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A NEWSLETTER FOR EPIGENETIC RESEARCHERS

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Next Generation DNA Bisulfite Conversion

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The new BisulFlash™ method sustains DNA denaturation status throughout the entire bisulfite conversion process in order to retain 100% of the DNA in single stranded form, resulting in a faster, complete conversion.

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EPIGENTEK
Real Solutions For Epigenetic Research

Epigenetic change, gene regulation, and disease

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 microRNA-associated silencing are currently considered to initiate and sustain epigenetic change [1]. These systems interact and stabilize each other (Fig. 1), and a disruption of one or more of these interacting systems can lead to inappropriate activation or silencing of genes.

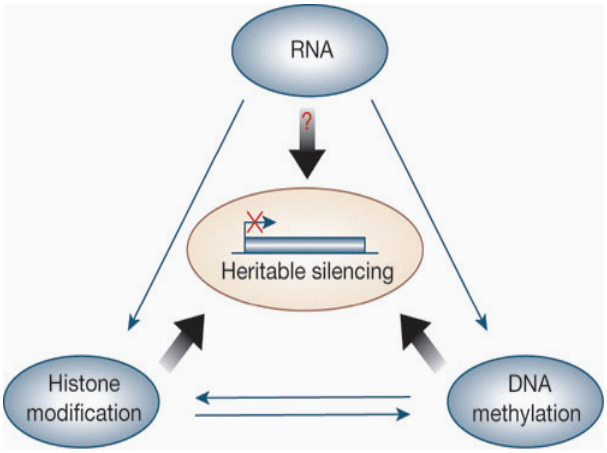

Figure 1 | Histone deacetylation and other modifications, particularly the methylation of lysine 9 within histone H3 (H3-K9) residues located in the histone tails, cause chromatin condensation and block transcriptional initiation. Histone modification can also attract DNA methyltransferases to initiate cytosine methylation, which in turn can reinforce histone modification patterns conducive to silencing. Experiments in yeast and plants have clearly shown the involvement of RNA interference in the establishment of heterochromatic states and silencing. RNA triggering of heritable quiescence might therefore also be involved in higher organisms [Egger, G *et al. Nature* **429**, 457-463, (2004)].

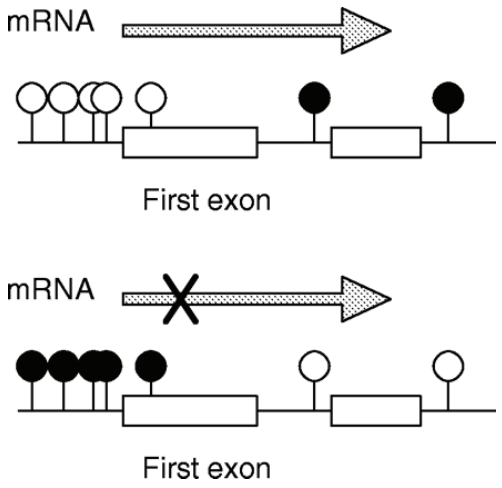

Figure 2 | Methylation status of promoter CGIs and gene expression. Promoter CGIs are generally kept unmethylated, and the downstream gene is expressed (upper panel). When the CGI is methylated, it causes repression of mRNA expression (lower panel). This mode of inactivation is observed in various tumor-suppressor genes. Filled and open circles represent methylated and unmethylated CpG sites, respectively [Miyamoto K. *et al. Jpn. J. Clin. Oncol.* **35**, 293-301, (2005)].

DNA Methylation

DNA methylation occurs by the covalent addition of a methyl group at the 5-carbon of the cytosine ring, resulting in 5-methylcytosine. These methyl groups project into the major groove of DNA and inhibit transcription. In human DNA, 5-methylcytosine is found in approximately 1.5% of genomic DNA, primarily at CpG sites [2]. There are clusters of CpG sites at 0.3-2 kb stretches of DNA known as CpG islands that are typically found in or near the promoter regions of genes where transcription is initiated. In the bulk of genomic DNA, most CpG sites are heavily methylated while CpG islands in germ-line tissues and in promoters of normal somatic cells remain unmethylated, thus allowing gene expression to occur. When a CpG island in the promoter region of a gene is methylated, expression of the gene is repressed (**Fig. 2**). The repression can be caused by directly inhibiting the binding of specific transcription factors, and indirectly by recruiting methyl-CpG-binding proteins that have associated repressive chromatin remodeling activity [3, 4]. In addition to the effect on gene transcription, DNA methylation is also involved in genomic imprinting, which refers to parental origin specific expression of a gene, as well as the formation of a chromatin domain.

DNA methylation is controlled at several different levels in normal and diseased cells. The addition of methyl groups is carried out by a family of enzymes, DNA methyltransferases (Dnmts). Chromatin structure in the vicinity of gene promoters also affects DNA methylation and transcriptional activity [3]. Three Dnmts (Dnmt1, Dnmt3a and Dnmt 3b) are required for establishment and maintenance of DNA methylation patterns. Two additional enzymes (Dnmt2 and Dnmt3L) may also have more specialized but related functions. Dnmt1 appears to be responsible for the maintenance of established patterns of DNA methylation, while Dnmt3a and 3b seem to mediate establishment of new or *de novo* DNA methylation patterns. Diseased cells such as cancer cells may be different in that Dnmt1 alone is not responsible for maintaining abnormal gene hypermethylation and both Dnmts 1 and 3b may cooperate for this function. The local chromatin structure also contributes to the control of DNA methylation. It was observed that histone H2B deubiquitination decreases the di-methylation on lysine 9 of H3, suppress siRNA-directed methylation of DNA, and release heterochromatic silencing of transgenes as well as transposons [5]. Also, DNA methylation can be enhanced by the complex of Dnmt1 and G9a, a histone methyltransferase [6].

Histone Modification

Histone modifications have also been defined as epigenetic modifiers. Post-translational modifications of histones include acetylation on specific lysine residues by histone acetyltransferases (HATs), deacetylation by histone deacetylase (HDACs), methylation of lysine and arginine residues by histone methytrans-

ferases (HMTs), demethylation of lysine residues by histone demetylases (DMTs), 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 (**Fig. 3**) [7].

Next to DNA methylation, histone acetylation and histone methylation are the most well-characterized epigenetic marks. Generally, tri-methylation at H3-K4, H3-K36, or H3-K79 results in an open chromatin configuration and is therefore a characteristic of euchromatin. Euchromatin is also characterized by a high level of histone acetylation, which is mediated by histone acetyl transferases. Conversely, histone deacetylases have the ability to remove this epigenetic mark, leading to transcriptional repression. Condensed heterochromatin is enriched in the tri-methylation of H3-K9, H3-K27, and H4-K20, and silencing of euchromatin loci caused by histone deacetylation involves the recruitment of specific K9 histone methyltransferases. Methylated H3-K9 provides a binding site for the chromodomain-containing heterochromatin protein 1 (HP1), which induces transcriptional repression and heterochromatinization. At euchromatic loci, this process is mediated by co-repressors, such as retinoblastoma protein pRb or KAP1.

Histone demethylases have the opposite effect on transcription—for example, the histone demethylase LSD1 is responsible for H3-K4 demethylation, which leads to transcriptional inactivation. Other histone demethylases, such as jumonji (JHDM2A), are responsible for H3-K9 demethylation whereas JHDM1 has the ability to convert active chromatin marks, such as H3-K36me2, to an unmodified state [8].

Lysine residues can be mono-, di-, or tri-methylated, each of which can differentially regulate chromatin structure and transcription. Along with other histone modifications such as phosphorylation, this enormous variation leads to a multiplicity of possible combinations of different modifications. This might constitute a "histone code," which can be read and interpreted by different cellular factors.

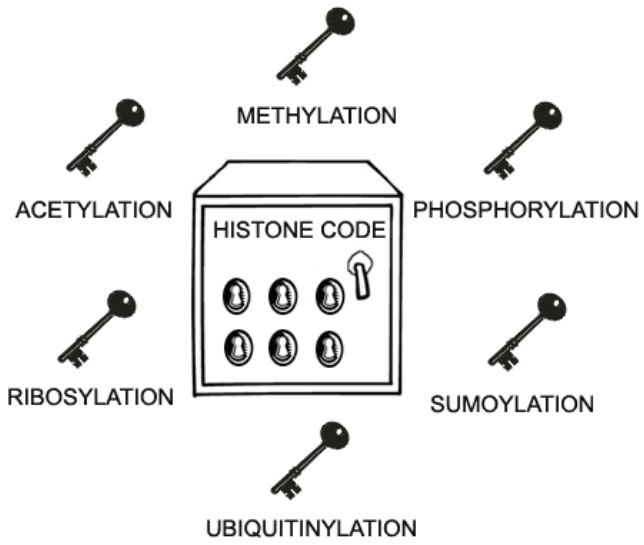

Figure 3 | Abstract representation of various histone modifications used as a cellular vocabulary for the regulation of different transcription-based processes.

MicroRNA-Associated Gene Silencing

MicroRNAs (miRNAs) are small RNA molecules, about 22 nucleotides long, that can negatively control their target gene expression posttranscriptionally. They are transcribed by RNA polymerase II (Pol II) into primary miRNAs, and are then processed in the nucleus by RNase III Drosha and DGCR8 (microprocessor complex) into the precursor miRNAs. Precursor miRNAs are structured as imperfect stem-loops, and they are exported into the cytoplasm by Exportin-5. The precursor miRNAs are further processed in the cytoplasm by another RNase III, Dicer into the final functional mature miRNAs [9]. MiRNAs bind to their target mRNAs and down-regulate their stabilities and/or translation. When binding to its target mRNA with complete complementarity, the miRNA can lead to degradation of the target. MiRNAs can also bind to their targets with incomplete complementarity, often in the 3′ UTR regions, and this leads to the translational suppression of their target genes by a mechanism that has yet to be completely elucidated [9]. Each miRNA is predicted to have many targets, and each mRNA may be regulated by more than one miRNA. Currently, there are more than 800 human miRNAs known.

