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THE DECODER

A NEWSLETTER FOR EPIGENETIC RESEARCHERS

2012 • Issue 2

DNA Methylation Analysis of 5mC, 5hmC, and 5fC Bases

ChIP & Protein-DNA Interaction

High Throughput DNA & Chromatin Shearing

Histone Modification Studies

"Rosetta Stone"

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Advances

in Technologies & Tools for Epigenetic Analysis

A brief look into technological developments in epigenetics over the recent years as the constantly and rapidly evolving landscape of epigenetic research is shaped by new discoveries, novel techniques, and better technologies.

Advances in technologies and tools for epigenetic analysis

The methods and technologies for epigenetic analysis have significantly advanced in the past decade. The most important advancement is the combination of DNA bisulfite modification and ChIP with next generation sequencing, allowing epigenetic analysis, especially in DNA methylation and chromatin/histone modifications, to be carried out at the genome-scale and a base-resolution level. Another key advancement is the emergence of antibody-based immunoassays to measure global (total) DNA methylation or histone modifications in a simpler, high throughput format.

The term epigenetics refers to a heritable change in phenotype that does not involve a change in the underlying DNA sequence. At least three systems including DNA methylation, histone modification, and non-coding RNA (ncRNA)-associated gene silencing are currently considered to initiate and sustain epigenetic change (Fig. 1). DNA methylation occurs by the covalent addition of a methyl group at the 5-carbon of the cytosine ring by DNA methyltransferases, resulting in 5-methylcytosine (5-mC). When a CpG island in the promoter region of a gene is methylated, expression of the gene is repressed. A number of human diseases such as cancer are known to occur when DNA methylation is not properly established and/or maintained. The recent discovery of TET enzyme-mediated hydroxylation of 5-mC to 5-hydroxymethylcytosine (5-hmC), and further modification to 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC) demonstrates that DNA methylation is a dynamic and reversible process [1, 2, 3]. Post translational modifications of either histones or chromatin are associated with transcriptional repression or activation, depending on the modified sites. The histone modifications include acetylation on specific lysine residues by histone acetyltransferases (HATs), deacetylation by histone deacetylases (HDACs), methylation of lysine and arginine residues by histone methyltransferases (HMTs), demethylation of lysine residues by histone demethylases (HDMTs), and phosphorylation of specific serine groups by histone kinases (HKs). Additional histone modifications include the attachment of ubiquitin (Ub), small ubiquitin-like modifiers (SUMOs), and poly-ADP-ribose (PAR) units. These modifications might constitute a “histone code” which can be read and interpreted by different cellular factors. Epigenetic-related non-coding RNA can be divided into two classes: long ncRNA (>200 nts) and short ncRNA (<30 nts), the latter of which includes miRNA, siRNA, and Piwi interacting RNA. These ncRNAs are shown to play a role in heterochromatin formation as well as in DNA methylation targeting and gene silencing. Epigenetic research has seen explosive growth in recent years. Accordingly, the technologies of epigenetic analysis have also significantly advanced.

Global DNA Methylation Analysis. One of the most commonly used methods for quantification of global DNA methylation is digestion of DNA followed by HPLC analysis. This method generally requires relatively large amounts of DNA (50 µg), which may be difficult to obtain in tissues that have low DNA yields. An antibody-based immunoassay for global DNA methylation quantification was recently developed. This method overcomes the drawbacks of HPLC which include low throughput and high costs. The antibody-based method uses a 96-well plate format to provide accurate differential global DNA methylation absorbance readings with as little as 50 ng of genomic DNA. Both HPLC and an immunoassay method can also be used for global hydroxymethylation detection [4, 5].

Another method recently developed for global DNA methylation measurement is the long interspersed nuclear element-1 (LINE-1) assay [6]. The level of LINE–1 methylation can be measured using bisulfite treatment of DNA followed by PCR or pyrosequencing of multiple DNA repetitive elements.

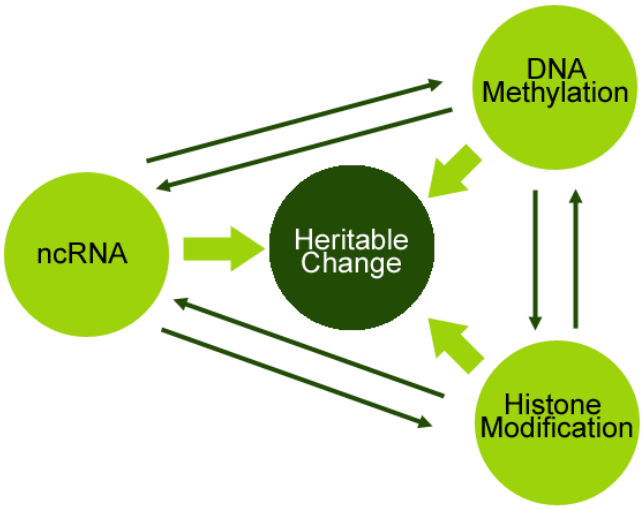

Figure 1 | Schematic of epigenetic alterations initiated and sustained by DNA methylation, histone modification, and non-coding RNA.

Gene/Sequence Specific DNA Methylation Analysis. Many methods for the gene/sequence-specific detection of DNA methylation have been developed over the past decade. Most of these methods involve bisulfite conversion of DNA, followed by PCR or sequencing. Bisulfite conversion of DNA distinguishes methylated cytosine from unmethylated cytosine and enables amplification of DNA methylation to be more specific and reliable. Some well-established methods for gene/sequence-specific detection of DNA methylation include methylation-specific PCR (MS-PCR), methylation-specific single-nucleotide primer extension (MS-SNuPE), combined bisulfite restriction analysis (COBRA), bisulfite sequencing/pyrosequencing technology, methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA), and oligonucleotide based microarray.

