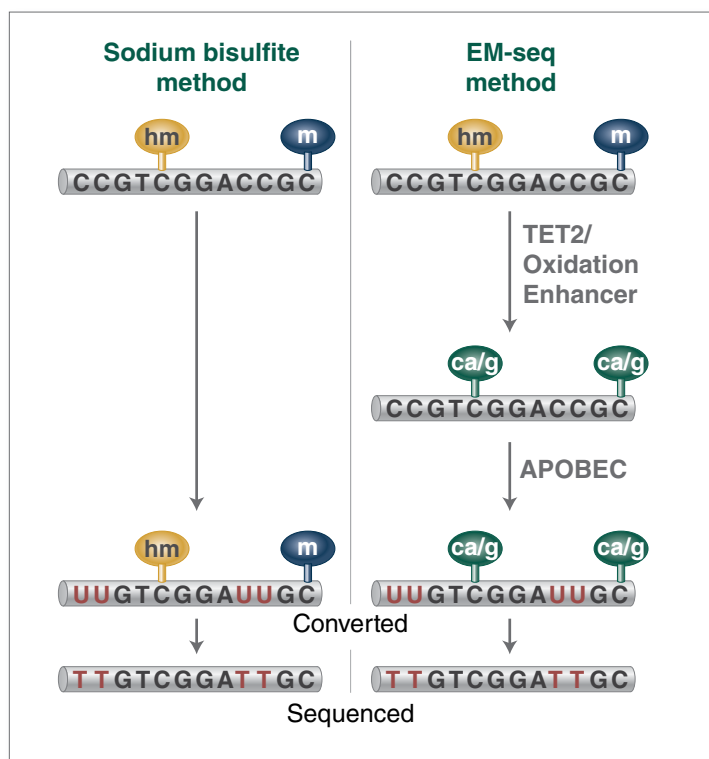
The NEBNext Enzymatic Methyl-seq Kit provides a high-performance enzyme-based alternative to bisulfite conversion for methylome analysis using Illumina® sequencing.

Libraries are prepared using as little as 10 ng input DNA and the supplied NEBNext Ultra II reagents and the optimized EM-seq Adaptor. TET2 then oxidizes 5-mC and 5-hmC, providing protection from deamination by APOBEC in the next step. In contrast, unmodified cytosines are deaminated to uracils. Libraries are then amplified using a NEBNext master mix formulation of Q5U® (a modified version of Q5® High-Fidelity DNA Polymerase), and sequenced using Illumina instrumentation.

The consistently high conversion performance and minimized DNA damage with the EM-seq protocol, in combination with highly efficient Ultra II library prep, result in superior detection of CpGs with fewer sequencing reads.

NEBNext Enzymatic Methyl-seq Kit .....E7120S/L  
NEBNext Enzymatic Methyl-seq Conversion Module .....E7125S/L

### EM-seq and sodium bisulfite conversion methods



### ADVANTAGES

- Superior sensitivity of detection of 5mC and 5hmC
- Greater mapping efficiency
- More uniform GC coverage
- Detect more CpGs with fewer sequence reads
- Uniform dinucleotide distribution
- Larger library insert sizes
- High-efficiency library preparation
- Conversion module also available separately

## What users are saying:

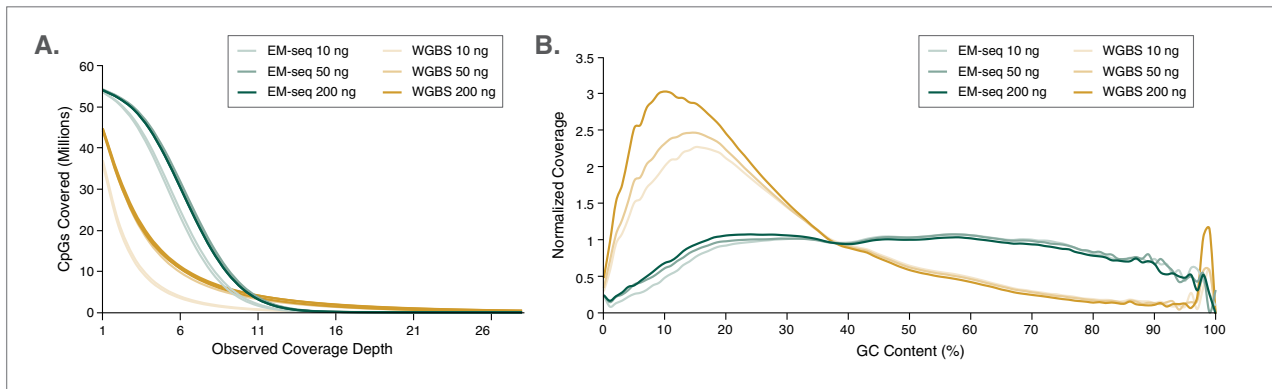
*“We’ve been testing EM-seq on a variety of inputs, platforms, and samples, and it shows more even coverage across CpG islands, the whole genome, and also greater detection of CpG sites across the genome vs. WGBS.”*

— Christopher Mason,  
Weill Cornell Medical School  
New York

*“Whole genome bisulfite sequencing is the workhorse technique in our laboratory and we have tested range of different kits. NEB’s EM-seq Kit provides an excellent alternative that causes far less damage to the DNA and results in larger fragments which make the process of sequencing more cost effective. We found that the kit also produces libraries with very low biases in nucleotide coverage and methylation estimates.”*

— Duncan Sproul,  
MRC Human Genetics Unit  
Edinburgh

EM-seq identifies more CpGs than WGBS, at lower sequencing coverage depth with superior uniformity of GC coverage.



10, 50 and 200 ng Human NA12878 genomic DNA was sheared to 300 bp using the Covaris S2 instrument and used as input into EM-seq and WGBS protocols. For WGBS, NEBNext Ultra II DNA was used for library construction, followed by the Zymo Research EZ DNA Methylation-Gold Kit for bisulfite conversion. Libraries were sequenced on an Illumina NovaSeq™ 6000 (2 x 100 bases). Reads were aligned to hg38 using bwa-meth 0.2.2.

A: Coverage of CpGs with EM-seq and WGBS libraries was analyzed using 324 million paired end reads, and each top and bottom strand CpGs were counted independently, yielding a maximum of 56 million possible CpG sites. EM-seq identifies more CpGs at lower depth of sequencing.

B: GC coverage was analyzed using Picard 2.17.2 and the distribution of normalized coverage across different GC contents of the genome (0-100%) was plotted. EM-seq libraries have significantly more uniform GC coverage, and lack the AT over-representation and GC under-representation typical of WGBS libraries.

EM-seq identifies more CpGs than WGBs, at lower sequencing coverage depth



C. Coverage of CpGs with EM-seq and WGBS libraries was analyzed using 324 million paired end reads. The number of unique and common CpGs identified by EM-seq and WGBS at 1X and 8X minimum coverage for each input amount are shown. EM-seq covers at least 20% more CpGs than WGBS at 1X minimum coverage threshold. The difference in CpG coverage increases to two-fold at 8X minimum coverage threshold.

“We were very excited by an opportunity to use the new EM-seq system launched now by NEB. In addition to its attractive features, such as user-friendliness and cleanliness of the process, for example, we have realized that it enables us to determine in precise and DNA sparing way the cytosine methylation status even at low integrity DNA. If bisulfite conversion were the only approach to apply, we would definitely fail to generate relevant results. The cool, biochemical approach to analyse cytosine methylation the system is utilizing, it also opens new avenues to explorations of methylation at intact long DNA fragments.”