Small interfering RNAs (siRNAs), often considered to be closely related to miRNAs, have been shown to be involved in both DNA methylation and histone modifications. The processing pathways of siRNAs and miRNAs share many of the enzymes involved in the RNA interference (RNAi) pathway. Recent evidence also suggests that they affect histone modifications. It was shown that RNase treatment can abolish the localization of methylated H3 lysine 9 and HP1 to pericentromeric chromatin, and that Dicer-related RNAi machinery is necessary for the formation of heterochromatin structure. Because siRNAs and miRNAs are closely related, miRNAs could also play important roles in controlling DNA methylation and histone modifications.

MiRNAs can be involved in establishing DNA methylation—for example, miR-165 and miR-166 have been shown to be required for the methylation at the PHABULOSA (PHB) gene in Arabidopsis. They interact with the newly processed PHB mRNA to change the chromatin of the template PHB gene. This presents an exciting new mechanism by which miRNAs can control gene expression in addition to the RNAi pathway, though similar findings in mammalian cells have yet to be shown.

In addition, key DNA methylation enzymes DNMT1, 3a, and 3b are all predicted to be potential targets of miRNAs, although it remains to be experimentally determined whether the DNMTs can indeed be regulated by miRNAs. Also, miRNAs may regulate chromatin structure by regulating key histone modifiers. MiR-140, which is cartilage-specific, can target histone deacetylase 4 in mice and it was also suggested that miRNAs may be involved in meiotic silencing of unsynapsed chromatin in mice. Taken together, miRNAs can be considered important players in the epigenetic control of gene expression (**Fig. 4**).

Epigenetic Diseases

The importance of epigenetics is emphasized by the growing number of human diseases that are known to occur when this epigenetic information is not properly established and/or maintained (**Fig. 5**) [10].

Cancer. Accumulation of genetic and epigenetic errors transforms a normal cell into an invasive or metastatic tumor cell, such that altered DNA methylation patterns cause abnormal expression of cancer-associated genes. DNA hypomethylation activates oncogenes and initiates chromosome instability, whereas DNA hypermethylation initiates silencing of tumor suppressor genes. Global histone modification patterns are also found to correlate with cancers such as prostate, breast, and pancreatic cancer. Altered miRNA expression is also thought to contribute to cancer. A global decrease in miRNA levels has been observed in human cancers, indicating that small RNAs may have an intrinsic function in tumor suppression. However, some miRNAs such as miRNA-27a and miRNA-372 function as oncogenes. Amplification or overexpression of these miRNAs can down-regulate tumor suppressors or other genes involved in cell differentiation, thereby contributing to tumor formation by stimulating proliferation, angiogenesis, and invasion. These epigenetic changes can be used as biomarkers for the molecular diagnosis of early cancer.

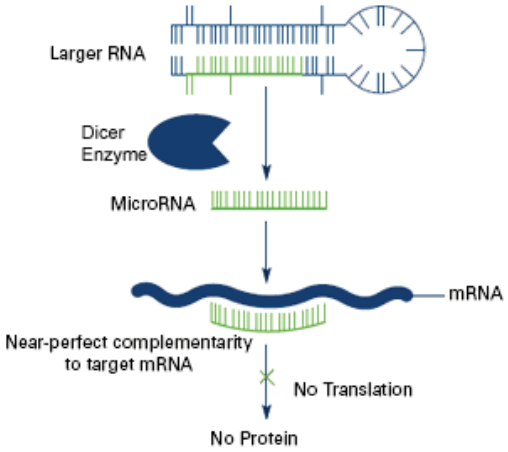

Figure 4 | The enzyme Dicer generates microRNAs by chopping larger RNA molecules into tiny Velcro®-like pieces. MicroRNAs stick to mRNA molecules and prevent the mRNAs from being made into proteins.

Imprint Disorders. Several inherited syndromes are due to faulty genomic imprinting defined as parent-specific, monoallelic expression of a gene. These include Angelman's syndrome, Prader-Willi syndrome, and Beckwith-Wiedemann syndrome (BWS). In these conditions, an abnormal phenotype is established as a result of the absence of the paternal or maternal copy of an imprinted gene or because of deregulation of an imprinted gene. For example, a cluster of imprinted genes at 11p15.5 is involved in the pathology of BWS, a syndrome characterized by organ over-growth and association with embryonal tumors such as Wilms' tumor. Loss of methylation in imprinting control regions can cause a deregulation of imprinting and either biallelic expression (such as IGF2) or silencing (such as CDKN1C) of imprinted genes, which is found in most sporadic cases of BWS.

Immunity & Related Disorders. There are several pieces of evidence showing that loss of epigenetic control over complex immunity processes contributes to autoimmune disease. Abnormal DNA methylation has been observed in patients with lupus whose T cells exhibit decreased methyltransferase activity and hypomethylated DNA. Disregulation of this pathway apparently leads to overexpression of methylation-sensitive genes such as the leukocyte function-associated factor (LFA1), which causes lupus-like autoimmunity. Interestingly, LFA1 expression is also required for the development of arthritis, which raises the possibility that altered DNA methylation patterns may contribute to other diseases displaying idiopathic autoimmunity. Abnormal histone modification patterns (histone H3 and

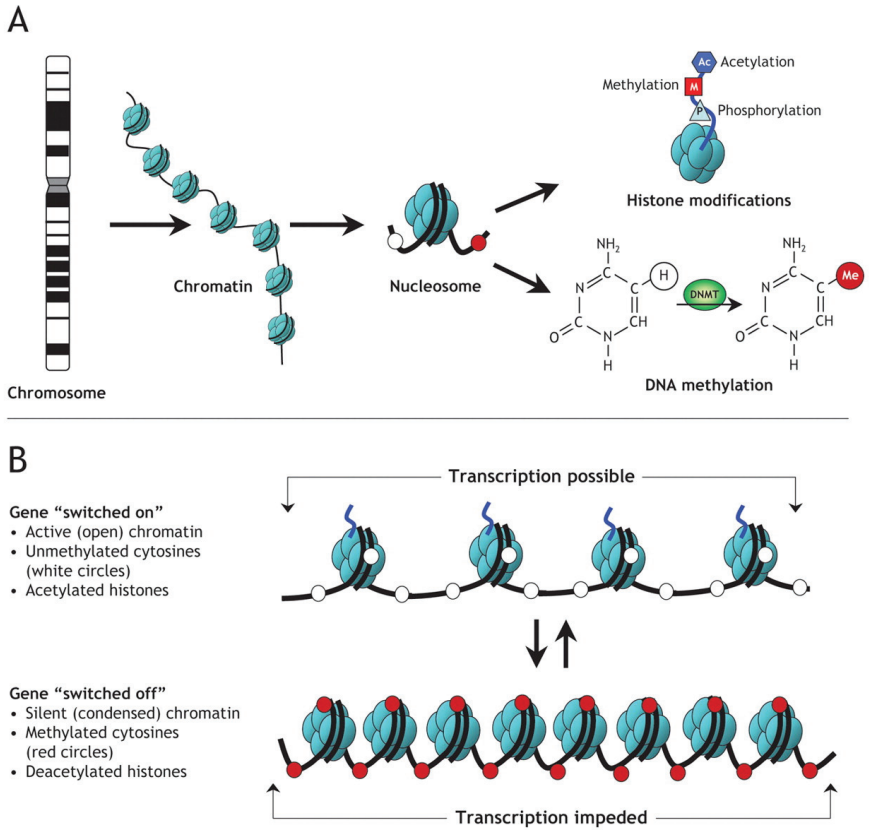

Figure 5 | Schematic of epigenetic modifications and the reversible changes in chromatin organization that influence gene expression.

H4 hypoacetylation) were also observed in lupus CD4+ T cells. Recent evidence showed roles of miRNA regulation in immune functions and in the development of autoimmunity and autoimmune disease. For example, miRNA -146a was observed to contribute to abnormal activation of the type I interferon pathway in human lupus by targeting the key signaling proteins.

Neuropsychiatric Disorders. Epigenetic errors also play a role in the causation of complex adult psychiatric, autistic, and neurodegenerative disorders. Several reports have associated schizophrenia and mood disorders with DNA rearrangements that include DNMT genes. DNMT1 is selectively overexpressed in gamma-aminobutyric acid (GABA)-ergic interneurons of schizophrenic brains, whereas hypermethylation has been shown to repress the expression of Reelin (a protein required for normal neurotransmission, memory formation, and synaptic plasticity) in brain tissue from patients with schizophrenia and patients with bipolar illness and psychosis. A role for aberrant methylation mediated by folate levels has been suggested as a factor in Alzheimer's disease.

Also, preliminary evidence supports a model that incorporates both genetic and epigenetic contributions in the causation of autism. Autism has been linked to the region on chromosome 15 that is responsible for Prader-Willi syndrome and Angelman syndrome. Findings in the autopsies of brain tissues from patients with autism have revealed deficiency in MECP2 expression that appears to account for the reduced expression of several relevant genes. Studies in numerous Huntington's disease (HD) models have shown that mutant huntingtin alters histone acetyltransferase activity, and indicate that aberrant activity of this enzyme might be an underlying mechanism of transcriptional dysregulation in HD.

Pediatric Syndromes. In addition to epigenetic alterations, specific mutations affecting components of the epigenetic pathway have been identified as being responsible for several syndromes: DNMT3B in the ICF (immunodeficiency, centromeric instability, and facial anomalies) syndrome, MECP2 in Rett syndrome, ATRX in ATR-X syndrome (a-thalassemia/mental retardation syndrome, X linked), and DNA repeats in facioscapulohumeral muscular dystrophy. In Rett syndrome, for example, MECP2 encodes a protein that binds to methylated DNA; mutations in this protein cause abnormal gene expression patterns within the first year of life. Girls with Rett syndrome display reduced brain growth, loss of developmental milestones and profound mental disabilities. Similarly, the ATR-X syndrome also includes severe developmental deficiencies due to loss of ATRX, a protein involved in maintaining the condensed, inactive state of DNA. Together, this constellation of clinical pediatric syndromes is associated with alterations in genes and chromosomal regions necessary for proper neurologic and physical development.