Recently, technically improved methods have also been developed. These assays include methylated DNA immunoprecipitation microarray (MeDIP-chip) and next generation sequencing (NGS)-based MeDIP-seq or bisulfite-seq [7, 8]. MeDIP-seq combines MeDIP with massively parallel DNA sequencing and gives full genome coverage, although it does not obtain nucleotide resolution. Bisulfite-seq allows genome-wide or whole genome DNA methylation to be analyzed at single base-resolution. Most recently, hMeDIP-seq and oxBisulfite-seq have been developed for genome-wide hydroxymethylation analysis [9, 10, 11].

Global Histone Modification Analysis. For a lengthy period of time Western blot was used as a major method in the detection of global histone modification. Within the last 10 years different technical approaches have become available to quantify the amount of modified histones. These methods include ELISA-based assays, mass spectrometry analysis, and antibody arrays for multiplexed histone modification detection.

Gene Specifc Chromatin/Histone Modification Analysis. Chromatin immunoprecipitation (ChIP) offers an advantageous tool for studying Protein-DNA interaction and detecting whether a specific protein binds to the specific sequences of a gene in living cells. When studying Protein-DNA interaction most commonly used methods utilize a combination of ChIP with PCR (ChIP-PCR), microarray (ChIP-on-chip), and sequencing (ChIP-seq). Recently, the ChIP

method itself was significantly improved in a number of ways. The assay is now much faster (one-hour ChIP), is capable of high-throughput (96-well plate format-ChIP), has a high enrichment efficiency using as low as 100 cells (carrier ChIP) [12], and allows for precise binding locations (ChIP-exo). ChIP-exo uses exonucleases to degrade strands of the protein-bound DNA in the 5'-3' direction to within a small number of nucleic acids of the protein binding site and allows a single base pair resolution in identifying protein binding locations [13].

New ChIP-seq methods have also been developed for analyzing transcription factors and site-specific histone modifications on a genome-wide scale by combining ChIP with NGS. Furthermore, a method has been developed to detect cross talk between DNA methylation and chromatin states by combining bisulfite conversion, ChIP, and NGS [14]. These new techniques can be used to directly interrogate the genomic relationship between allele-specific DNA methylation, histone modification, or other important epigenetic regulators.

Epigenetic Enzyme Activity Detection. There are several methods for detecting the activity of epigenetic enzymes such as DNMTs, HMTs/HDMTs, HATs and HDACs. These methods include antibody-free fluorescent assays, 3H-release assays, and immunoassays. Immunoassays are still the most commonly used method when measuring the activity of epigenetic enzymes.

Non-coding RNA Detection. Standard Northern blot hybridization is the most commonly used technique when detecting miRNAs and other small ncRNAs. However this method requires the design and synthesis of many sequence-specific, complementary DNA probes, which limits its use in large-scale miRNA detection strategies. Recently, new methods for ncRNA analysis have been developed, including miRNA qRT-PCR and RNA-sequencing [15]. miRNA qRT-PCR significantly increases the detection sensitivity and throughput. Meanwhile RNA-seq allows for genome-scale mapping of ncRNA at base-wise resolution.

Summary

There are many different technologies that have been developed for epigenetic analysis in the past decades, especially for DNA methylation and chromatin/histone modifications. The most important advancement in these technologies is the combination of DNA bisulfite modification and ChIP with next generation sequencing, allowing epigenetic analysis to be carried out at a genome-scale and a base-resolution level. Advances in these technologies allows for a better understanding of epigenetic processes, specifically gene regulation and human diseases, and will lead to the development of new approaches for molecular diagnosis and targeted therapy across the clinical spectrum.

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4. Li W et al: J Nucleic Acids. 2011;2011:870726
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15. Isakov O et al: Nucleic Acids Res. 2012;40(11):e86.

High Throughput DNA & Chromatin Shearing

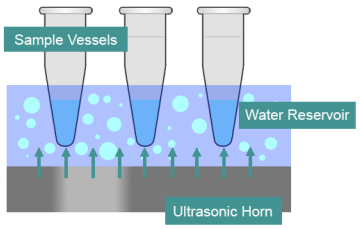
Non-probe based ultrasonic processing is becoming increasingly popular for shearing DNA and chromatin with higher precision and throughput. New technologies have been developed to improve the number of samples that can be simultaneously processed, thereby increasing productivity, while still maintaining reproducibility, consistency, and precision.

The EpiSonic™ 1100 by Epigentek is a high-throughput capable sonication instrument of the latest generation for use in a wide range of biological applications, in particular for DNA and chromatin shearing. This completely digital instrument allows for simultaneous processing of 96 samples or more and can be easily integrated into existing lab workflows.

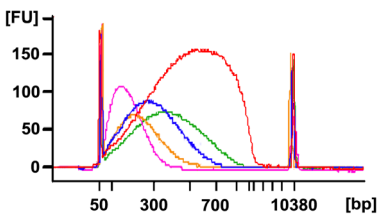
Through the use of Digitally Adaptive Sonocavitation™ (DAS) the EpiSonic™ is able to shear DNA or chromatin as well as lyse cells and tissues with ideal results. DAS technology allows for highly targeted shearing forces to break up DNA, chromatin, and tissues in a non-contact, non-invasive manner. The included circulating chiller ensures that chilled water is automatically recirculated in order to maintain sample integrity and prevent degradation.