— Vladimir Benes,  
Head Genomics Core Facility  
at EMBL Heidelberg

# Bisulfite Conversion

## EpiMark Bisulfite Conversion Kit

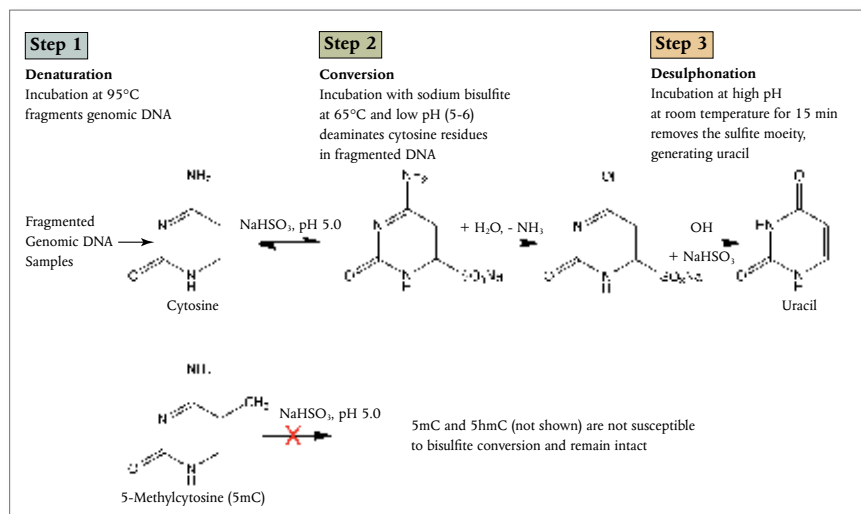
Bisulfite conversion, involves the conversion of unmodified cytosines to uracil, leaving the modified bases (5mC and 5hmC). The EpiMark Bisulfite Conversion Kit is designed for the detection of methylated cytosine, using a series of alternating cycles of thermal denaturation, followed by incubation with sodium bisulfite. This kit includes all the reagents necessary for complete bisulfite conversion, including spin columns. Amplification of bisulfite-treated samples can then be performed using EpiMark Hot Start *Taq* DNA Polymerase.

EpiMark Bisulfite Conversion Kit .....E3318S

### BENEFITS

- Conversion of unmodified cytosines to uracil
- All reagents, including purification columns, are provided

### Overview of bisulfite conversion

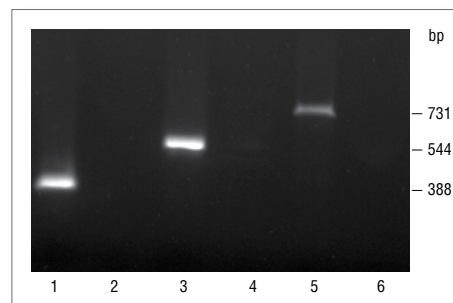


## EpiMark Hot Start *Taq* DNA Polymerase

EpiMark Hot Start *Taq* DNA Polymerase is a mixture of *Taq* DNA Polymerase and a temperature sensitive, aptamer-based inhibitor. This inhibitor binds reversibly to the enzyme, inhibiting polymerase activity below 45°C, but releases the enzyme during normal PCR cycling conditions. This permits PCR reactions to be assembled at room temperature and eliminates an activation step. This aptamer-based hot start activity combined with the supplied reaction buffer, that has been optimized for amplification of converted DNA, makes EpiMark Hot Start *Taq* an excellent choice for use on bisulfite-treated DNA.

EpiMark Hot Start *Taq* DNA Polymerase.....M0490S/L

EpiMark Kit enables complete DNA conversion



1 µg of genomic DNA was bisulfite-treated using the EpiMark Bisulfite Conversion Kit, and 2 µl of eluted DNA was analyzed by end-point PCR using EpiMark Hot Start *Taq*. Amplification with primer pairs for bisulfite converted DNA (lanes 1, 3, and 5), or with primer pairs for unconverted DNA (lanes 2, 4, and 6) were performed; lanes 2, 4, and 6 show no amplification product, indicating complete conversion.



# Enrichment of Methylated DNA

## EpiMark Methylated DNA Enrichment Kit

The EpiMark Methylated DNA Enrichment Kit enables the enrichment of double-stranded CpG methylated DNA based on CpG methylation density. It utilizes the methyl-CpG binding domain of human MBD2a protein as a capture agent. The protein is fused to the Fc tail of human IgG1 (MBD2a-Fc), which is coupled to Protein A Magnetic Beads (MBD2a-Fc/Protein A Bead). This stable complex will selectively bind double-stranded methylated CpG containing DNA. The high binding affinity of the beads coupled with optimized reagents increases sensitivity and accuracy. This kit contains all the individual components necessary to achieve enrichment in less than two hours using a four-step process:

- Step I. Fragment genomic DNA by sonication, nebulization or enzymatic treatment to an average size of less than 1,000 bp
- Step II. Generation of bead mixture by combining MBD2a-Fc, Protein A Magnetic Beads and 1X Bind/Wash Reaction Buffer
- Step III. Capture of methylated CpG DNA by incubation with MBD2a-Fc/Protein A Magnetic Bead mixture
- Step IV. Elute enriched methylated CpG DNA from beads

In the final step, enriched fractions are eluted in small volumes, simplifying downstream applications, including adaptor ligation for next generation sequencing.

EpiMark Methylated DNA Enrichment Kit .....E2600S

### ADVANTAGES

- Increased sensitivity
- Easy-to-use protocol yields enriched methylated DNA in less than 2 hours
- Amenable to downstream applications, including next generation sequencing
- Suitable for low levels of input DNA

# RNA Methylation

Just as epigenetic information can be conveyed via DNA modifications, so too can it be conveyed via RNA modifications. Common modifications of messenger RNAs (mRNAs) include methylation of cytosines and adenosines. The most common mRNA modification in mammals is N6-methyladenosine (m6A), and it is thought to be involved in RNA stability, splicing, transport, and tolerance (1,2).

## EpiMark® N6-Methyladenosine Enrichment Kit

The EpiMark N6-Methyladenosine Enrichment Kit can be used to enrich m6A modified RNA in immunoprecipitation protocols for downstream analysis by next-generation RNA sequencing or RT-qPCR. The kit contains a rabbit monoclonal antibody specific for N6-Methyladenosine (m6A). The kit also contains two control RNAs, one with m6A modification (*Gaussia* luciferase) and one without (*Cypridina* luciferase) to monitor enrichment and depletion. The GLuc RNA control was transcribed in the presence of 20% m6ATP and 80% ATP. This kit can be used to enrich m6A modified RNA in immunoprecipitation protocols for downstream analysis by next-generation RNA sequencing or RT-qPCR. Modified RNA is isolated from a fragmented RNA sample by binding to the N6-Methyladenosine antibody attached to Protein G Magnetic Beads. After multiple wash and clean-up steps, the enriched RNA is eluted in nuclease-free water and is ready for further analysis.

EpiMark N6-Methyladenosine Enrichment Kit .....E1610S

### ADVANTAGES

- Complete protocol for enrichment of m6A-modified – RNA and analysis by RT-qPCR included
- RNA controls (m6A modified and unmodified RNA) enable monitoring of enrichment and depletion
- Antibody supplied in a ready-to-use solution form

### References

1. Bokar, J.A. (2005) *Fine-tuning of RNA Functions by Modification and Editing*, Springer-Verlag, Berlin pp. 141–178.
2. Kariko, K., Buckstein, M., Ni, H. and Weissman, D. (2005), *Immunity*, 23, pp. 165–175.