Other Diseases. More human diseases that are involved by epigenetic modifications may include atherosclerosis, hypertension, glaucoma and macular degeneration, and also diabetes.

The increased knowledge and technologies in epigenetics over the last 10 years allow us to better understand the interplay between epigenetic change, gene regulation, and human diseases, and will lead to the development of new approaches for molecular diagnosis and targeted treatments across the clinical spectrum.

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2. Lister, R. *et al*. Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* **462**, 315-322 (2009).
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Achieving DNA bisulfite treatment perfection with a next generation DNA bisufite conversion tool for DNA methylation analysis

Because bisulfite conversion only works effectively on single stranded DNA while DNA denaturation is critical to retain single stranded DNA form, Epigentek developed a proprietary method to truly combine the bisulfite conversion and DNA denaturation process into one, without DNA degradation. Essentially, this method sustains DNA denaturation status throughout the entire bisulfite conversion process in the thermal cycler at a single temperature in order to retain 100% of the DNA in single stranded form. Therefore, with 100% ssDNA, one could convert all cytosines into uracil with almost no methylcytosine deamination and, in turn, finish the experiment faster (in just 30 minutes).

DNA methylation occurs by the covalent addition of a methyl group at the 5-carbon of the cytosine ring, resulting in 5-methylcytosine. There are various methods used to assess DNA methylation states. However, 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. By treating DNA with bisulfite, cytosine residues are deaminated to uracil while leaving 5-methylcytosine intact:

	Unmethylated DNA	Methylated DNA
Original Sequence	C-C-G-T-C-G-A-C-G-T	C-^{M}C-G-T-^{M}C-G-A-^{M}C-G-T
After Conversion	U-U-G-T-U-G-A-U-G-T	U-^{M}C-G-T-^{M}C-G-A-^{M}C-G-T

The traditional bisulfite conversion method needs 12-16 hours for bisulfite treatment, resulting in heavy DNA degradation (>80%), high inappropriate methylcytosine deamination (>3.5%) and low cytosine conversion rate (<95%). In March 2005, Epigentek became the first company to develop a fast DNA bisulfite modification method to overcome these problems—shortening the entire bisulfite process from 16 hours to just 1.5 hours, significantly improving cytosine conversion efficiency (>99.9%), and effectively preventing converted DNA degradation.

To effectively and efficiently prepare converted DNA for use in various downstream analyses, an ideal DNA bisulfite modification method should be: (1) highly accurate to allow for the complete conversion of cytosine to uracil (correct conversion) without deamination of methylcytosine to thymine (inappropriate conversion); and (2) rapid enough to enable the bisulfite process to be as short as possible, as rapid DNA methylation analysis is highly demanded for basic research and particularly for clinical applications.

Epigentek continues to innovate with the development of the BisulFlash™ DNA Modification Kit, perfecting DNA bisulfite treatment for better DNA methylation analysis. This kit greatly improves the currently used methods/kits for DNA bisulfite modification. With the novel and optimized bisulfite composition, the BisulFlash™ DNA Modification Kit allows for the DNA modification step to be just 20 minutes with a complete cytosine conversion. More importantly, it greatly reduces inappropriate conversion of 5-methylcytosine to thymine (<0.1%). The BisulFlash™ DNA Modification Kit is suitable for MS-PCR, real time MS-PCR, methylation microarray, and pyrosequencing. Additionally, based on its ability for a complete cytosine conversion, it is specifically suitable for next generation methylation sequencing/pyrosequencing.

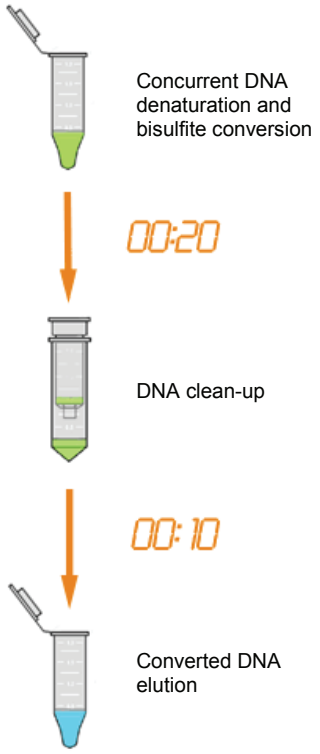

Figure 1 | Schematic procedure of the BisulFlash™ DNA Modification Kit to obtain converted DNA.

The BisulFlash™ DNA Modification Kit has the following advantages and features:

- Convenient single temperature incubation without the need for a separate DNA denaturation step.
- Ready-to-use, powder-free DNA conversion mix solution allows you to do just 1 reaction or all 50 reactions in a single run.
- The fastest and most convenient protocol that can be finished in as short as 30 minutes.
- Completely converts unmethylated cytosine into uracil (>99.99%) with negligible inappropriate or error conversion of methylcytosine to thymine (<0.1%).
- Powerful protection against DNA degradation, with over 90% of DNA loss prevented.
- Extremely low requirement of input DNA for modification—only 0.2 ng or 50 cells.

Principle and Procedure

As a next generation bisulfite conversion tool, the BisulFlash™ DNA Modification Kit contains all reagents required for an ultra-fast bisulfite conversion on a DNA sample (**Fig. 1**). With the unique and ready-to-use conversion mix solution which contains

powerful DNA protection reagents, DNA denaturation status is sustained throughout the entire bisulfite DNA conversion process, thereby enabling 100% of DNA to be modified in single stranded form without chemical and thermophilic degradation. Thus, this novel approach leads to an accelerated conversion of all cytosine to uracil with negligible methylcytosine deamination. The non-toxic DNA capture solution enables DNA to tightly bind to the column filter, thus DNA cleaning can be carried out on the column to effectively remove residual bisulfite and salts.

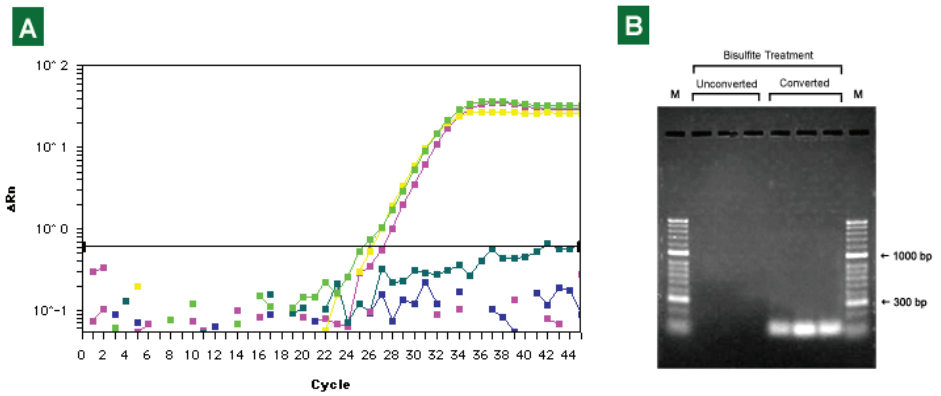

Figure 2 | 200 ng of genomic DNA isolated from 3 cancer cell lines was treated with the BisulFlash™ DNA Modification Kit. Next, the unconverted and converted DNA in each treated sample was determined using unconverted DNA-specific and converted DNA-specific primers (β-actin, 110 bps), respectively. (**a**) Real time PCR with Methylamp™ MS-qPCR Fast Kit (Cat. No. P-1028). (**b**) End-point PCR. The BisulFlash™ kit treated DNA was completely converted and no unconverted DNA in the treated samples was determined after 45 cycles.

Rapid Results

Only 30 minutes are required for the entire BisulFlash™ procedure—from your sample DNA to converted DNA eluate. This is significantly faster than any currently used kits (2-8 hours) or homebrew methods (>16 hours). The BisulFlash™ kit provides everything required for a successful bisulfite conversion and DNA clean-up in the shortest time with the fewest steps possible, outperforming other available kits on the market (**Table 2**).

Perfect DNA Conversion

Each reaction with the BisulFlash™ kit can use 0.2 ng – 1 µg of DNA. For optimal conversion, the DNA amount is 20-200 ng. The novel procedure and proprietary ready-to-use DNA conversion mix solution allow DNA denaturation and bisulfite conversion to occur at the same time and enable all cytosines to be converted to uracil (>99.99%), while inappropriate conversion of 5-methylcytosine to thymine is negligible (<0.1%) (**Fig. 2** and **Fig. 3**). The highly efficient cytosine conversion is proven by the low CT values obtained from converted DNA amplified using real-time PCR (**Table 1**) and real-time PCR followed by sequencing (**Fig. 3**). This perfect conversion rate is superior to other bisulfite kits available on the market and provides repeatable and dependable downstream analysis.