Epigentek's EpiSonic™ 1100 has been extensively tested and optimized for shearing samples in a precise, consistent, and reproducible manner. Desired sample sizes are able to be achieved due to the digital precision of the amplitude controls by allowing for user adjustment in intensity intervals of 1% at a time. Samples that are placed in different positions with the included vessel holder are able to maintain consistency and equal sizes between each other. Most importantly, samples are sheared in reproducible lengths between the first run and the next run, creating predictable results and eliminating optimization time and labor.

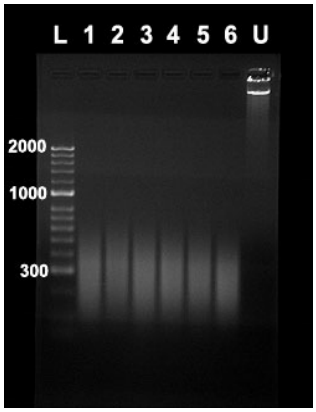
Learn more by visiting www.epigentek.com/episonic.



▲ EpiSonic's sonciation mechanism.



▲ 3 µg of placenta DNA in 0.2 ml tubes were sheared with the EpiSonic™ 1100 for different sonication durations (10, 15, 20, 45, and 62.5 min). The resulting lengths of the sheared DNA were analyzed with an Agilent Bioanalyzer 2100.



▲ Demonstration of consistent DNA shearing in different positions. 2 µg of HeLa DNA in 0.2 ml PCR tubes were inserted into the 0.2ml Tube Rack and processed with 20 sec on 30 sec off for 60 cycles at 20% amplitude. An average size of 250 bp is generated from DNA samples placed in different positions.

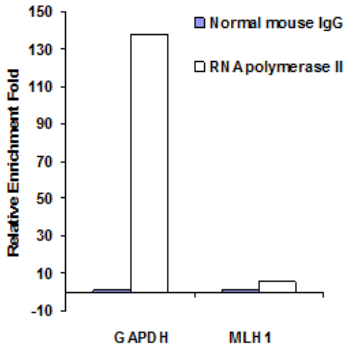
Chromatin Immunoprecipitation

Protein-DNA interaction plays a critical role in various cellular functions such as signal transduction, gene transcription, chromosome segregation, DNA replication and recombination, and epigenetic silencing. Identifying the genetic targets of DNA binding proteins and knowing the mechanisms of protein-DNA interaction is crucial for understanding the cellular process.

General ChIP

Chromatin immunoprecipitation (ChIP) offers an advantageous tool for studying protein-DNA interactions by detecting whether a specific protein binds to the specific sequences of a gene in living cells by PCR (ChIP-PCR), microarrays (ChIP-chip), or sequencing (ChIP-seq).

Currently used ChIP methods have several drawbacks of which the most critical weakness is a lengthy procedure, often involving overnight processes. Additionally, the labor-intensive procedure involves an excessive amount of steps, inconsistency in results, and sub-optimized chromatin shearing. In order to overcome these limitations Epigentek offers the microplate-based ChromaFlash™ One-Step ChIP Kit and the ChromaFlash™ One-Step Magnetic ChIP Kit, each of which can be completed within 3 hours and require as little as 10,000 cells per ChIP reaction. In order to be integrated into workflows the kits have been designed to be compatible with various downstream analyses including ChIP-PCR, ChIP-on-chip, and ChIP-seq. The assays are extremely flexible, allowing for either a single reaction each time or high throughput with 96 reactions each time.

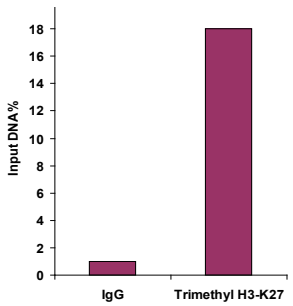


▲ The data above shows the analysis of enrichment of RNA polymerase II in GAPDH and MLH1 promoters by the ChromaFlash™ One-Step ChIP Kit, with chromatin extract prepared from formaldehyde fixed colon cancer cells. Captured DNA was used for analyzing levels of RNA polymerase II enriched in the GAPDH and MLH1 promoters.

Product Name	Size	Cat. No.
ChromaFlash One-Step ChIP Kit	48 reactions	P-2025-48
	96 reactions	P-2025-96
ChromaFlash One-Step Magnetic ChIP Kit	48 reactions	P-2026-48
	96 reactions	P-2026-96

Plant ChIP

The EpiQuik™ Plant ChIP Kit is a convenient microwell based solution for efficiently investigating *in vivo* protein-DNA interaction. Chromatin from the cells is extracted, sheared, and added into the microwell immobilized with the antibody. DNA is released from the antibody-captured protein-DNA complex, reversed, and purified through the specifically designed Fast-Spin Column. The entire procedure can be completed within 6 hours and is compatible with all DNA amplification-based approaches.



▲ DNA was immunoprecipitated from 2-week-old icu2-1/icu2-1 seedlings using the EpiQuik™ Plant ChIP Kit. PCR was used to amplify the ORNITHINE TRANSCARBAMILASE (OTC) gene and regions of the AGAMOUS gene.