# 5-Hydroxymethylcytosine and 5-methylcytosine Identification and Quantification

## EpiMark 5-hmC and 5-mC Analysis Kit

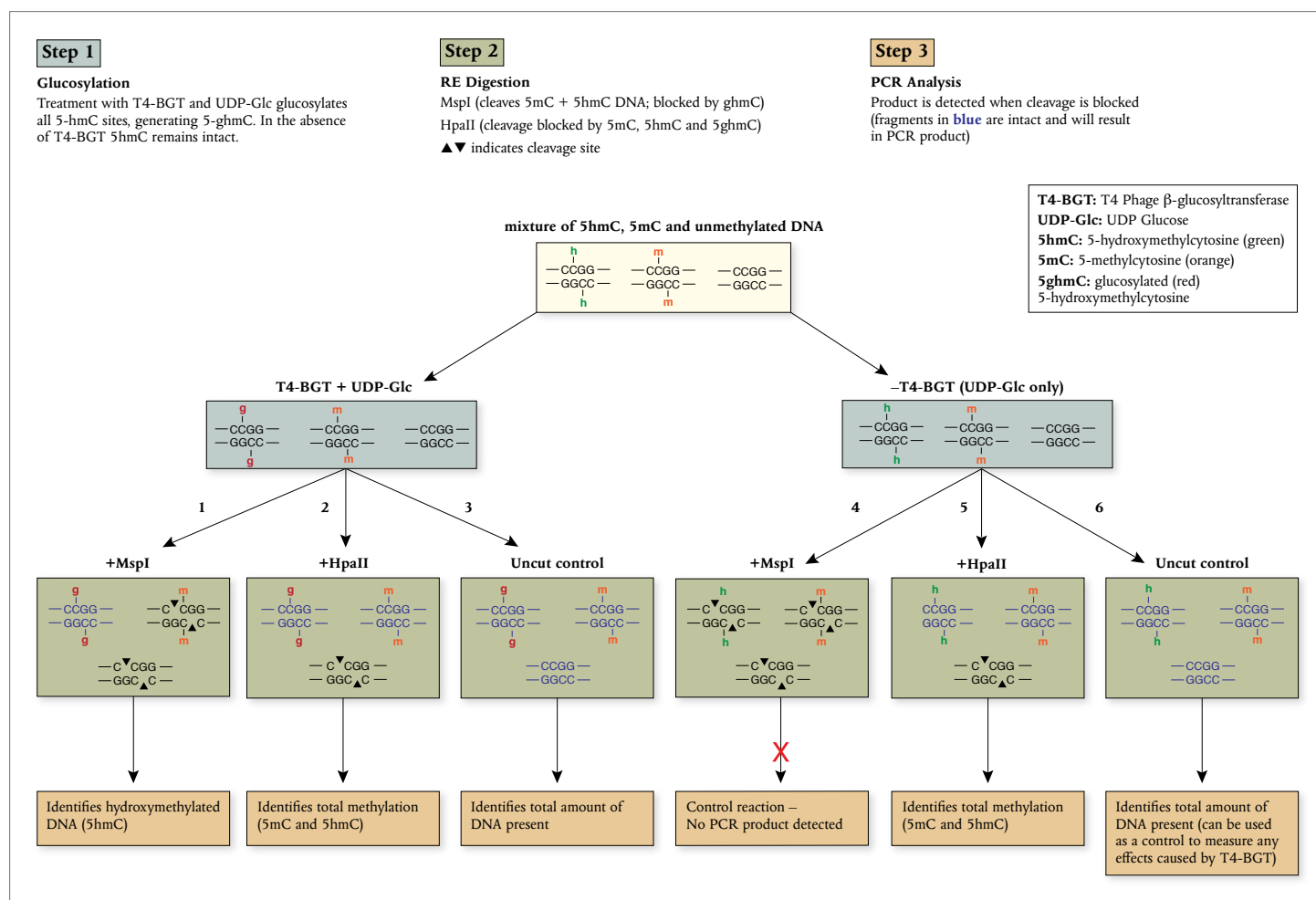
The EpiMark 5-hmC and 5-mC Analysis Kit can be used to analyze and quantitate 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC) within a specific locus. The kit distinguishes 5mC from 5hmC by the addition of glucose to the hydroxyl group of 5hmC via an enzymatic reaction utilizing T4 phage  $\beta$ -glucosyltransferase (T4-BGT). When 5-hmC occurs in the context of CCGG, this modification converts a cleavable MspI site to a noncleavable one.

### ADVANTAGES

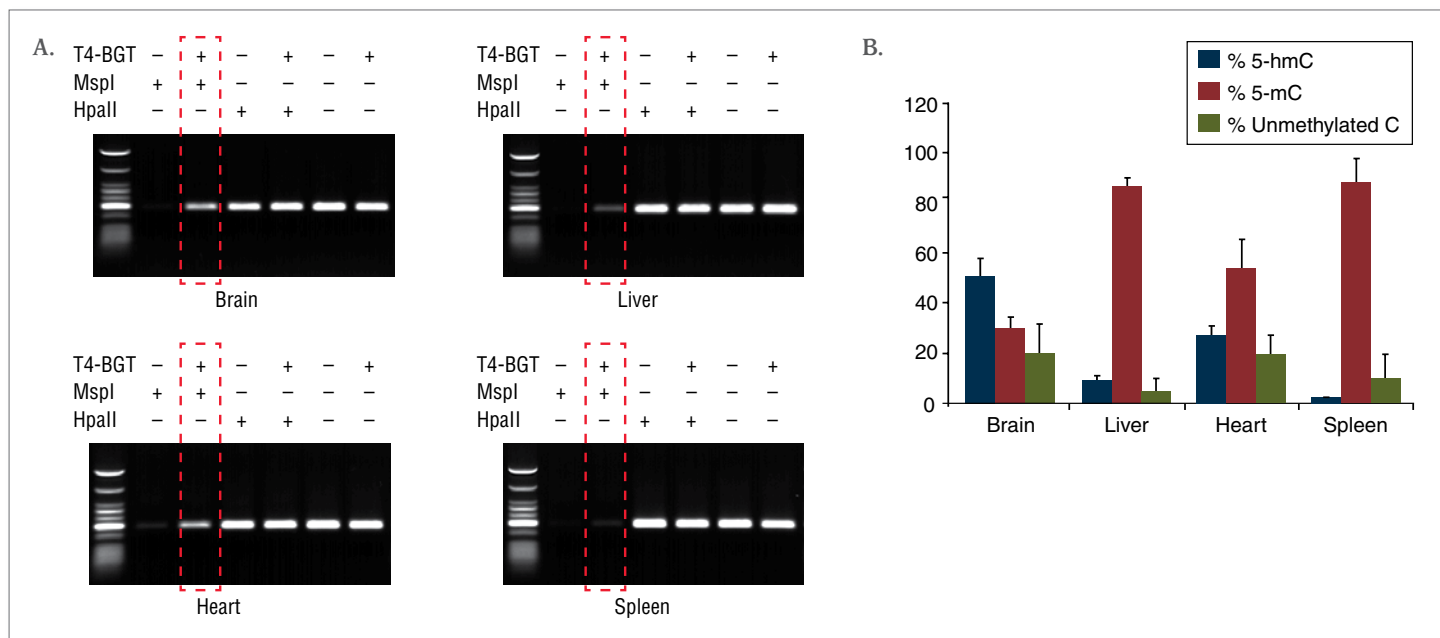
- Reproducible quantitation of 5hmC and 5mC
- Easy-to-use protocols
- Compatible with existing techniques
- Amenable to high throughput

EpiMark 5-hmC and 5-mC Analysis Kit .....E3317S

### Overview of 5-hmC and 5-mC identification using the EpiMark 5-hmC and 5-mC Analysis Kit



Analysis of the different methylation states in Balb/C mouse tissue samples using the EpiMark 5-hmC and 5-mC Analysis Kit



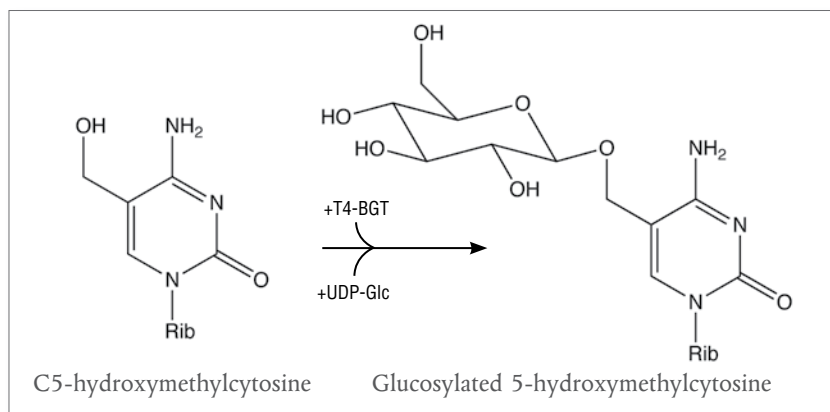
A) Endpoint PCR of the 6 different reactions needed for methylation analysis. The boxed lanes indicate the presence of 5hmC. B) Real-time PCR data was used to determine amounts of 5hmC and 5mC present. The results demonstrate a variation in 5hmC levels in the tissue sources indicated.