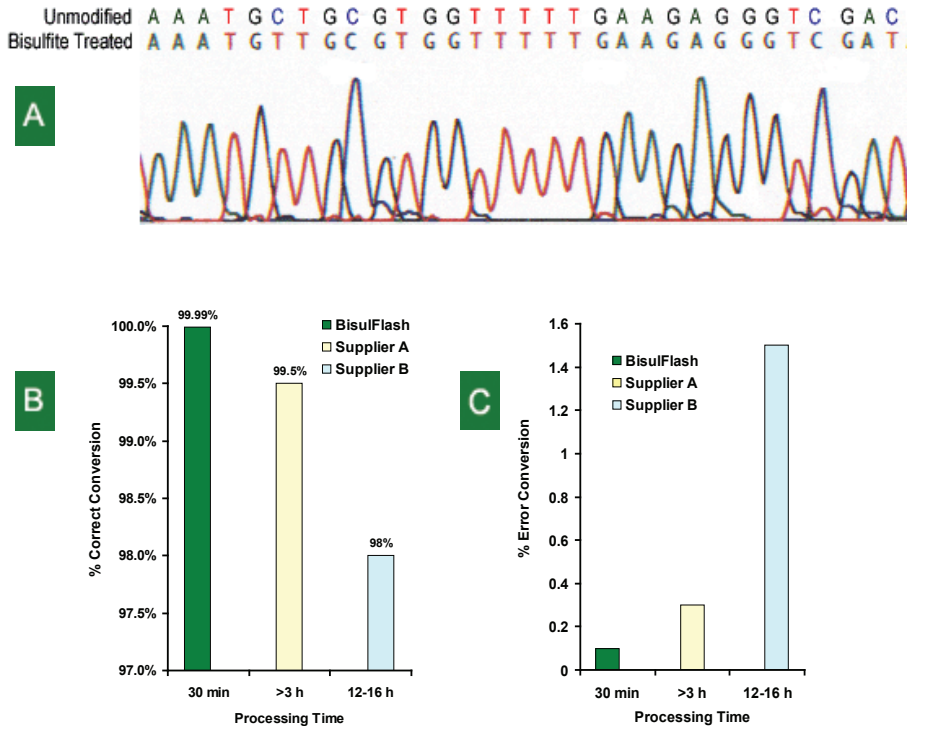

Figure 3 | Demonstration of high accuracy of DNA conversion achieved by the BisulFlash™ kit. 50 ng of genomic DNA methylated in all CpG sites by DNA methylase was treated with the BisulFlash™ DNA Modification Kit. Converted DNA was amplified by real time qPCR using primers for multiple promoters containing numerous CpG sites and then directly sequenced. Correct conversion (C-U) and inappropriate or error conversion (mC-T) rates were culcalated as percentage of total cytosines or mCpGs. (**a**) Representative bisulfite sequencing analysis of converted DNA. (**b**) Correct conversion rate. (**c**) Inappropriate or error conversion rate.

Powerful DNA Protection

DNA protection reagents are added into the DNA conversion mix solution to prevent DNA from chemical and thermophilic degradation in the bisulfite treatment and provide effective DNA denaturation, resulting in single-stranded DNA necessary for complete cytosine conversion. The prevention of DNA degradation enables subsequent amplification and analysis of large PCR fragments (**Fig. 4**). The efficient integrated DNA clean-up buffer and unique elution buffer allow for long-term storage (>6 months) and multiple freezing/thawing of the converted DNA without affecting

the DNA quality.

It is well known that bisulfite conversion only works effectively on single stranded DNA while DNA denaturation is critical for retaining DNA in single stranded form. Yet current methods process bisulfite reaction under DNA non-denaturation status because it was considered that bisulfite conversion is not possible under DNA denaturing status without siginificant DNA degradation. The BisulFlash™ method conceptually breaks this limitation by sustaining DNA denaturation throughout the entire bisulfite conversion process with powerful DNA protection, enabling 100% of the DNA to be modified in single stranded form without DNA degradation. As a result, this maximizes conversion rate and speed.

	Total Time	CT Value
BISULFLASH	30 min	28.2
SUPPLIER A	>3 hours	30.5
SUPPLIER B	12-16 hours	30.8

Table 1 | The highly efficient cytosine conversion is shown by the low CT values obtained from converted DNA by using the BisulFlash™ DNA modification Kit. 100 ng of genomic DNA isolated from cancer cells were treated with the BisulFlash™ kit and with DNA modification kits from two other suppliers. 1 µl from 20 µl of eluate was used for real time qPCR.

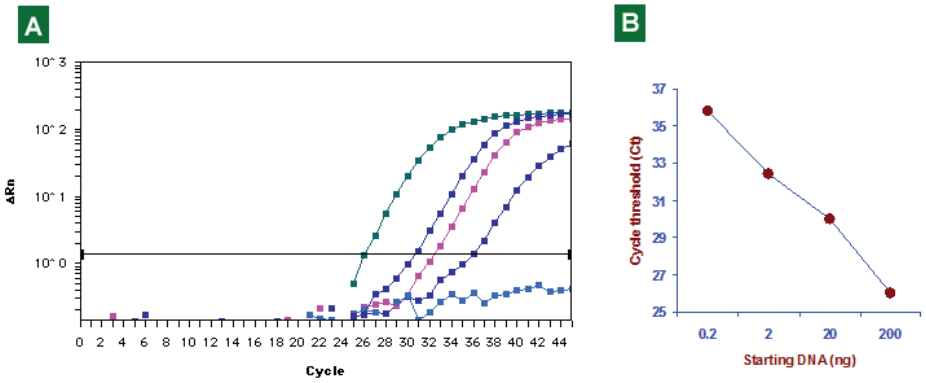

Figure 4 | Effective DNA protection. Fully methylated human genomic DNA at various amounts (0.2 ng-200 ng) were converted using the BisulFlash™ DNA Modification Kit. 1 µl of 20 µl eluate was used for real time qPCR and a pair of primers was used to amplify converted DNA. As little as 0.2 ng DNA is sufficient for bisulfite conversion using the BisulFlash™ DNA Modification Kit. (**a**) Real time PCR with Methylamp™ MS-qPCR Fast Kit (Cat. No. P-1028). (**b**) Starting DNA amount-CT value curve.

	BISULFLASH	SUPPLIER 1	SUPPLIER 2	SUPPLIER 3
Processing Time	30 min	>3 hours	>6 hours	12-16 hours
Correct Conversion	99.99%	99.5%	99.4%	98%
Error Conversion	<0.1%	>0.3%	N/A	>1.5%
Correct/Error Conv. Ratio	~1000	~330	N/A	~65
DNA Degradation	Very low	Medium	Low	High
Min. Starting DNA	0.2 ng	0.5 ng	1 ng	1 ng
Convenience	Very High	High	Medium	Low

Table 2 | Comparative overview of commercial kits. Data was obtained through actual use of the kit, customer feedback, or information provided by the supplier's datasheet or website.

DNA Bisulfite Conversion Products	Size	Cat. No.	US Price
BisulFlash DNA Modification Kit	50 reactions	P-1026-050	$99
Methylamp Coupled DNA Isolation & Modification Kit	40 samples	P-1002-40	$198
Methylamp Whole Cell Bisulfite Modification Kit	40 samples	P-1016-40	$138
	80 samples	P-1016-80	$259

DNA Methylation Products	Size	Cat. No.	US Price
TuMinute PCR Clean-Up Kit	50 samples	P-1005-1	$58
	100 samples	P-1005-2	$109
Methylamp Universal Methylated DNA Kit	10 modifications	P-1011-1	$198
	20 modifications	P-1011-2	$299
Methylamp Universal Methylated DNA Preparation Kit	for 40 µg of DNA	P-1019-1	$175
Methylamp MS-qPCR Fast Kit	100 reactions	P-1028-100	$126

Sample Preparation Products	Size	Cat. No.	US Price
FitAmp General Tissue Section DNA Isolation Kit	50 samples	P-1003-1	$178
	100 samples	P-1003-2	$329
FitAmp Plasma/Serum DNA Isolation Kit	50 samples	P-1004-1	$98
	100 samples	P-1004-2	$179
DNA Concentrator Kit	50 samples	P-1006-1	$58
	100 samples	P-1006-2	$109
FitAmp Gel DNA Isolation Kit	50 samples	P-1007-1	$68
	100 samples	P-1007-2	$119
FitAmp Paraffin Tissue Section DNA Isolation Kit	50 samples	P-1009-1	$158
	100 samples	P-1009-2	$299
FitAmp Urine DNA Isolation Kit	50 samples	P-1017-050	$108
	100 samples	P-1017-100	$199
FitAmp Blood and Cultured Cell DNA Extraction Kit	50 samples	P-1018-050	$109
	100 samples	P-1018-100	$199

Please see www.epigentek.com for additional products, product information, and available sizes.

Microplate-based chromatin immunoprecipitation tools

Protein-DNA interaction plays a critical role in 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 important for understanding cellular processes.

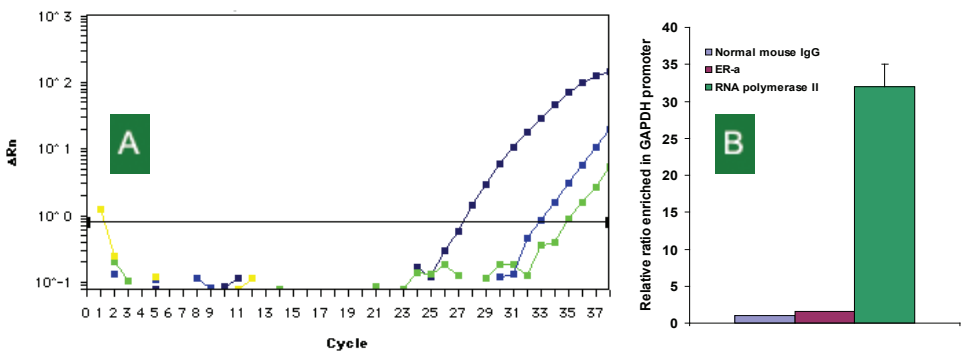

Figure 1 | Microplate-based ChIP. Nuclear extract prepared from formaldehyde fixed colon tissues (1 mg/each reaction) was added into the microwell. RPII protein- and ERα protein-DNA complexes were captured by affinity antibodies pre-bound to the microwells. Captured DNA was used for analyzing levels of RPII and ERα enriched in the GAPDH promoter with the use of primers and probes specific to the GAPDH promoter. (**a**) Real time quantitative PCR using EpiQuik™ Fast qPCR Kit (Cat. No. P-1030). (**b**) Analysis of enrichment of RNA polymerase II and ERα in GAPDH promoter by microwell-based ChIP.