Product Name	Size	Cat. No.
EpiQuik Plant ChIP Kit	24 reactions	P-2014-24
	48 reactions	P-2014-48

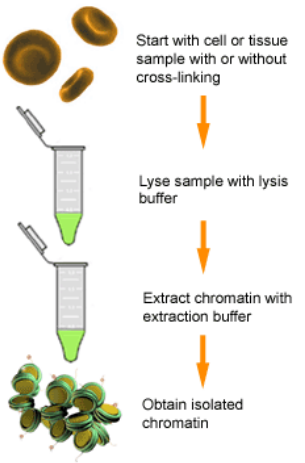
General Protein-DNA Binding Assays

Measurement of direct interactions between protein and DNA *in vitro* has an advantage for analyzing the binding of different transcription factors to specific DNA consensus sequences located in the gene promoters. Several methods have been developed to analyze direct interactions between protein and DNA *in vitro*, however, these methods are time consuming, labor-intensive, and produce radioactive waste. The EpiQuik™ General Protein-DNA Binding Assay Kit uses radioactive-free materials to efficiently measure the transcription factor of DNA binding activity in nuclear extracts within 3 hours.

Product Name	Size	Cat. No.
EpiQuik General Protein-DNA Binding Assay Kit (Colorimetric)	96 assays	P-2004-96

Chromatin Extraction

The ChromaFlash™ Chromatin Extraction Kit is a complete set of optimized buffers and reagents for isolating chromatin or DNA-protein complex from mammalian cells or tissues in a simple and rapid format. Epigentek also offers the ChromaFlash™ Plant Chromatin Extraction Kit for the isolation of chromatin or DNA-protein complexes from plants in a simple and rapid format. These kits are suitable for preparing both native chromatin and cross-linked chromatin from monolayer or suspension cells, or from tissues. Once the Chromatin is extracted it can be used in a variety of standard chromatin immunoprecipitation methods as well in *in vitro* protein-DNA binding assays, or nuclear enzyme assays.

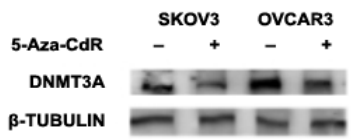


▲ Schematic procedure of chromation isolation with the ChromaFlash Chromatin Extraction Kit.

Product Name	Size	Cat. No.
ChromaFlash Chromatin Extraction Kit	100 extractions	P-2001-100
ChromaFlash Plant Chromatin Extraction Kit	50 extractions	P-2022-050

ChIP-Grade Antibodies

Antibodies are used in chromatin immunoprecipitation (ChIP) to capture the DNA and the DNA protein-complex for further epigenetic study. The quality of the antibody is critical in achieving a successful ChIP experiment. ChIP antibodies must be fully characterized with a high level of affinity and specificity towards a target. Epigentek's ChIP-grade antibodies have been validated for use in chromatin immunoprecipitation with optimal results through extensive testing. They have been tested for specificity and affinity towards various protein targets. To view an entire list of our ChIP grade antibodies visit www.epigentek.com and search for the keyword "ChIP-grade antibody."



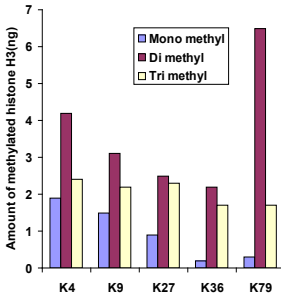
▲ Western blot analysis of DNMT3A in cancer cells treated and untreated with 5-Aza-CdR.

Histone Modification

Histones are primary protein components of eukaryotic chromatin and play a role in gene regulation. H3 and H4 histones have tails protruding from the nucleosome that can be modified posttranslationally to alter the histone's interactions with DNA and nuclear proteins, leading to epigenetic changes for regulating many normal and disease-related processes.

Histone Methylation

Histone methylation can be defined as the modification of certain amino acids through the addition of one, two, or three methyl groups by histone methyltransferases (HMTs). In the cell nucleus, DNA is wound around histones and when methylation of these histones takes place specific genes in DNA will consequently be silenced. Histones H3 and H4 are involved in the structure of chromatin in eukaryotic cells and can undergo several different types of epigenetic modifications that influence cellular processes such as transcription activation, chromosome packaging, and DNA damage/repair.



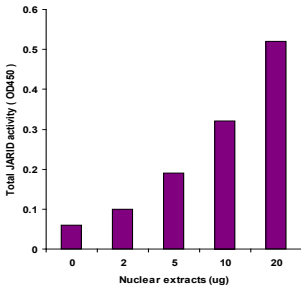
▲ Histone H3 methylation quantification. Histone H3 mono-, di-, and tri-methylation at different sites was quantified with the EpiQuik™ Histone H3 Methylation Quantification Kit series (Fluorometric).

Epigentek offers a complete series of ELISA-like kits, in 48 and 96 well assay sizes, for the quantification of H3 and H4 methylation at all lysines, as well as for the quantification of total histone H3 and *in situ* H3 methylation. Our complete line of histone methylation kits can be found at www.epigentek.com/catalog/histone-modification.php.

Product Name	Cat. No.
EpiQuik Total Histone H3 Quantification Kit (Colorimetric)	P-3062

Histone Demethylases

Histone demethylation is the removal of methyl groups in modified histone proteins via histone demethylases. The discovery of histone demethylases demonstrates that histone methylation is not a permanent modification, but rather a more dynamic process. Two major families of histone demethylases have been discovered: Lysine Specific Demethylase 1 (LDS1) and Jumomji domain containing (JmjC) histone demethylases such as JMJD2, JMJD3/UTX, and JARID1.



▲ Demonstration of high sensitivity of a JARID activity assay achieved by using the Epigenase™ JARID Demethylase Activity/Inhibition Assay Kit (Colorimetric) (Cat. No. P-3082) with A549 nuclear extracts.