## T4 Phage $\beta$ -glucosyltransferase

T4 Phage  $\beta$ -glucosyltransferase (T4-BGT) is also available as a stand-alone enzyme for the glucosylation of 5hmC in DNA. This is the same enzyme included in the EpiMark 5-hmC and 5-mC Analysis Kit.

T4 Phage  $\beta$ -glucosyltransferase .....M0357S

### Glucosylation with T4-BGT



Treatment of DNA with T4-BGT and UDP-Glc glucosylates all 5-hydroxymethylcytosine (5hmC) sites, generating glucosylated 5-hydroxymethylcytosine (5ghmC).

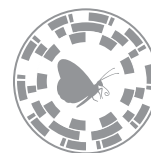
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#### APPLICATIONS

- Glucosylation of 5hmC in DNA (1)
- Immunodetection of 5hmC in DNA (3)
- Labeling of 5hmC by incorporation of [ $^3\text{H}$ ]- or [ $^{14}\text{C}$ ]- glucose into 5hmC-containing DNA acceptor after incubation with [ $^3\text{H}$ ]- or [ $^{14}\text{C}$ ]- UDP-Glc (4)
- Detection of 5hmC in DNA by protection from endonuclease cleavage

Learn about 5-hmC detection in Balb/C brain tissue.



# Methylation-Sensitive Restriction Enzymes

Some restriction enzymes are methylation-sensitive (i.e., digestion is impaired or blocked by methylated DNA). When used in conjunction with an isoschizomer that has the same recognition site but is methylation insensitive, information about methylation status can be obtained. Table 4A lists methylation sensitive restriction enzymes that can be used in epigenetic studies.

Table 4A: Methylation Sensitive Restriction Enzymes

	METHYLATION SENSITIVITY	SEQUENCE	NEB#	ISOSCHIZOMER
<b>DpnII</b>	Cleaves dam sites** which lack adenomethylation and is blocked by complete dam methylation and probably by hemi-methylation	5'... <b>▼</b> GATC...3' 3'...CTAG <b>▲</b> ...5'	R0543	Mbol DpnII
<b>HpaII</b>	Will not cleave methylated CpG sites	5'... <b>▼</b> CCGG...3' 3'...GGCC <b>▲</b> ...5'	R0171	MspI
<b>MspI</b>	Not methylation sensitive	5'... <b>▼</b> CCGG...3' 3'...GGCC <b>▲</b> ...5'	R0106	HpaII

\*\* dam sites: methylation at the N6 position of the adenine in the sequence GATC (GmATC).

# Methylation-Dependent Restriction Enzymes

Some restriction enzymes are dependent on methylation or hydroxymethylation for cleavage to occur, making them particularly useful for DNA methylation studies.

## McrBC

McrBC is an endonuclease which only cleaves DNA containing methylcytosine (5-methylcytosine, 5-hydroxymethylcytosine or N4 methylcytosine) on one or both strands (2). McrBC will not act upon unmethylated DNA (3) and will not recognize HpaII/MspI sites (CCGG) in which the internal cytosine is methylated. McrBC requires GTP for cleavage, but in the presence of a non-hydrolyzable analog of GTP, the enzyme will bind to methylated DNA specifically, without cleavage (4).

McrBC makes one cut between each pair of half-sites, cutting close to one half-site or the other, but cleavage positions are distributed over several base pairs approximately 30 base pairs from the methylated base (5). Therefore, the enzyme does not produce defined DNA ends upon cleavage. Also, when multiple McrBC half-sites are present in DNA (as is the case with cytosine-methylated genomic DNA) the flexible nature of the recognition sequence results in an overlap of sites and a smeared, rather than a sharp, banding pattern is produced.

## APPLICATIONS

- Differentiation of methylation patterns

## RESTRICTION ENZYME DIGESTION PROTOCOL

- Add the following components to a sterile microcentrifuge tube (restriction enzyme should be added last):

COMPONENT	25 µl REACTION	50 µl REACTION
DNA	0.5 µg	1 µg
10X NEBuffer	2.5 µl	5 µl
Nuclease-free water	to 25 µl	to 50 µl
Restriction Enzyme*	5 units	10 units

\* Restriction enzymes can be diluted using the recommended diluent buffer.

- Gently mix the reaction by pipetting up and down and microfuge briefly
- Incubate at the recommended temperature for 1 hour or 5 minutes for Time-Saver™ qualified restriction enzymes (see [www.neb.com/TimeSaver](http://www.neb.com/TimeSaver) for more information)
- Terminate the reaction by heat inactivation or DNA purification according to product recommendations

## APPLICATIONS

- CpG methylation studies (6–10)
- Methylated cytosine detection
- Methylated DNA enrichment (11)

## MspJI Family of Restriction Enzymes

Scientists at NEB recently identified the MspJI family of restriction enzymes, which are dependent on methylation and hydroxymethylation for cleavage to occur (12). These enzymes excise DNA fragments containing a centrally located 5hmC or 5mC modified residue that can be extracted and sequenced. Due to the known position of this epigenetic modification, bisulfite conversion is not required prior to downstream analysis.

Table 4B: Methylation Dependent Restriction Enzymes

	METHYLATION SENSITIVITY	SEQUENCE	NEB #	ISOSCHIZOMER
<b>AbaSI</b> (1, 2, 3)	Recognizes 5-glucosylhydroxy-methylcytosine ( <sup>ghm</sup> C) in double-stranded DNA and cleaves 11–13 bases 3' from the modified C	$  \begin{array}{c}  5' \dots \text{ghmC} \text{N}_{11-13} \text{N}_{9-16} \text{G} \dots 3' \\  3' \dots \text{G} \text{N}_{9-10} \text{N}_{11-13} \text{xC} \dots 5' \\  \text{xC} = \text{ghmC}, \text{hmC}, \text{mC} \text{ or } \text{C}  \end{array}  $	R0665	N/A
<b>DpnI</b>	Cleaves fully-adenomethylated dam** sites (hemi-adenomethylated dam sites 60X more slowly). Cleavage of mammalian genomic DNA is blocked by overlapping CpG methylation.	$  \begin{array}{c}  \text{CH}_3 \\    \\  5' \dots \text{GATC} \dots 3' \\  3' \dots \text{CTAG} \dots 5' \\    \\  \text{CH}_3  \end{array}  $	R0176	DpnII
<b>FspEI</b> (6)	Cleaves DNA containing 5-methylcytosine and 5-hydroxymethylcytosine	$  \begin{array}{c}  5' \dots \text{C}^{\text{m}}\text{C}(\text{N})_{12} \dots 3' \\  3' \dots \text{G} \text{G}(\text{N})_{16} \dots 5'  \end{array}  $	R0662	N/A
<b>LpnPI</b> (6)	Cleaves DNA containing 5-methylcytosine and 5-hydroxymethylcytosine	$  \begin{array}{c}  5' \dots \text{C}^{\text{m}}\text{C D G}(\text{N})_{10} \dots 3' \\  3' \dots \text{G G H C}(\text{N})_{14} \dots 5'  \end{array}  $	R0663	N/A
<b>McrBC</b>	Cleaves DNA containing 5-methylcytosine, 5-hydroxymethylcytosine or N4-methylcytosine on one or both strands.	$  \begin{array}{c}  5' \dots \text{PumC(N40-3000)PumC} \dots 3' \\  \text{Optimum spacing is N55-103} \\  \text{Pu} = \text{A or G} \\  \text{Cleavage site is between the half-sites and } \sim 30 \text{ bp from one of the half-sites}  \end{array}  $	M0272	N/A
<b>MspJI</b> (4,5,6,7,8)	Cleaves DNA containing 5-methylcytosine and 5-hydroxymethylcytosine	$  \begin{array}{c}  5' \dots \text{mC N N R}(\text{N})_9 \dots 3' \\  3' \dots \text{G N N Y}(\text{N})_{13} \dots 5'  \end{array}  $	R0661	N/A

\*\* dam sites: methylation at the N6 position of the adenine in the sequence GATC (GmATC).