Chromatin immunoprecipitation (ChIP) offers an advantageous tool for studying protein-DNA interaction. It allows for detecting that a specific protein binds to the specific sequences of a gene in living cells through the combination of PCR (ChIP-PCR), microarray (ChIP-chip), and sequencing (ChIP-Seq). For example, measurement of the amount of methylated histone H3 at lysine 9 (meH3-K9) associated with a specific gene promoter region under various conditions can be achieved through a ChIP-PCR assay, while recruitment of meH3-K9 to the promoters on the genome-wide scale can be detected by ChIP-chip. Particularly, ChIP with antibodies directly against modified histones and various transcriptional factors is widely demanded. However, the conventional ChIP is time consuming (2-3 days) with low throughput.

Epigentek provides a series of microplate-based ChIP kits that allows the experimenter to perform chromatin immunoprecipitation at extraordinarily rapid speeds and consistency. Superior to all other current ChIP methods available, the kits are ready-to-use and provide all the essential components needed to carry out a successful ChIP experiment. The kits are suitable for combining the specificity of immunoprecipitation with qualitative and quantitative PCR, DNA sequencing, and DNA microarray.

Principle and Procedure

This kit includes a positive control antibody (RNA polymerase II), a negative control normal mouse IgG, and GAPDH primers that can be used as a positive control to demonstrate the efficacy of the kit reagents and protocol. RNA polymerase II is considered to be enriched in the GAPDH gene promoter that is expected to be undergoing transcription in most growing mammalian cells and can be immunoprecipitated by RNA polymerase II but not by normal mouse IgG. In this ChIP, cells are cross-linked with formaldehyde and chromatin is extracted. The chromatin is then sheared and added into the microwell immobilized with affinity antibodies. Cross-linked DNA is released from antibody-captured protein-DNA complex, reversed and purified through the specifically designed F-Spin™ Column. Eluted DNA can be used for various down-stream applications.

The EpiQuik™ ChIP Kit series has the following features:

- **Fast** - only 5 hours are needed from cells/tissues to eluted DNA.
- **Flexible** - Strip microplate format allows for either manual or high throughput, with either the included and ready-to-use ChIP antibody or your own antibody
- **Convenient** - works with multi-channel pipetting; columns included for DNA purification, saving time and labor.
- **Compatibile** - suitable with ChIP-PCR, ChIP-on-chip, ChIP-WGA, and ChIP-seq methodologies.
- **Reliable** - consistent assay conditions.

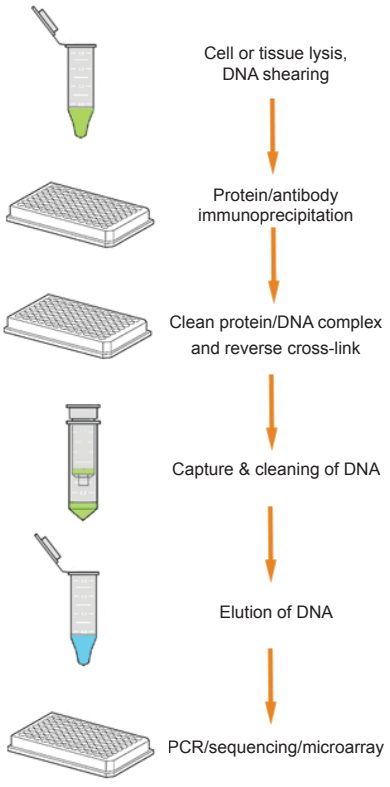

Figure 2 | Schematic procedure of the EpiQuik™ Chromatin Immunoprecipitation Kit.

Chromatin Immunoprecipitation from Plant Cells

Epigentek also offers an EpiQuik™ Plant ChIP kit, which contains all reagents required for carrying out a successful chromatin immunoprecipitation from plant cells. Particularly, this kit includes a ChIP-grade dimethyl-histone H3-K9 antibody and a negative control normal mouse IgG. 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 F-Spin™ Column. The eluted DNA can be used for various down-stream applications.

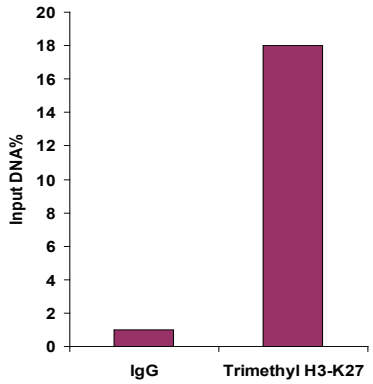

Figure 3 | Microplate-based plant ChIP. DNA was immunoprecipitated from 2-week-old icu2-1/icu2-1 seedlings using the EpiQuik™ Plant ChIP Kit (Cat. No. P-2014). PCR was used to amplify the ORNITHINE TRANSCARBAMILASE (OTC) gene and regions of the AGAMOUS gene.

Chromatin Immunoprecipitation	Size	Cat. No.	US Price
EpiQuik Chromatin Immunoprecipitation Kit	48 reactions	P-2002-2	Inquire
	96 reactions	P-2002-3	Inquire
EpiQuik Tissue Chromatin Immunoprecipitation Kit	48 reactions	P-2003-2	Inquire
	96 reactions	P-2003-3	Inquire
EpiQuik Plant ChIP Kit	24 reactions	P-2014-24	$278
	48 reactions	P-2014-48	$459
EpiQuik Methyl-Histone H3-K4 ChIP Kit	24 reactions	P-2007-24	$267
	48 reactions	P-2007-48	$438
EpiQuik Tissue Methyl-Histone H3-K4 ChIP Kit	24 reactions	P-2009-24	$288
	48 reactions	P-2009-48	$469
EpiQuik Methyl-Histone H3-K9 ChIP Kit	24 reactions	P-2006-24	$297
	48 reactions	P-2006-48	$488
EpQuik Tissue Methyl-Histone H3-K9 ChIP Kit	24 reactions	P-2008-24	$298
	48 reactions	P-2008-48	$499
EpQuik Tri-Methyl-Histone H3-K9 ChIP Kit	24 reactions	P-2006T-24	$297
	48 reactions	P-2006T-48	$488
EpiQuik Acetyl-Histone H3 ChIP Kit	24 reactions	P-2010-24	$287
	48 reactions	P-2010-48	$468

Post Reaction Analysis	Size	Cat. No.	US Price
EpiQuik ChIP DNA Clean-Up Kit	50 samples	P-2023-050	$65
EpiQuik Fast PCR Kit	100 reactions	P-1029-100	$109
EpiQuik Fast Quantitative PCR Kit	100 reactions	P-1030-100	$129

Chromatin Immunoprecipitation	Size	Cat. No.	US Price
EpiQuik Tissue Acetyl-Histone H3 ChIP Kit	24 reactions	P-2012-24	$288
	48 reactions	P-2012-48	$469
EpiQuik Acetyl-Histone H4 ChIP Kit	24 reactions	P-2011-24	$287
	48 reactions	P-2011-48	$468
EpiQuik Tissue Acetyl-Histone H4 ChIP Kit	24 reactions	P-2013-24	$288
	48 reactions	P-2013-48	$469
EpiQuik Methyl-CpG Binding Domain Protein 2 ChIP Kit	24 reactions	P-2017-24	$327
	48 reactions	P-2017-48	$498
EpiQuik Tissue Methyl-CpG Binding Domain Protein 2 ChIP Kit	24 reactions	P-2018-24	$328
	48 reactions	P-2018-48	$499

Sample Preparation	Size	Cat. No.	US Price
EpiQuik Chromatin Preparation Kit	100 preps	P-2022-100	$116

Please see www.epigentek.com for additional products, product information, and available sizes.

Microplate-based enrichment of methylated DNA

Highly specific capture of methylated DNA provides an advantage for the convenient and comprehensive identification of methylation status of normal cells and diseased cells (i.e., cancer cells) that may lead to the development of new diagnostic and therapeutic methods in cancer. Several methods have been used for enriching methylated DNA such as agarose beads-based methylated DNA capture. However, these methods so far are considerably time consuming, labor intensive, and have low throughput.

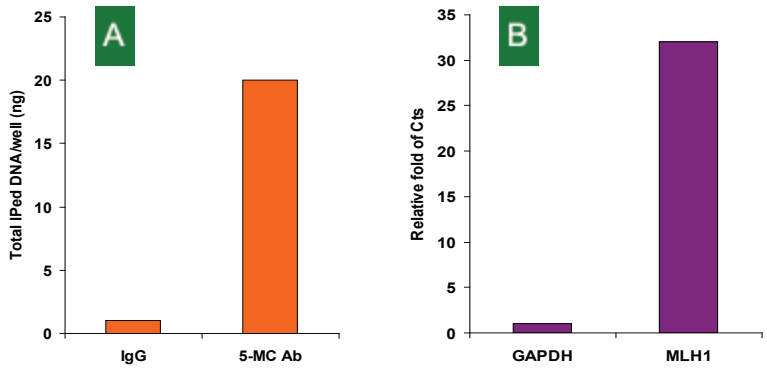

Figure 1 | Enrichment of methylated DNA using the Methylamp™ Methylated DNA Capture Kit (Cat. No. P-1015), DNA (0.5 ug) isolated from MCF-7 cells was added into the microwells. (**a**) Methylated DNA was captured by a 5-mC antibody. (**b**) Captured methylated DNA was used for analyzing methylation level of GAPDH and MLH1 promoter with the use of primers and probes specific to GAPDH and MLH1 promoters, respectively.

Epigentek's methylated DNA immunoprecipitation (MeDIP) series of kits use a proprietary and unique procedure and composition to enrich methylated DNA in a convenient 96-well plate format. In the assay, an antibody specific to methylcytosine is used to capture methylated genomic DNA. The enriched methylated fractions can then be used for a standard DNA detection.

The kit has the following features:

- Highly efficient enrichment of methylated DNA: > 98%
- Directly immunoprecipitate the methylated fractions of DNA from cell lysates.
- Extremely fast procedure: from cells to eluted DNA in just 3-4 hours.
- Strip microplate format makes the assay flexible: manual or high throughput.
- Columns for DNA purification are included: save time and reduce labor.
- Compatible with all DNA amplification-based approaches.
- Simple, reliable, and consistent assay conditions.