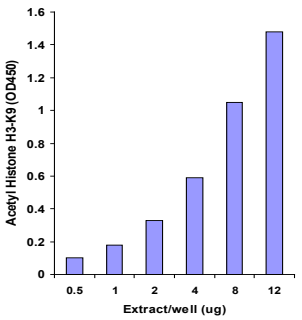
Epigentek's histone demethylase activity/inhibition assay kits for the Jumonji domain-containing histone demethylase enzyme family and for LSD1 are designed for an easy and fast measurement of JMJD2, JMJD3/UTX, JARID, or LSD1 activity or inhibition. The antibody-based, immunospecific kits directly detect converted demethylated products, rather than by-products, in a 96 stripwell microplate format.

Each of these kits are up to 200 times more sensitive than formaldehyde release-based assays. Furthermore our line of histone demethylase kits can be completed in as little as 3 hours.

Product Name	Cat. No.
Epigenase LSD1 Demethylase Activity/Inhibition Assay Kit (Colorimetric)	P-3078
Epigenase JMJD2 Demethylase Activity/Inhibition Assay Kit (Colorimetric)	P-3080
Epigenase JARID Demethylase Activity/Inhibition Assay Kit (Colorimetric)	P-3082
Epigenase JMJD3/UTX Demethylase Activity/Inhibition Assay Kit (Colorimetric)	P-3084

Histone Acetylation

Acetylation of histones H3 and H4 are involved in the regulation of chromatin structure and recruitment of transcription factors to gene promoters. Histone acetylation is tightly involved in cell cycle regulation, cell proliferation, and apoptosis. An imbalance in the equilibrium of histone acetylation has been associated with tumorigenesis and cancer progression.



▲ Histone H3 acetylation quantification. Histone extracts were prepared from MCF-7 cancer cells using the EpiQuik™ Total Histone Extraction Kit. Acetyl H3-K9 was quantified with the EpiQuik™ Global Acetyl Histone H3-K9 Quantification Kit (Colorimetric) (Cat. No. P-4010).

Epigentek's ELISA-based histone acetylation quantification kits can measure total histone H3 or H4 acetylation levels as well as modified histones at specific lysines. Procedures can be finished within 3-4 hours and all assays are available in both 48 and 96 well sizes. Visit www.epigentek.com/catalog/histone-modification.php for a complete line of histone acetylation kits.

Product Name	Cat. No.
EpiQuik Total Histone H3 Acetylation Detection Fast Kit (Colorimetric)	P-4030
EpiQuik Total Histone H4 Acetylation Detection Fast Kit (Colorimetric)	P-4032

HATs and HDACs

Histone acetyltransferases (HATs) and histone deacetylases (HDACs) play a critical role in controlling histone acetylation. Epigentek offers a complete line of kits to quantify each of the HDACs, 1 through 11, or measure total HDAC activity/inhibition using a simple and highly accurate 4-hour ELISA-based assay. Also offered is the HAT activity/inhibition assay kit, which can detect HATs within 3 hours without the need for radioactivity, extraction, or chromatography.

Product Name	Cat. No.
EpiQuik HAT Activity/Inhibition Assay Kit	P-4003
Epigenase HDAC Activity/Inhibition Direct Assay Kit (Colorimetric)	P-4034
EpiQuik HDAC1 Assay Kit	P-4005
EpiQuik HDAC2 Assay Kit	P-4006
EpiQuik HDAC3 Assay Kit	P-4040
EpiQuik HDAC4 Assay Kit	P-4042
EpiQuik HDAC5 Assay Kit	P-4044
EpiQuik HDAC6 Assay Kit	P-4046
EpiQuik HDAC7 Assay Kit	P-4048
EpiQuik HDAC8 Assay Kit	P-4007
EpiQuik HDAC9 Assay Kit	P-4050
EpiQuik HDAC10 Assay Kit	P-4052
EpiQuik HDAC11 Assay Kit	P-4054

Histone Protein Extraction

The EpiQuik™ Total Histone Extraction Kit provides a simple and selective method for extracting total core histone proteins from mammalian cells and tissues in just 60 minutes, while keeping the post-translational modifications in the extracts intact.

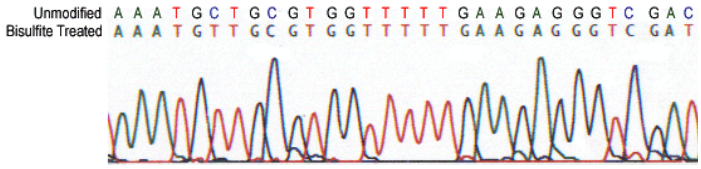
Product Name	Size	Cat. No.
EpiQuik Total Histone Extraction Kit	100 extractions	OP-0006-100

5-Methylcytosine, 5-Hydroxymethylcytosine, & 5-Formylcytosine

DNA methylation occurs by the covalent addition of a methyl group at the 5-carbon of the cytosine ring by DNA methyltransferases, resulting in 5-methylcytosine (5-mC). When a CpG island in the promoter region of a gene is methylated, expression of the gene is repressed. A number of human diseases are known to occur when DNA methylation is not properly established and/or maintained. The recent discovery of TET enzyme-mediated hydroxylation of 5-mC to 5-hydroxymethylcytosine (5-hmC), and further modification to 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC) demonstrates that DNA methylation is a dynamic and reversible process.