H = A or C or T, not G

D = A or G or T, not C

### ADVANTAGES

- Specificity to epigenetically relevant DNA modifications (5mC and 5hmC)
- Easy-to-use protocols (enzymatic digestion followed by gel extraction)
- Less harsh than bisulfite conversion
- Simplified data analysis

### GENOMIC DNA DIGESTION (MspJI) PROTOCOL

1. Set up the following reaction in a sterile microcentrifuge tube (it is important to add the recommended amount of MspJI last):

COMPONENT	STANDARD REACTION
DNA (0.5 to 1 µg)	1–5 µg
10X NEBuffer 4	3 µl
BSA	1 µl
MspJI	0.5–1 µl (2 to 4 units)
Nuclease-free water	to 30 µl

2. Incubate at 37°C for 16 hours.

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# DNA Methyltransferases

NEB offers a selection of DNA methyltransferases that can be used to generate methylated DNA at specific sites for gene expression studies. Our selection includes CpG methyltransferases, which is especially useful for studying CpG methylation effects.

PRODUCT	NEB #	SEQUENCE
<b>CYTOSINE-C5 METHYLTRANSFERASES</b>		
Human DNA (cytosine-5) Methyltransferase (DNMT1)	M0230S/L	$  \begin{array}{c}  \text{CH}_3 \\    \\  5' \dots \text{C G} \dots 3' \\  3' \dots \text{G C} \dots 5'  \end{array}  \xrightarrow{\text{Human DNMT1}}  \begin{array}{c}  \text{CH}_3 \\    \\  5' \dots \text{C G} \dots 3' \\  3' \dots \text{G C} \dots 5'  \end{array}  $
CpG Methyltransferase (M.SssI)	M0226S/L	$  \begin{array}{c}  \text{CH}_3 \\    \\  5' \dots \text{C G} \dots 3' \\  3' \dots \text{G C} \dots 5'  \end{array}  $
GpC Methyltransferase (M.CviPI)	M0227S/L	$  \begin{array}{c}  \text{CH}_3 \\    \\  5' \dots \text{G C} \dots 3' \\  3' \dots \text{C G} \dots 5'  \end{array}  $
AluI Methyltransferase	M0220S/L	$  \begin{array}{c}  \text{CH}_3 \\    \\  5' \dots \text{A G C T} \dots 3' \\  3' \dots \text{T C G A} \dots 5'  \end{array}  $
HaeIII Methyltransferase	M0224S/L	$  \begin{array}{c}  \text{CH}_3 \\    \\  5' \dots \text{G G C C} \dots 3' \\  3' \dots \text{C C G G} \dots 5'  \end{array}  $
HhaI Methyltransferase	M0217S/L	$  \begin{array}{c}  \text{CH}_3 \\    \\  5' \dots \text{G C G C} \dots 3' \\  3' \dots \text{C G C G} \dots 5'  \end{array}  $
HpaII Methyltransferase	M0214S/L	$  \begin{array}{c}  \text{CH}_3 \\    \\  5' \dots \text{C C G G} \dots 3' \\  3' \dots \text{G G C C} \dots 5'  \end{array}  $
MspI Methyltransferase	M0215S/L	$  \begin{array}{c}  \text{CH}_3 \\    \\  5' \dots \text{C C G G} \dots 3' \\  3' \dots \text{G G C C} \dots 5'  \end{array}  $
<b>CYTOSINE-N4 METHYLTRANSFERASE</b>		
BamHI Methyltransferase	M0223S/L	$  \begin{array}{c}  \text{CH}_3 \\    \\  5' \dots \text{G G A T C C} \dots 3' \\  3' \dots \text{C C T A G G} \dots 5'  \end{array}  $
<b>ADENINE-N6 METHYLTRANSFERASES</b>		
<i>dam</i> Methyltransferase	M0222S/L	$  \begin{array}{c}  \text{CH}_3 \\    \\  5' \dots \text{G A T C} \dots 3' \\  3' \dots \text{C T A G} \dots 5'  \end{array}  $
EcoRI Methyltransferase	M0211S/L	$  \begin{array}{c}  \text{CH}_3 \\    \\  5' \dots \text{G A A T T C} \dots 3' \\  3' \dots \text{C T T A A G} \dots 5'  \end{array}  $
TaqI Methyltransferase	M0219S/L	$  \begin{array}{c}  \text{CH}_3 \\    \\  5' \dots \text{T C G A} \dots 3' \\  3' \dots \text{A G C T} \dots 5'  \end{array}  $

## APPLICATIONS

- Blocking restriction enzyme cleavage
- Generating positive control DNA samples for methylation-specific PCR or bisulfite sequencing experiments
- Studying CpG methylation-dependent gene expression [CpG Methyltransferase (M.SssI), NEB #M0226]
- Probing sequence-specific contacts within the major groove of DNA
- Nucleosome footprinting
- Uniform [<sup>3</sup>H]-labeling of DNA
- Altering the physical properties of DNA [e.g., methylcytosines lower the free energy of Z-DNA formation (1), increase the helical pitch of DNA (2), alter the kinetics of cruciform extrusion (3) and decrease reactivity to hydrazine (4)]

## Genomic DNA Methylation Using CpG Methyltransferase (*M. SssI*)

CpG Methyltransferase (*M. SssI*) may be useful for studying the function of cytosine methylation in higher eukaryotes as its specificity mimics the pattern of modification found in their genomes (1). In contrast to the mammalian enzymes (2,3), both unmethylated and hemi-methylated DNA substrates are methylated with equal efficiency by this CpG methyltransferase (4), making it a more useful tool for modifying DNA.

CpG Methyltransferase can be used to block cleavage by a variety of restriction endonucleases whose recognition sites either contain the sequence CG, or overlap the dinucleotide. It should be noted that DNAs methylated by the CpG Methyltransferase are subject to Mcr and Mrr restriction in *E. coli*, and thus should be transformed into Mcr- Mrr- *E. coli* strains.

The high density of CpG dinucleotides in DNA substrates should be taken into account when methylating DNAs *in vitro*. For example, lambda DNA (48,502 bp) contains 3,112 CpG sites, and thus a 0.1 mg DNA/ml solution is 19  $\mu$ M with respect to methyl acceptor sites for the methyltransferase. This is significant because the recommended concentration of methyl donor, S-adenosylmethionine (SAM, AdoMet), is 160  $\mu$ M, an 8-fold excess over acceptor sites. Reducing the DNA concentration (<0.02 mg/ml) gives two advantages. First, the SAM concentration remains high enough to drive the reaction. Second, potential end-product inhibition, arising from S-adenosyl-L-homocysteine (SAH, AdoHcy) generated during the reaction, is limited.

### Protocol:

- For the standard reaction in step 2, dilute SAM to 1600  $\mu$ M using the supplied 32 mM stock. (1  $\mu$ l SAM, 19  $\mu$ l Nuclease-free water).
- Add the following to a sterile microcentrifuge tube, in the order listed:

	STANDARD REACTION	REPRESENTATIVE LARGE-SCALE REACTION
Nuclease-free water	14 $\mu$ l	220 $\mu$ l
10X NEBuffer 2	2 $\mu$ l	50 $\mu$ l
SAM	2 $\mu$ l from step 1	10 $\mu$ l (32 $\mu$ M SAM)
Genomic DNA	1 $\mu$ l (1 $\mu$ g)	200 $\mu$ l (500 $\mu$ g/ml $\lambda$ DNA)
CpG methylase ( <i>M. SssI</i> )	1 $\mu$ l (4 U/ $\mu$ l)	20 $\mu$ l (20 U/ $\mu$ l)

- Mix by pipetting up and down at least six times.
- Incubate for one hour at 37°C.
- Stop the reaction by heating at 65°C for 20 minutes.
- DNA can be purified by phenol extraction followed by ethanol precipitation or by using a commercial DNA purification kit. For long-term storage at -20°C, suspend in TE.