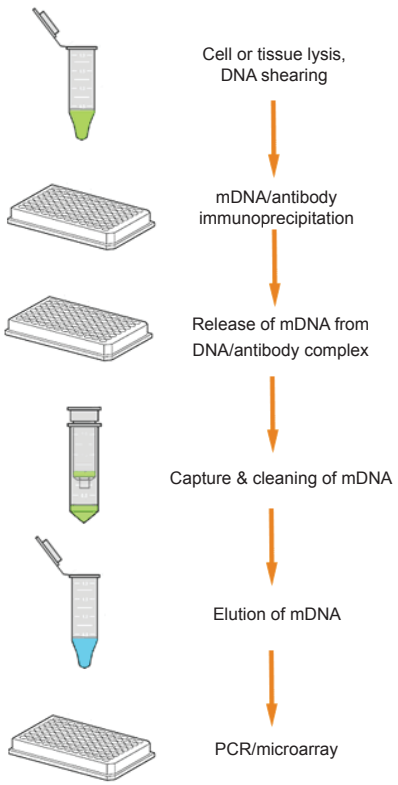

Figure 2 | Schematic procedure of Epigentek's Methylated DNA Immunoprecipitation kits.

Methylated DNA Immunoprecipitation	Size	Cat. No.	US Price
Methylamp Methylated DNA Capture Kit *(from purified DNA)*	24 reactions	P-1015-24	$298
	48 reactions	P-1015-48	$489
EpiQuik Methylated DNA Immunoprecipitation Kit *(from nuclear extract)*	24 reactions	P-2019-24	$297
	48 reactions	P-2019-48	$498
EpiQuik Tissue Methylated DNA Immunoprecipitation Kit *(from tissues)*	24 reactions	P-2020-24	$298
	48 reactions	P-2020-48	$499

Sample Preparation	Size	Cat. No.	US Price
FitAmp Genomic DNA Isolation Kit	50 samples	P-1024-050	$79
EpiQuik Chromatin Preparation Kit	100 preps	P-2022-100	$116

Post Reaction Analysis	Size	Cat. No.	US Price
EpiQuik ChIP DNA Clean-Up Kit	50 samples	P-2023-050	$65
EpiQuik Fast PCR Kit	100 reactions	P-1029-100	$109
EpiQuik Fast Quantitative PCR Kit	100 reactions	P-1030-100	$129

Please see www.epigentek.com for additional products, product information, and available sizes.

Quantifying global DNA methylation through an ELISA-like method

It has been well demonstrated that DNA methylation plays a critical role in the regulation of gene expression. A decrease in global DNA methylation has been observed in various cancers, neurological disorders, autoimmune diseases, and aging. In particular, the measurement of global DNA methylation in cancer cells could provide very useful information for detection and analysis of cancer.

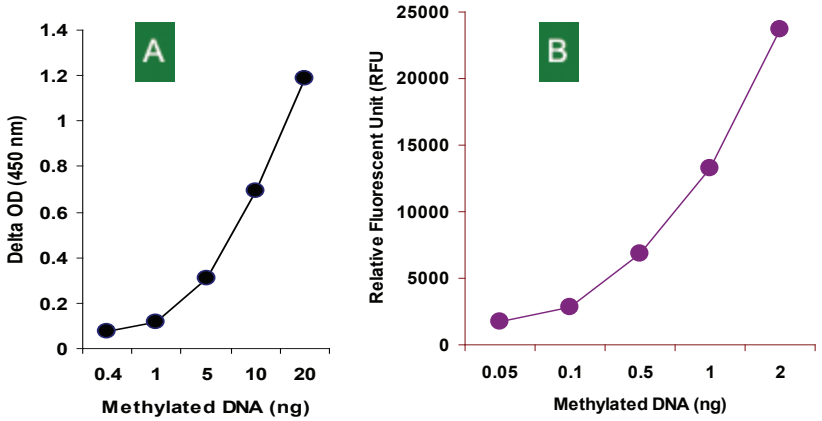

Figure 1 | Quantification of methylated DNA. (**a**) Colorimetric Methylamp™ Global DNA Methylation Quantification Ultra Kit. (**b**) Fluorometric SuperSense™ Methylated DNA Quantification Kit.

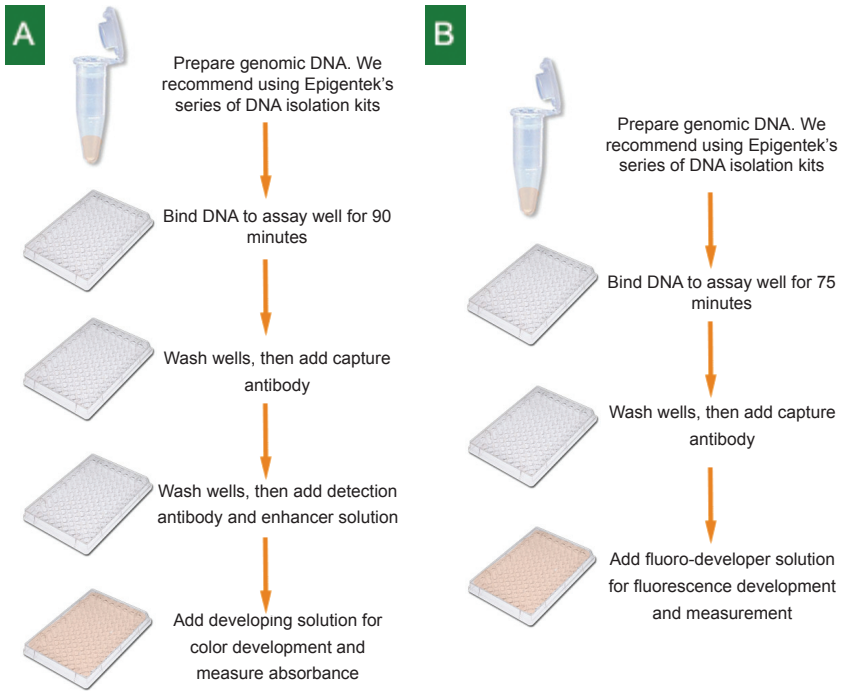

Figure 2 | Schematic procedure. (**a**) Colorimetric Methylamp™ Global DNA Methylation Quantification Ultra Kit. (**b**) Fluorometric SuperSense™ Methylated DNA Quantification Kit.

There are several methods to measure global DNA methylation levels, including mass spectrometry, enzymatic degradation and analysis, and immunohistostaining. However, such methods have many disadvantages including the need for special equipment, long protocols, low sensitivity, and/or radioactivity.

To address these problems, Epigentek pioneered and created an ELISA based colorimetric kit, *Methylamp Global DNA Methylation Quantification Kit* in 2006 and improved upon it in 2007 with the *Methylamp Global DNA Methylation Quantification Ultra Kit.* These kits are faster, more accurate, and much more convenient than conventional methods. Epigentek now also offers the fluorometric ability to quantify global DNA methylation levels with the *SuperSense Methylated DNA Quantification Kit.*

By creating these kits, we have improved the way one can gather DNA methylation information through speed, convenience, and effectiveness. Epigentek's kits for measuring global DNA methylation shifts is available in both colorimetric (*Methylamp*) and fluorometric (*SuperSense*) formats.

Principle and Procedure

DNA is immobilized to the strip well specifically coated with a DNA affinity substance. The methylated fraction of DNA can then be recognized by a 5-methylcytosine antibody. With the colorimetric kit, the amount of methylated DNA, which is proportional to the OD intensity, is quantified through an ELISA-like reaction. With the fluorometric kit, the ratio or amount of methylated DNA, which is proportional to fluorescence intensity, is fluorometrically quantified.

Easy, Fast, and Flexible

- 4-step colorimetric procedure within 3.5 hours or 3-step fluorometric procedure within 2.5 hours.
- 96 well plate format allows for single sample or high throughput studying.
- Methylated DNA is quantified through an ELISA-like reaction either colorimetrically or fluorometrically.

Safe and Convenient

- All reagents, including universal positive control (suitable for quantifying methylated DNA from any species), are included in the kit.
- Colorimetric or fluorometric quantification—without radioac-

tivity, extraction, or chromatography—replaces obsolete or inferior methods.

Accurate and Responsive

- Detection limit is as low as 50 pg of methylated DNA with the input DNA being as low as 2 ng.
- Can use genomic DNA isolated from any species such as mammalian, plant, fungal, bacterial, and virus in a variety of forms including cultured cells, fresh and frozen tissues, paraffin-embedded tissues, plasma/serum samples, and body fluid samples.

	METHYLAMP ULTRA	SUPERSENSE
Format	Colorimetric	Fluorometric
Speed	3.5 Hours	2.5 Hours
Protocol	4 Easy Steps	3 Easy Steps
Detection Limit	0.2 nanograms	50 picograms
DNA Origin	Mammal, Plant, Fungus, Bacteria, Virus	Mammal, Plant, Fungus, Bacteria, Virus
High Throughput	Strip-based 96 well-plate assay format	Strip-based 96 well-plate assay format

Table 1 | Differences between the colorimetric Methylamp™ Ultra kit and the fluorometric SuperSense™ kit.

DNA Methylation Quantification	Size	Cat. No.	US Price
Methylamp Global DNA Methylation Quantification Ultra Kit	48 assays	P-1014B-48	$298
	96 assays	P-1014B-96	$539

DNA Methylation Quantification	Size	Cat. No.	US Price
SuperSense Methylated DNA Quantification Kit	48 assays	P-1021-48	$298
	96 assays	P-1021-96	$539

Please see www.epigentek.com for additional products, product information, and available sizes.

Quantifying all sites/ patterns of histone H3 and H4 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. The quantitative detection of these histones provides useful information for a better understanding of epigenetic regulation of gene activation, and for developing HMT, HAT, or HDAC targeted drugs.