DNA Bisulfite Modification

Only bisulfite modification of genomic DNA, followed by PCR amplification, cloning, and sequencing of individual PCR amplimers yields reliable information on the methylation states of individual cytosines on individual DNA molecules. Epigentek's BisulFlash™ DNA Modification Kit is a complete set of optimized buffers and reagents to perform DNA modification using an ultra-fast, next generation DNA bisulfite conversion technology. The entire procedure can be completed in just 30 minutes with a 99.9% conversion rate of cytosine to uracil.

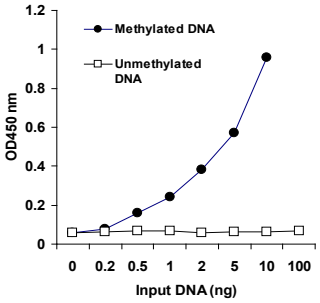


▲ High accuracy of DNA conversion is achieved by the BisulFlash™ kit. 50 ng of genomic DNA methylated in all CpG sites by DNA methylases was treated with the BisulFlash™ DNA Modification Kit. Converted DNA was amplified by real time qPCR using primers for multiple promoters containing numerous CpG sites and then directly sequenced.

Product Name	Size	Cat. No.
BisulFlash DNA Modification Kit	50 reactions	P-1026-050

Global 5-mC Quantification

The biological importance of 5-mC as a major epigenetic modifier of both phenotype and gene expression has been widely recognized. A decrease in 5-mC content (DNA hypomethylation) is likely caused by methyl-deficiency due to a variety of environmental influences, and has been proposed as a molecular marker in multiple biological processes. It has also been well demonstrated that a decrease in global DNA methylation is an important characteristic of cancer.



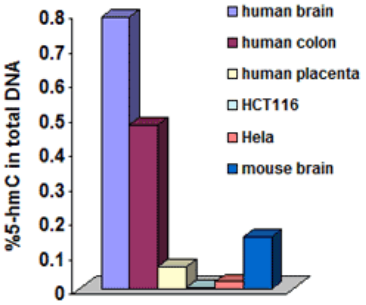
▲ Demonstration of high sensitivity and specificity of methylated DNA detection achieved by the MethylFlash™ kit. Synthetic unmethylated DNA (containing 50% cytosine) and methylated DNA (containing 50% 5-methylcytosine) were added into the assay wells at different concentrations and then measured with the MethylFlash™ Methylated DNA Quantification Kit (Colorimetric).

Epigentek provides the MethylFlash™ Methylated DNA Quantification Kit which uses an ELISA-like method to quantify 5-mC or methylated DNA in DNA samples. The whole procedure can be finished within 4 hours through the use of a unique kit composition that enables background signals to be extremely low, which eliminates the need for plate blocking and allows the assay to be simple, accurate, and reliable. The kit is also highly sensitive, providing a detection limit of as low as 0.2 ng of methylated DNA from just 50 ng of input genomic DNA.

Product Name	Size	Cat. No.
MethylFlash Methylated DNA Quantification Kit (Colorimetric)	48 assays	P-1034-48
	96 assays	P-1034-96

Global 5-hmC Quantification

5-hydroxymethylcytosine (5-hmC) is a modified form of cytosine recently discovered in animal tissues. The function of 5-hmC in epigenetics may be different from its forerunner 5-methylcytosine (5-mC) and may be involved in DNA demethylation. It is believed that 5-hmC plays an important role in switching genes on and off.



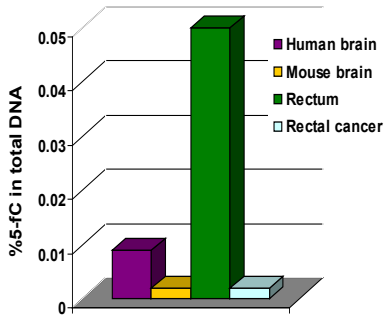
▲ Demonstration of high sensitivity of 5-hydroxymethylcytosine detection achieved by the MethylFlash™ kit. Synthetic hydroxymethylated DNA was added into the assay wells at different concentrations and then measured with the MethylFlash™ Hydroxymethylated DNA Quantification Kit.

Epigentek offers the MethylFlash™ Hydroxymethylated DNA Quantification Kit which provides a cost-effective way to measure levels of 5-hydroxymethylcytosine and to distinguish between 5-hmC, 5-mC, and C. The entire procedure can be completed within 3 hours and 45 minutes and has a detection limit that can be as low as 40 pg of hydroxymethylated DNA.

Product Name	Size	Cat. No.
MethylFlash Hydroxymethylated DNA Quantification Kit (Colorimetric)	48 assays	P-1036-48
	96 assays	P-1036-96

Global 5-fC Quantification

5-formylcytosine (5-fC) has been recently found and classified as a "seventh" DNA base in mammalian cells and tissue. It is formed by the oxidation of 5-hydroxymethylcytosine through TET hydroxylase and has been demonstrated to be an intermediate of the DNA demethylation process. The detection of 5-fC in various tissues and cells is important because 5-fC could be a marker to indicate active DNA demethylation.



▲ Percentage of 5-fC in different tissues measured with the MethylFlash™ 5-Formylcytosine DNA Quantification Kit.

The MethylFlash™ 5-Formylcytosine (5-fC) DNA Quantification Kit uses a unique ELISA-like procedure to directly quantify 5-fC within just 3 hours and 45 minutes. The microplate-based kit utilizes a high quality antibody which does not cross react with 5-mC, 5-hmC or 5-caC, allowing for high specificity and a detection limit that can be as low as 1 pg of 5-fC. Furthermore DNA isolated from cells or tissues can be directly used which eliminates the need for DNA digestion or hydrolysis.