### TIPS

- MgCl<sub>2</sub> is not required as a cofactor. In the presence of Mg<sup>2+</sup>, methylation by *M. SssI* becomes distributive rather than processive and also exhibits topoisomerase activity (5).
- Adding more AdoMet after 4 hours can improve results, and using more enzyme for less time may improve methylation. Methylation reactions, however, are greatly affected by AdoHcy (6), which is a by-product of the methylation reaction and binds more tightly to methylases than does AdoMet. Inhibition by AdoHcy greatly reduces the reaction rate.
- The incubation time can be increased to 4 hours. Overnight incubations do not give significant increases in methylation.
- The volume of DNA can be increased to 5  $\mu$ l. When using more dilute DNA, increase the reaction volume to 50  $\mu$ l. Using too much DNA volume in the reaction can cause inhibition by changing the pH or salt concentration of the reaction.
- Up to 4  $\mu$ g of DNA can be methylated in a 20  $\mu$ l reaction. The SAM concentration should be adjusted to 640  $\mu$ M. Concentrated *SssI* (NEB #M0226) (1  $\mu$ l of 20,000 U/ml) should be used.
- The protocol can also be used for other types of DNA, including plasmids and purified PCR products.

### TOOLS & RESOURCES

Visit [www.neb.com](http://www.neb.com) to find:



A protocol for labeling genomic DNA with [<sup>3</sup>H] using methyltransferases

### References

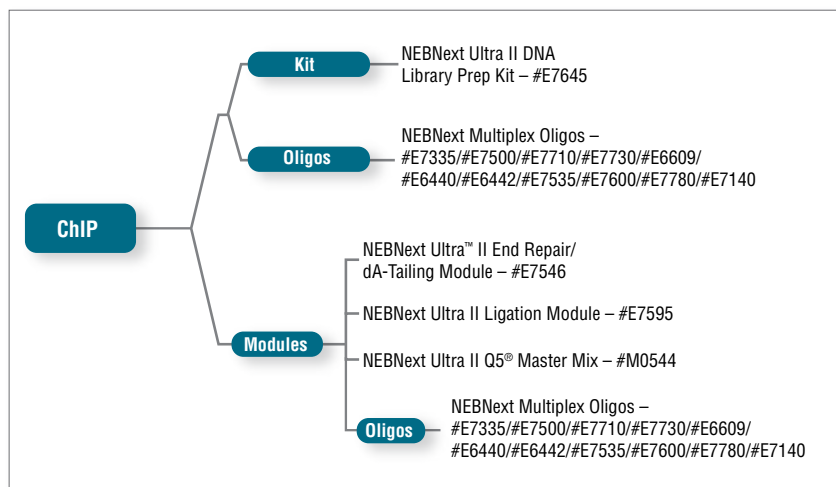
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# Sample Preparation for ChIP-Seq

## NEBNext Reagents

NEBNext reagents are a series of highly pure reagents that facilitate library preparation of DNA or RNA for downstream applications, such as next generation sequencing and expression library construction. These reagents undergo stringent quality controls and functional validation, ensuring maximum yield, convenience and value.

For sample preparation of a ChIP-Seq DNA library, NEB offers kits, oligos and modules that support standard or fast workflows. To decide which products to choose, use the selection chart below.



### ADVANTAGES

- Fast high-performance workflows with minimal hands-on-time
- Convenient formats include kits and modules
- All reagents undergo stringent quality controls, plus sequencing validation
- Value pricing

### TOOLS & RESOURCES

Visit [www.NEBNext.com](http://www.NEBNext.com) to find:



Complete list of NEBNext reagents for sample prep of DNA or RNA for next generation sequencing

Protocols & FAQs

Online tutorials to help with product selection, general handling tips and more

Access to NEBNext Selector Tool, our online tool for help with selecting the right NEBNext product

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WHY CHOOSE NEBNext REAGENTS FOR NGS LIBRARY PREP?

# Methylated and Hypomethylated DNA

Positive and negative control DNAs are especially important for studies using sensitive PCR-based assays. NEB offers three sets of genomic DNA that are untreated or treated with CpG Methylase (*M. SssI*), which methylates cytosine residues (C5) within the double-stranded dinucleotides recognition sequence 5'...CG...3'. The methylation-positive DNAs are extensively tested for complete methylation by an additional methyl group transfer assay and methylation-specific PCR.

A partially demethylated DNA control has also been created by treating Jurkat cells with a potent methyltransferase inhibitor (5-Aza-2-deoxycytidine, 5-Aza-dc). Hypomethylation is verified using bisulfite conversion and sequencing to analyze a section of intergenic (IGS) repetitive DNA, which is normally highly methylated.

CpG Methylated Jurkat Genomic DNA .....	N4002S
5-Aza-dc-Treated Jurkat Genomic DNA .....	N4003S
NIH 3T3 Mouse Genomic DNA .....	N4004S
HeLa Genomic DNA .....	N4006S
CpG Methylated HeLa Genomic DNA .....	N4007S

## APPLICATIONS

- PCR
- SNP analysis
- Southern blotting
- Genomic DNA library construction
- Methylation-specific PCR (MSP)
- Enzymatic Methyl-seq (EM-seq)
- Bisulfite sequencing
- Methylation-sensitive single-nucleotide primer extension (ms-SNUPE)
- Combined bisulfite restriction analysis (COBRA)
- Bisulfite treatment and PCR single-stranded confirmation polymorphism analysis (Bisulfite-PCR-SSCP/BiPS)

# Chromatin and Histones

In eukaryotes, chromatin is organized into nucleosome core particles (NCPs) that consist of approximately 147 bp of DNA and an octamer complex made up of two molecules of each histone (H2A, H2B, H3 and H4). The linker histone H1 further condenses chromatin by binding to DNA between the nucleosome core particles (1). Chromatin can be generally classified as condensed, transcriptionally silent heterochromatin or less-condensed, transcriptionally active euchromatin. The dynamic nature of the chromatin predicts different conformational forms exist in the nucleus at a given time. Furthermore, chromatin structure is influenced by the modification of DNA or histones that comprise it and by its transcriptional state (2). Although, most genomic DNA is believed to be packed into heterochromatin (telomeres, pericentric regions and areas rich in repetitive sequences), looping of large stretches of chromatin from a chromosome to generate local secondary structure poised for transcription is observed (3).

New England Biolabs offers a selection of unmodified, recombinant human histones that function as substrates for histone-modifying enzymes. Seven human histones, including three histone H3 variants, have been individually cloned in *E. coli* expression vectors and then purified from *E. coli* cell extracts. Mass spectrometry analysis demonstrates that these histones are free of post-translational modifications. To aid in studying intact nucleosomes, NEB also offers the EpiMark Nucleosome Assembly Kit. The precise mixing of preformed recombinant Human Histone H2A/H2B Dimer and Histone H3.1/H4 Tetramer generates a human histone octamer, and in the presence of DNA, forms nucleosomes (4,5). Enzymes that are unable to modify individual histone or DNA may be active on these nucleosome core particles, the histone dimer or the histone tetramer (6).

## EpiMark Nucleosome Assembly Kit

This kit contains the components necessary to form an unmodified recombinant human nucleosome using experimental DNA of interest or the supplied control DNA. The protocol requires the mixing of already formed and purified recombinant human histone H2A/H2B dimer and histone H3.1/H4 tetramer in the presence of DNA at high salt, followed by dialysis down to low salt to form nucleosomes. One tetramer associates with two dimers to form the histone octamer on the DNA, generating a nucleosome. A method for assaying nucleosome formation by gel shift assay is also provided. These nucleosomes may serve as better substrates for enzymes that are inactive on the DNA or one of the core histones alone. Each described reaction creates nucleosomes from ~50 pmol of a 208 bp DNA and may be scaled depending on the experiment.

EpiMark Nucleosome Assembly Kit.....E5350S

### APPLICATIONS

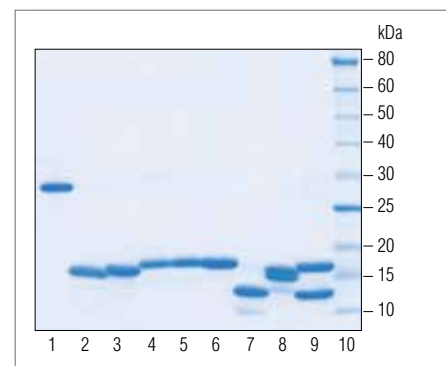
- Purification and characterization of enzymes that modify histone proteins
- Formation of unmodified nucleosome core particles, which may be modified by enzymes that are inactive on individual histones or DNA

### TOOLS & RESOURCES



Visit [www.epimark.com](http://www.epimark.com) for more information on histone modifications

## Highly Purified Histones from NEB



### Experience the purity of Histones from NEB.

SDS-PAGE analysis of the histones available from NEB.