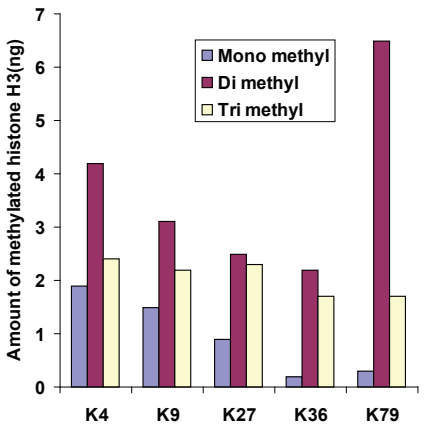

Figure 1 | Histone H3 methylation quantification. Histone extracts were prepared from MDA-231 breast cancer cells using the EpiQuik™ Total Histone Extraction Kit (Cat. No. OP-0006). Histone H3 mono-, di-, and tri-methylation at different sites was quantified with the EpiQuik™ Histone H3 Methylation Quantification Kit series (Fluorometric).

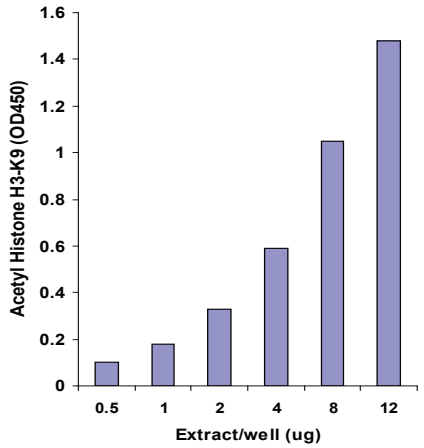

Figure 2 | Histone H3 acetylation quantification. Histone extracts were prepared from MCF-7 cancer cells using the EpiQuik™ Total Histone Extraction Kit (Cat. No. OP-0006). Acetyl H3-K9 was quantified with the EpiQuik™ Global Acetyl Histone H3-K9 Quantification Kit (Colorimetric) (Cat. No. P-4010).

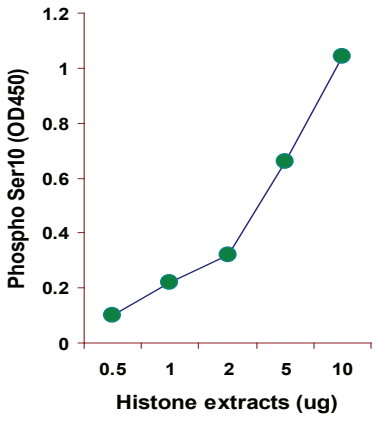

Figure 3 | Histone H3 phosphorylation quantification. Histone extracts were prepared from MDA-231 cancer cells using the EpiQuik™ Total Histone Extraction Kit (Cat. No. OP-0006). Phospho H3-S10 was quantified with the EpiQuik™ Global Histone H3 Phosphorylation (Ser10) Quantification Kit (Colorimetric) (Cat. No. P-7002).

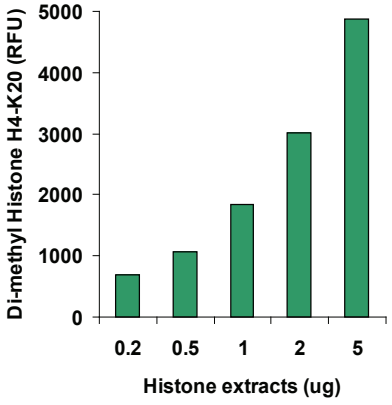

Figure 4 | Histone H4 methylation quantification. Histone extracts were prepared from MDA-231 cancer cells using the EpiQuik™ Total Histone Extraction Kit (Cat. No. OP-0006). Di-methyl H4-K20 was quantified with the EpiQuik™ Global Di-Methyl Histone H4-K20 Quantification Kit (Fluorometric) (Cat. No. P-3067).

Epigentek offers a complete series of kits available for the quantification of histone H3 and H4 modifications at all sites and degrees. These modifications include H3 methylation at lysine K4, K9, K27, K36 and K79; H3 acetylation at K9, K14, K18, K23, K36 and K56; H3 phosphorylation at S10, S28 and T11; H4 acetylation at K5, K8, K12, and K16; and H4 methylation at K20.

The EpiQuik™ kit series for the quantification of histone H3 or H4 modifications comes in both colorimetric and fluorometric versions. In an assay with one of the kits, the methylated, acetylated, or phosphorylated histone H3 or H4 at various sites of interest is captured to the strip wells, which are coated with a corresponding antibody. The captured modified histone H3 or H4 can then be detected with a labeled detection antibody followed by a color or fluorescent development reagent. The ratio of the modified histone H3 or H4 is proportional to the intensity of absorbance or fluoroscence. The absolute amount of modified histone H3 or H4 can be quantitated by comparing to the standard control.

Epigentek's histone H3 and H4 modification kit series is:

- **Fast** - Complete procedure in 2 hours and 30 minutes.
- **Convenient** - Fluorometric or colorimetric assay without the need for radioactivity, electrophoresis, or chromatography. The control is conveniently included for the quantitation of the methylated or acetylated histones.
- **Sensitive** - Specifically captures methylated or aceylated histones with the detection limit as low as 2 ng/well colorimetrically and 0.2 ng/well fluorometrically. Detection range from 20 ng to 5 µg/well of histone extracts.
- **Flexible** - Strip microplate format allows either manual or high throughput assays.
- **Reliable** - Consistent assay conditions and superior results.

Product	No. of Kits	Cat. No.
Histone Methylation Quantification Kits	58	P-3014 to P-3071
Histone Acetylation Quantification Kits	26	P-4008 to P-4033
Histone Phosphorylation Quantification Kits	5	P-7001 to P-7005
EpiQuik Total Histone Extraction Kit	Price: $99	OP-0006-100

Please see www.epigentek.com for additional products, product information, and available sizes.

Rapid enzymatic preparation of single nucleosides for 5-mC quantification

Digesting DNA to deoxyribonucleosides is a common process and a requirement for mass spectrometry and HPLC-based DNA analyses. These analyses include examination of the effects of gene polymorphisms and nutritional status on DNA metabolism, determination of the base composition of DNA, investigation of epigenetic modifications such as deoxycytosine methylation, and oxidative damage. The currently used method for digesting DNA with tri-enzymes has several drawbacks including: (1) complicated procedure that includes adjusting pH twice, boiling samples, and separately incubating with different enzymes. This complicated procedure is time-consuming and limits the number of samples that can be prepared; and (2) the enzyme solution used in such a method has high buffer concentrations that can interfere with the enzyme/DNA reaction and the downstream DNA analysis. To address these issues, Epigentek offers the EpiQuik™ One-Step DNA Hydrolysis Kit. This kit is designed for rapidly hydrolyzing DNA to deoxynucleosides with a process that can be performed in a single incubation. Epigentek also provides DNA digestase that can be used for rapid and flexible DNA hydrolysis.

The EpiQuik™ One-Step DNA Hydrolysis Kit simply applies our proprietary enzymatic DNA digestion solution to DNA or oligonucleotides. After treatment with the DNA digestion buffer the DNA is easily digested into single nucleosides without phosphate groups. The kit has the following features:

- A fast one-step procedure, which can be finished in as short as 1 hour.
- Performed in a single incubation, without the need for DNA denaturation.
- Highly efficient enzymatic hydrolysis—non-specifically digests DNA or oligonucleotides into single nucleosides.
- 96-well microplate format makes the sample preparation flexible for use with high throughput formats.

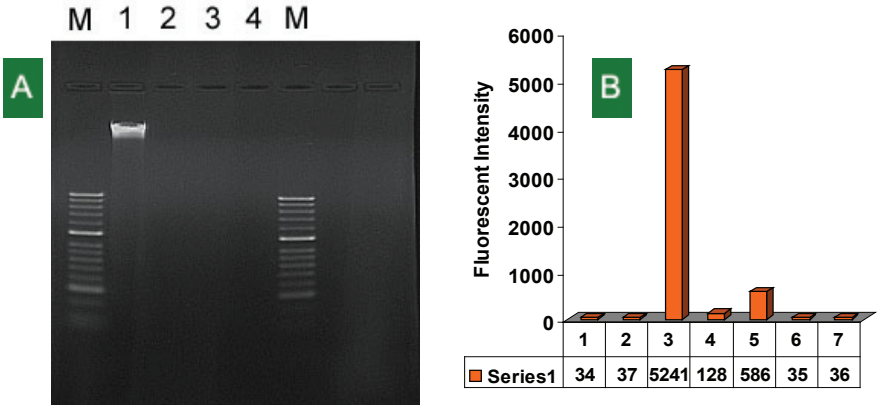

Figure 1 | The EpiQuik™ One-Step DNA Hydrolysis Kit rapidly hydrolizes DNA to individual deoxynuycleosides. (**a**) 0.5 µg and 2 µg of fully methylated HeLa DNA were treated with the kit at 37°C for 1 h and then analyzed by gel electrophoresis. *1*: 2 µg of undigested DNA; *2*: 0.5 µg of digested DNA; *3*: 2 µg of digested DNA; *4*: 1 µg of deoxynucleoside; "M" is a 2 kb DNA ladder. (**b**) 1 µg of fully methylated HeLa DNA was digested with the kit at 37°C for 1 and 2 h, respectively. The digested DNA was fluorescently quantified. *1*: blank; *2*: deoxynucleoside control; *3*: undigested DNA; *4*: undigested tri-oligos (3 nt); *5*: undigested penta-oligos (5 nt); *6*: DNA digested for 1 h; *7*: DNA digested for 2 h.