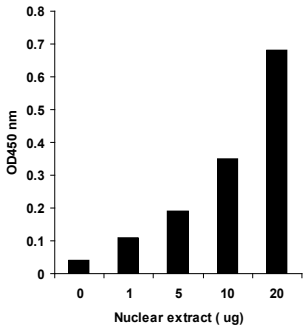
Product Name	Size	Cat. No.
MethylFlash 5-Formylcytosine DNA Quantification Kit (Colorimetric)	48 assays	P-1041-48
	96 assays	P-1041-96

DNMTs, TDG, & TET

While DNMTs are generally involved in the DNA methylation process, passive demethylation of DNA usually takes place on newly synthesized DNA strands via DNMTs during replication rounds. Active DNA demethylation mainly occurs by the removal of 5-methylcytosine through TET enzyme-mediated oxidation and the formation of further modified cytosine bases. Such further modified cytosines include 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine. These oxidation products have been shown to be repaired back into cytosine by TDG, a glycosylase which is involved in base excision repair.

DNMT Activity/Inhibition Assays

The addition of methyl groups during DNA methylation is carried out by a family of enzymes called DNA methyltransferases (DNMTs). Chromatin structure in the vicinity of gene promoters also affects DNA methylation and transcriptional activity. Three DNMTs (DNMT1, DNMT3A, and DNMT3B) are required for the establishment and maintenance of DNA methylation patterns. Two additional enzymes (DNMT2 and DNMT3L) may also have more specialized but related functions.



▲ High sensitivity and specificity achieved by using nuclear extracts with the EpiQuik™ DNA Methyltransferase Activity/Inhibition Assay Ultra Kit (Colorimetric); extracts were prepared from MCF-7 cells using the EpiQuik Nuclear Extraction Kit (Cat # OP-0002).

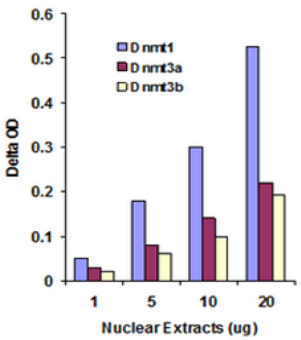
Epigentek's EpiQuik™ DNMT Activity/Inhibition Assay Ultra Kit uses an ELISA-like method to measure DNMT activity or inhibition at extremely fast speeds on a 96-strip-well microplate. The entire procedure can be completed within 3 hours and 45 minutes and has a detection limit as low as 0.5 µg of nuclear extract or 0.5 ng of purified enzymes.

Product Name	Size	Cat. No.
EpiQuik DNMT Activity/Inhibition Assay Ultra Kit (Colorimetric)	48 assays	P-3009-48
	96 assays	P-3009-96

DNMT Protein Quantification

At least three families of DNMTS have been identified in mammals: DNMT1, DNMT2, and DNMT3. DNMT1 prefers to methylate cytosine residues in hemimethylated DNA. DNMT3A and DNMT3B have been demonstrated to methylate both unmethylated and hemimethylated DNA equally.

The EpiQuik™ DNMT1, 3A, and 3B Assay Kits allow an experimenter to quantitatively measure DNMT proteins using an easy and rapid ELISA-like method. These kits provide all the essential components needed to carry out a successful DNMT assay experiment without the need for electrophoresis or transfer processes.

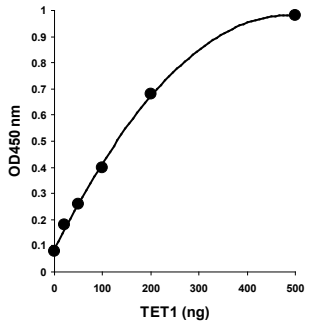


▲ Nuclear extracts were prepared from MCF-7 cells using the EpiQuik™ Nuclear Extraction Kit (Cat # OP-0002) and total amount of Dnmt was measured.

Product Name	Size	Cat. No.
EpiQuik DNMT1 Assay Kit	48 assays	P-3011-2
	96 assays	P-3011-3
EpiQuik DNMT3A Assay Kit	48 assays	P-3012-2
	96 assays	P-3012-3
EpiQuik DNMT3B Assay Kit	48 assays	P-3013-2
	96 assays	P-3013-3

TET Activity/Inhibition Assays

The ten-eleven translocation (TET) family of 5-mC hydroxylases includes TET1, TET2, and TET3. These TET proteins may promote DNA demethylation by binding to CpG-rich regions to prevent unwanted DNA methyltransferase activity, and by converting 5-mC to 5-hmC and further to 5-caC (5-carboxylcytosine) through hydroxylase activity.



▲ Demonstration of high sensitivity and specificity of the TET1 activity/inhibition assay achieved by using recombinant TET1 with the Epigenase™ 5mC Hydroxylase TET Activity/Inhibition Assay Kit (Colorimetric).

The Epigenase™ 5mC-Hydroxylase TET Activity/Inhibition Assay Kit is a complete set of optimized buffers and reagents that uses an ELISA-like procedure to measure activity/inhibition of total 5mC hydroxylase TET enzymes in nuclear extracts or purified TET isoforms (TETs 1-3) from a broad range of species. The microplate based kit can be finished within just 5 hours and can detect TET activity from as little as 20 ng of purified TET1 hydroxylase.

Product Name	Size	Cat. No.
Epigenase 5mC-Hydroxylase TET Activity/Inhibition Assay Kit (Colorimetric)	48 assays	P-3086-48
	96 assays	P-3086-96

TDG Activity/Inhibition Assays

Thymine DNA Glycosylase, or TDG, belongs to the TDG/mug DNA glycosylase family. Besides playing a critical role in active DNA methylation, TDG also removes thymine moieties from G/T mismatches by hydrolyzing the carbon-nitrogen bond between the sugar-phosphate backbone of DNA and the mismatched thymine.