1. Histone H1<sup>o</sup> (NEB #M2501) 1 µg
2. Histone H2A (NEB #M2502) 1 µg
3. Histone H2B (NEB #M2505) 1 µg
4. Histone H3.1 (NEB #M2503) 1 µg
5. Histone H3.2 (NEB #M2506) 1 µg
6. Histone H3.3 (NEB #M2507) 1 µg
7. Histone H4 (NEB #M2504) 1 µg
8. Histone H2A/H2B Dimer (NEB #M2508) 2 µg
9. Histone H3.1/H4 Tetramer (NEB #M2509) 2 µg
10. NEB Protein Ladder (NEB #P7703)

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# Recombinant Human Histones

## Histone H1°

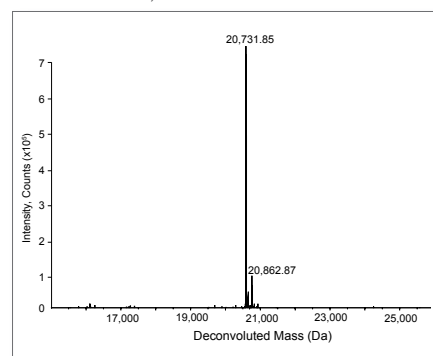
Histone H1 acts on the linker region of polynucleosome DNA to condense the chromatin into structures of ~30 nm (1) and is not necessary for octamer or nucleosome core particle formation.

Eight different histone H1 proteins have been identified in the human genome (2).

Histone H1° is a non replication-dependent histone that is highly expressed in cells that have terminally differentiated (3). Recombinant human histone H1 from NEB is expressed in *E. coli* using the H1F0 or H1FV gene (Genbank accession number: X03473).

Histone H1° Human, Recombinant .....M2501S

Mass Spectroscopy Analysis of Histone H1° Human, Recombinant



## Histones H2A & H2B

Histone H2A interacts with histone H2B to form the H2A/H2B heterodimer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer (1,4). Histones H2A and H2B are modified by various enzymes and have been shown to be important in gene transcription (5).

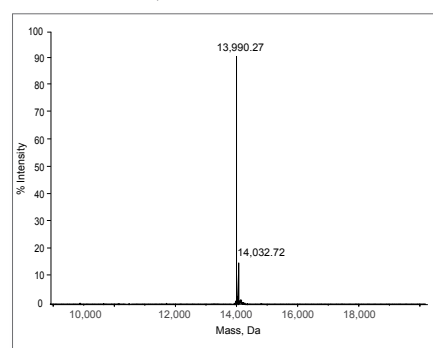
Recombinant human histones H2A and H2B are expressed in *E. coli* using the HIST3H2A gene (Genbank accession number: AY131974) and the HIST2H2BE or H2BFQ gene (Genbank accession number: AY131979), respectively. NEB also offers the preformed histone H2A/H2B dimer. This is generated by refolding the denatured, purified subunits H2A and H2B, followed by gel filtration.

Histone H2A Human, Recombinant .....M2502S

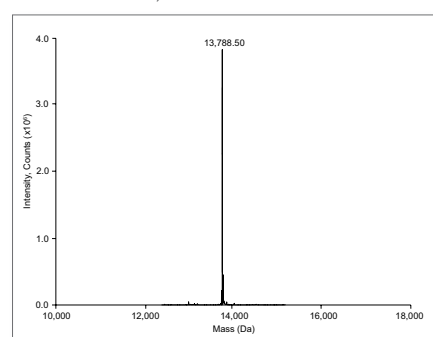
Histone H2B Human, Recombinant .....M2505S

Histone H2A/H2B Dimer Human, Recombinant.....M2508S

Mass Spectroscopy Analysis of Histone H2A Human, Recombinant



Mass Spectroscopy Analysis of Histone H2B Human, Recombinant



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## Histones H3 & H4

Histone H3 interacts with histone H4 to form the H3/H4 tetramer. Two H2A/H2B heterodimers interact with an H3/H4 tetramer to form the histone octamer (1,2).

Histone H3.1, an H3 variant that has thus far only been found in mammals, is replication-dependent and is associated with gene activation and gene silencing (3). Histone H3.2, an H3 variant that is found in all eukaryotes, except budding yeast, is replication-dependent and is associated with gene silencing (4). Histone H3.3, an H3 variant that is found in all eukaryotes from yeast to human, is replication and cell cycle phase-independent and is the most common H3 in non-dividing cells (5). It has been shown to be enriched in covalent modifications associated with gene activation (4,6).

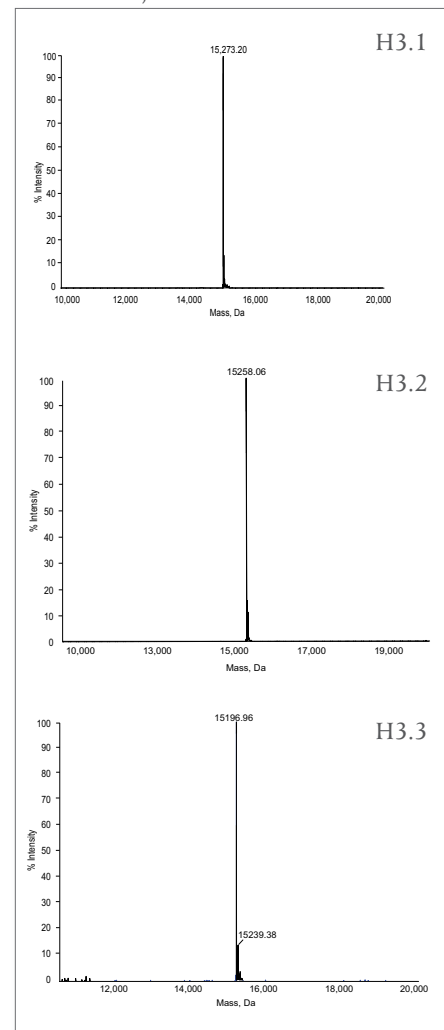
Recombinant human histones H3.1, H3.2 and H3.3 are synthesized in *E. coli* using the HIST1H3A or H3FA gene (Genbank accession number: AF531274), HIST2H3A or HIST2H3C gene (Genbank accession number: BC130637) and H3F3A or H3F3B gene (Genbank accession number: AK311905), respectively. Recombinant human histone H4 is synthesized in *E. coli* using the HIST2H4 gene (Genbank accession number: AF525682). NEB also offers preformed recombinant histone H3.1/H4 tetramer. This is generated by refolding the denatured, purified subunits H3.1 and H4, followed by gel filtration.

Histone H3.1 Human, Recombinant .....	M2503S
Histone H3.2 Human, Recombinant .....	M2506S
Histone H3.3 Human, Recombinant .....	M2507S
Histone H4 Human, Recombinant .....	M2504S
Histone H3.1/H4 Tetramer Human, Recombinant .....	M2509S

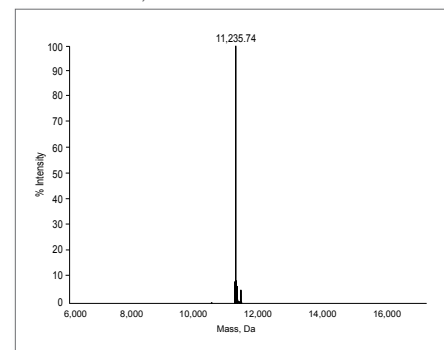
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## Mass Spectroscopy Analysis of Histone H3 Human, Recombinant



## Mass Spectroscopy Analysis of Histone H4 Human, Recombinant



# Histone Modifications

The core histones consist of a globular C-terminal domain and an unstructured N-terminal tail. Although a variety of modifications occur throughout the histone protein (see Table 1), they occur primarily on the N-terminal tail (1-5). Through their potential combinatorial modification on a given histone and its reversibility, these modifications dynamically restrict or recruit numerous other proteins or protein complexes onto chromatin (5). The study of their roles in gene regulation (6), cellular stress events (6), aging and DNA repair (7) is revealing the multiple functions of histone modifications in determining the fate of a cell. Additional variability is incorporated into the system by histone variants. Acting individually or in conjunction with DNA modification, histone modifications and histone variants are thought to establish an epigenetic code or epigenetic signature for gene regulation (5).