The Epigenase™ Thymine DNA Glycosylase (TDG) Activity/Inhibition Assay Kit, which eliminates the need for DNA cleavage, electrophoresis, and chromatography, uses a 5-hour ELISA-like procedure to detect activity/inhibition of TDG from as little as 20 ng of purified TDG protein.

Product Name	Size	Cat. No.
Epigenase Thymine DNA Glycosylase (TDG) Activity/Inhibition Assay Kit (Colorimetric)	48 assays	P-3094-48
	96 assays	P-3094-96

Nuclear Protein Isolation

The EpiQuik™ Nuclear Extraction Kit provides a simple and selective 60-minute method for extracting nuclear proteins used in a variety of applications. These applications may include western blotting, protein-DNA binding assays, nuclear enzyme assays, and any other procedures requiring optimized nuclear proteins. The protocol is fast and easy-to-use, and also isolates a very abundant yield of nuclear extract from mammalian cells or tissue samples.

Product Name	Size	Cat. No.
EpiQuik Nuclear Extraction Kit	100 assays	OP-0002-1

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A NEWSLETTER FOR EPIGENETIC RESEARCHERS

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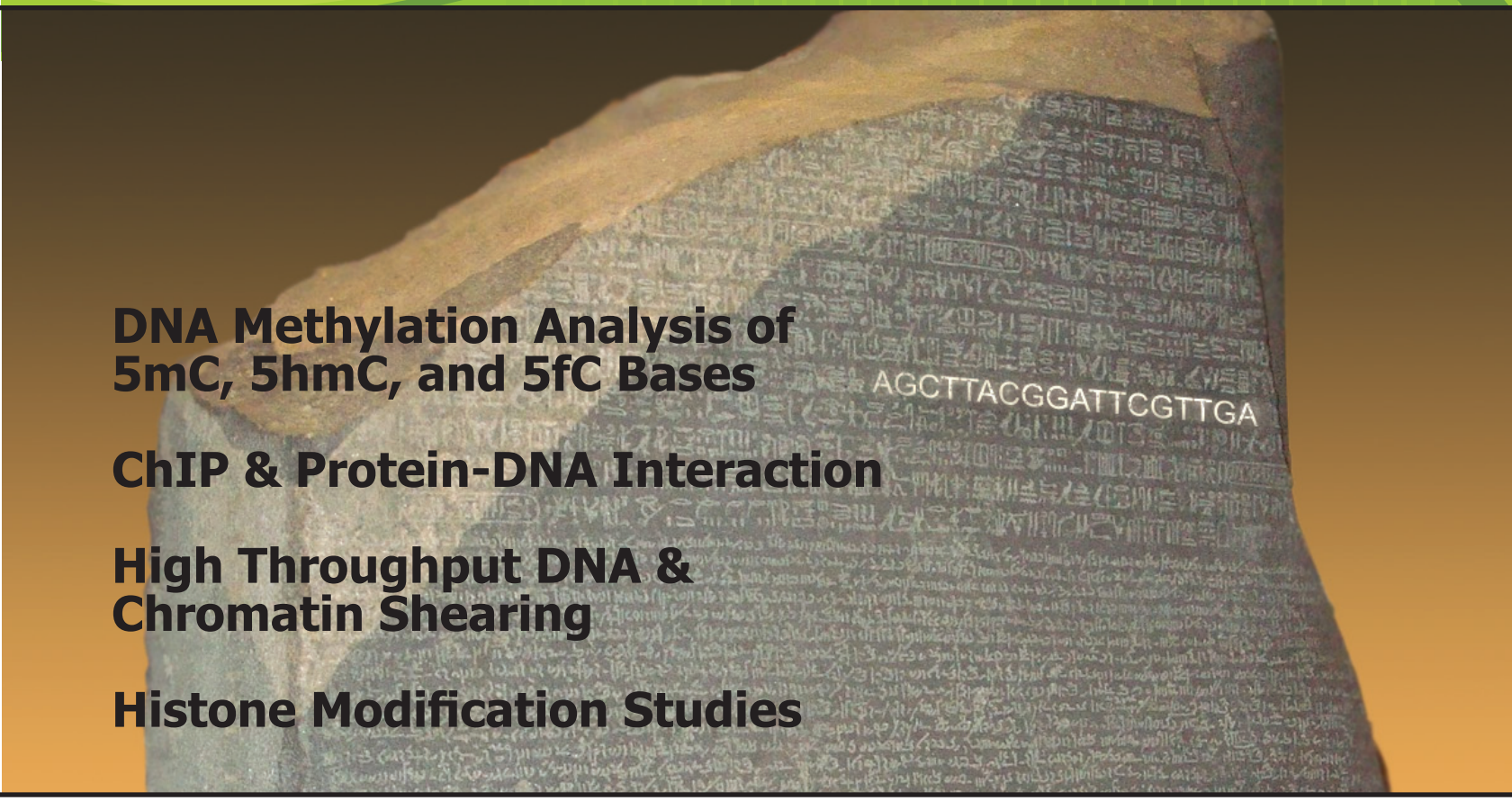

DNA Methylation Analysis of 5mC, 5hmC, and 5fC Bases

ChIP & Protein-DNA Interaction

High Throughput DNA & Chromatin Shearing

Histone Modification Studies