Table 1: Types of Histone Modifications

AMINO ACID	MODIFICATION
Lysine	Methylation, Acetylation, Ubiquitination, Sumoylation, ADP-Ribosylation
Arginine	Methylation
Serine	Phosphorylation
Threonine	Phosphorylation

## Methods for Studying Histone Modifications

One of the most widely used methods for studying histone modifications *in vivo* is chromatin immunoprecipitation (ChIP). In brief, protein and DNA are generally cross-linked by formaldehyde treatment. After the chromatin is fragmented by sonication, antibodies specific for a histone modification or chromatin binding protein are used to immunoprecipitate the DNA. The histones from NEB can be used as carrier chromatin in CChIP (Carrier Chromatin ImmunoPrecipitation) assays (8). For large-scale analyses, the isolated DNA can be analyzed on a microarray (ChIP-chip) or by sequencing (ChIP-seq, see page 20). The limitations of traditional ChIP (e.g., quality of the antibody, bias from fixation and fragmentation, and interference from other histone-binding proteins) are partially addressed by alternative methods, such as N-ChIP (Native-ChIP), biotin-tag affinity purification, and DamID (reviewed in 9).

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# Histone Methyltransferases

Lysine or arginine residues in histones undergo enzymatic methylation via the attachment of one, two or three methyl groups. The timing of the appearance of these modifications is often dynamic and will depend on the signaling condition of the cell. Histone modifications participate in transcription, repair, replication and chromatin condensation. NEB offers a selection of protein methyltransferases specific for histone H3.1, H3.2, H3.3 and histone H4.

## G9a Methyltransferase

G9a methyltransferase methylates lysine 9 (Lys 9) of histone H3 (1-3). Methylation occurs at the  $\epsilon$  amino group of lysine residues. Methylation of histone H3 Lys 9 is a hallmark of silent chromatin and is globally distributed throughout the heterochromatic regions, such as centromeres and telomeres (4,5). The G9a enzyme from NEB is expressed from mouse G9a cDNA (1,2).

## Human PRMT1 Methyltransferase

PRMT1 is a major protein arginine methyltransferase (6). It specifically methylates arginine 3 (Arg 3) of histone H4. Furthermore, methylation of histone H4 at Arg 3 facilitates transcriptional activation by nuclear hormone receptors (7). In addition, the ordered cooperative functions of PRMT1, p300 and CARM1 in transcriptional activation by p53 is observed on the GADD45 gene following ectopic p53 expression and/or UV irradiation (8). The PRMT1 enzyme from NEB is expressed from rat PMRT1 cDNA.

## SET7 Methyltransferase

SET7 Methyltransferase methylates lysine 4 (Lys 4) of histone H3 (9). Methylation occurs at the  $\epsilon$  amino group of lysine residues (10,11). Di- and tri- methylation of histone H3 Lys 4 is a hallmark of transcriptionally active chromatin and is globally distributed (12,13). The SET7 enzyme from NEB is expressed from human SET7 cDNA.

## SET8 Methyltransferase

SET8 (PR-Set7) Methyltransferase mono-methylates lysine 20 of histone H4 (H4-K20) at the  $\epsilon$  amino group of lysine residues. SET8-mediated histone H4 methylation is implicated in genome replication and stability; and plays an important role in the nodal pathways of embryo development.

## Human DNA (cytosine-5) Methyltransferase (DNMT1)

DNMT1 methylates cytosine residues in hemimethylated DNA at 5'...CG...3' sites (14,15). Mammalian DNA methylation afforded by DNMT1 is involved in carcinogenesis, embryonic development and several other biological functions (16-18).

G9a Methyltransferase .....	M0235S
Human PRMT1 Methyltransferase .....	M0221S
SET7 Methyltransferase.....	M0233S
SET8 Methyltransferase.....	M0428S
Human DNA (cytosine-5) Methyltransferase (DNMT1) .....	M0230S/L

### APPLICATIONS

- Purification and characterization of enzymes that modify histone proteins
- Octamer modification studies
- Carrier Chromatin Immunoprecipitation (CChIP)

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<b>METHYLOME ANALYSIS</b>		
NEBNext Enzymatic Methyl-seq (EM-seq) Kit	E7120	24/96 reactions
NEBNext Enzymatic Methyl-seq Module	E7125	24/96 reactions
NEBNext Multiplex Oligos for Enzymatic Methyl-seq (Unique Dual Index Primer Pairs)	E7140S/L	24/96 reactions
EpiMark 5-hmC and 5-mC Analysis Kit	E3317S	20 reactions
EpiMark Bisulfite Conversion Kit	E3318S	48 reactions
EpiMark Hot Start <i>Taq</i> DNA Polymerase	M0490S/L	100/500 reactions
EpiMark N6-Methyladenosine Enrichment Kit	E1610S	20 reactions
EpiMark Methylated DNA Enrichment Kit	E2600S	25 reactions
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DpnI	R0176S/L	1,000/5,000 units
DpnII	R0543S/T/L/M	1,000/1,000/5,000/5,000 units
FspEI	R0662S/L	200/1,000 units
HpaII	R0171S/M/L	2,000/10,000/10,000 units
LpnPI	R0663S/L	200/1,000 units
MspI	R0106S/T/M/L	5,000/5,000/25,000/25,000 units
MspJI	R0661S/L	200/1,000 units
5-Methyl-dCTP	N0356S	1 µmol
McrBC	M0272S/L	500/2,500 units
<b>METHYLTRANSFERASES &amp; ANTIBODIES</b>		
G9a Methyltransferase	M0235S	100 units
PRMT1 Methyltransferase	M0221S	50 units
SET7 Methyltransferase	M0233S	100 units
SET8 Methyltransferase	M0428S	100 units
Human DNA (cytosine-5) Methyltransferase (Dnmt1)	M0230S/L	50/250 units
CpG Methyltransferase (M.SssI)	M0226S/M/L	100/500/500 units
GpC Methyltransferase (M.CviPI)	M0227S/L	200/1,000 units
HpaII Methyltransferase	M0214S/L	100/500 units
MspI Methyltransferase	M0215S/L	100/500 units
EcoRI Methyltransferase	M0211S/L	10,000/50,000 units
dam Methyltransferase	M0222S/L	500/2,500 units
BamHI Methyltransferase	M0223S/L	100/500 units
HhaI Methyltransferase	M0217S/L	1,000/5,000 units
TaqI Methyltransferase	M0219S/L	1,000/5,000 units
AluI Methyltransferase	M0220S/L	100/500 units
HaeIII Methyltransferase	M0224S/L	500/2,500 units
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NEBNext Ultra II Ligation Module	E7595S/L	24/96 reactions
NEBNext Ultra II Q5 Master Mix	M0544S/L	50/250 reactions
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5-Aza-dc Treated Jurkat Genomic DNA	N4003S	15 µg
NIH 3T3 Mouse Genomic DNA	N4004S	15 µg
HeLa Genomic DNA	N4006S	15 µg
CpG Methylated HeLa Genomic DNA	N4007S	15 µg
<b>HISTONES</b>		
EpiMark Nucleosome Assembly Kit	E5350S	20 reactions
H1 <sup>9</sup> Human, Recombinant	M2501S	100 µg
H2A Human, Recombinant	M2502S	100 µg
H2B Human, Recombinant	M2505S	100 µg
H3.1 Human, Recombinant	M2503S	100 µg
H3.2 Human, Recombinant	M2506S	100 µg
H3.3 Human, Recombinant	M2507S	100 µg
H4 Human, Recombinant	M2504S	100 µg
Histone H3.1/H4 Tetramer Human, Recombinant	M2509S	1 nmol
Histone H2A/H2B Dimer Human, Recombinant	M2508S	2 nmol
Nucleosome Control DNA	N1202S	0.2 nmol





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