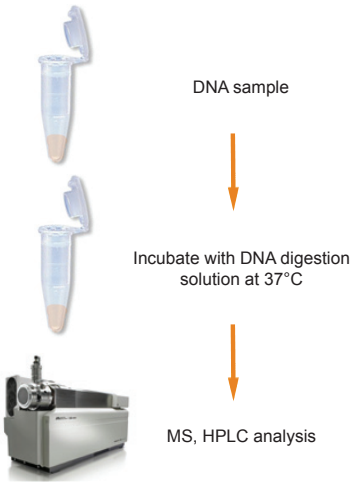

Figure 2 | Schematic procedure of the EpiQuik™ One-Step DNA Hydrolysis Kit

Product	Size	Cat. No.	US Price
EpiQuik One-Step DNA Hydrolysis Kit	96 samples	P-1023-96	$128
DNA Digestase	500 µg	R-1049-1	$198

Please see www.epigentek.com for additional products, product information, and available sizes.

DID YOU KNOW?

Epigenetics is the most rapidly growing field in biological sciences today. The world market for epigenetics is projected to reach $18.2 billion by the year 2015. *Epigentek* is identified as a key player in this market through its innovation, along with Celgene Corporation, CellCentric Ltd., Epigenomics AG, Genpathway Inc., Merck & Co. Inc., MGI Pharma Inc., Millipore Corporation, OncoMethylome Sciences S.A., and Orion Genomics.

Source: *Epigenetics: A Global Strategic Business Report* (Feb 2010) Global Industry Analysts, Inc.

Non-radioactive assays for epigenetic enzyme activity and inhibition

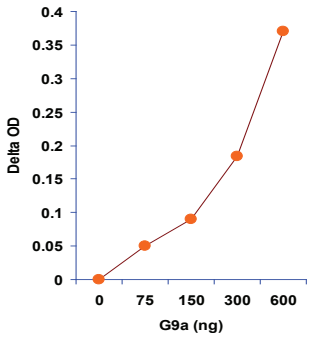

Figure 1 | G9a activity assay. Recombinant G9a was used and the enzyme activity was measued using the EpiQuik™ Histone Methytransferase Activity/Inhibition Assay Kit (H3-K9).

Epigenetic regulation of gene transcription has been the key biological determinant of cellular function with important pathogenic and therapeutic relevance. This regulation is mediated by selective, enzyme-catalyzed, covalent modification of DNA and of proteins (especially histones). These enzymes include DNA methyltrasferases (DNMTs) and DNA demethylases, histone methyltransferases (HMTs) and histone demethylases, histone acetyltransferases and histone deacetylases (HDACs).

Misregulation of these enzymes may lead to disease states such as cancer. Inhibitors that target to these enzymes such as HMTs, Dnmts, and HDACs have proven effective as novel cancer therapeutic agents. It has been found that different forms of HDACs or Dnmts play a distinct role in biological and pathological processes. Epigentek provides a series of epigenetic enzyme activity and inhibition assay kits, which not only allows for the testing of the activity of various HMTs (**Fig. 1**), Dnmts (**Fig. 2**), and HDACs (**Fig. 3**) but also for the screening of inhibitors against these enzymes.

Epigentek's epigenetic enzyme activity and inhibition kit series has the following features:

- **Fast** - the entire procedure can be completed within 3 hours.
- **Non-radioactive** - innovative colorimetric or fluorometric assay with microplate reader.
- **Sensitive and Specific** - only converted methylated DNA is recognized by a high affinity anti-5-methylcytosine antibody
- **Convenient** - strip microplate format makes the assay flexible: manual or high throughput analysis.

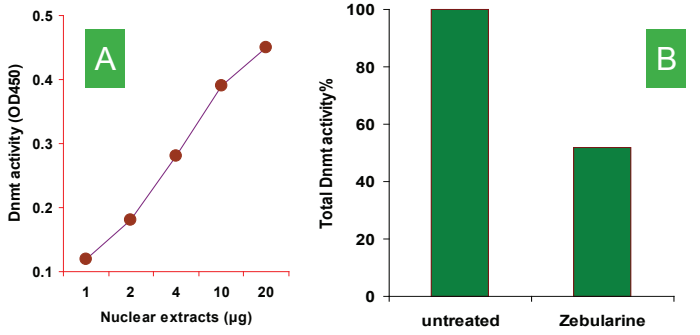

Figure 2 | Dnmt activity and inhibition assay. (**a**) Nuclear extracts were prepared from MCF-7 cells using EpiQuik Nuclear Extraction Kit and total Dnmt activity was measured. (**b**) HSC-3 cells were incubated with/without zebularine (220 uM) for 48 h. Nuclear proteins were extracted and total Dnmt activity was measured.

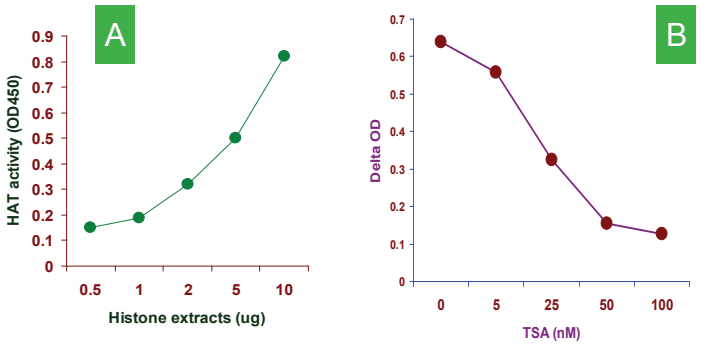

Figure 3 | HAT activity and HDAC activity inhibition assay. (**a**) Nuclear extracts were prepared from MCF-7 cells using EpiQuik™ Nuclear Extraction Kit and total HAT activity was measured. (**b**) HDAC3 enzyme inhibition by HDAC inhibitor TSA.

DNA Methyltransferase Assay	Size	Cat. No.	US Price
EpiQuik DNA Methyltransferase Activity/ Inhibition Assay Kit	96 assays	P-3001-2	$489
EpiQuik DNA Methyltransferase Activity/ Inhibition Assay Kit (Fluorometric)	96 assays	P-3004-96	$489
EpiQuik Dnmt1 Assay Kit	96 assays	P-3011-3	$499
EpiQuik Dnmt3A Assay Kit	96 assays	P-3012-3	$499
EpiQuik Dnmt3B Assay Kit	96 assays	P-3013-3	$499
EpiQuik DNA Demethylase Activity/ Inhibition Assay Kit	96 assays	P-3019-96	$499

Histone Methyltransferase Assay	Size	Cat. No.	US Price
EpiQuik Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K4)	96 assays	P-3002-2	$489
EpiQuik Histone Methyltransferase Activity/Inhibition Assay Kit (H3-K9)	96 assays	P-3003-2	$489

HDAC/HAT Assay	Size	Cat. No.	US Price
EpiQuik HDAC Activity/Inhibition Assay Kit (Fluorometric)	48 assays	P-4001-48	$168
	96 assays	P-4001-96	$299
EpiQuik HDAC Activity/Inhibition Assay Kit (Colorimetric)	48 assays	P-4002-48	$168
	96 assays	P-4002-96	$299
EpiQuik HAT Activity/Inhibition Assay Kit	96 assays	P-4003-96	$349

Nuclear Protein Extraction	Size	Cat. No.	US Price
EpiQuik Nuclear Extraction Kit I	100 assays	OP-0002-1	$138

Please see www.epigentek.com for additional products, product information, and available sizes.

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DNA methylation
Histone methylation/demethylation
Histone acetylation/deacetylation
Histone phosphorylation
ADP rybosylation
Sumoylation
Ubiquitin
Histone modification regulation
RNAi
DNA damage/repair
Chromatin assembly
Chromatin remodeling
Chromatin binding protein

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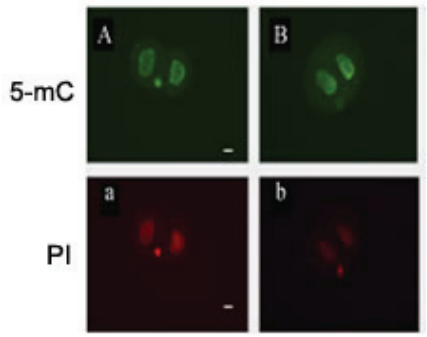

Figure 1 | Immunofluorescence staining with an Epigentek antibody against 5-methylcytosine of mouse tetraploid embryos (Cat. No. A-1014).

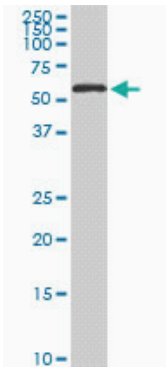

Figure 2 | HDAC1 mAb tested by western blot. Hela nuclear extract was used for western blot analysis. HDAC1 was detected with an HDAC1 mAb (Cat. No. A-4001) from Epigentek.

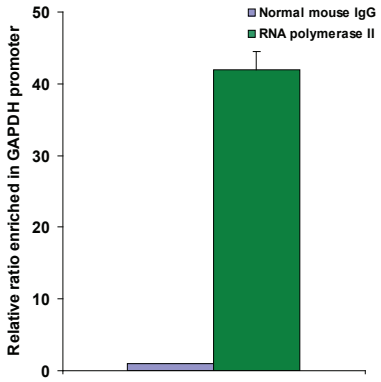

Figure 3 | RNA polymerase II mAb from Epigentek tested by ChIP. ChIP was performed using the EpiQuik™ Tissue Chromatin Immunoprecipitation Kit (Cat. No. P-2003). Captured DNA was used for analyzing levels of RPII and ERα enriched in the GAPDH promoter with the use of primers and probes specific to the GAPDH promoter.

Selected Epigenetic Antibodies	Size	Cat. No.	US Price
Anti-Dnmt1	50 µg	A-1001-050	$188
Anti-Dnmt3a	50 µg	A-1003-050	$188
Anti-Dnmt3b	50 µg	A-1004-050	$188
Anti-MBD2	50 µg	A-1007-050	$188
Anti-MDB4	50 µg	A-1009-050	$188
Anti-5-mC (5-methylcytosine)	50 µg	A-1014-050	$188
Anti-EZH2	50 µg	A-2019-050	$218
Anti-RNA Polymerase II (CTD4H8)	50 µg	A-2032-050	$218
Anti-HDAC1	50 µg	A-4001-050	$188
Anti-HDAC3	50 µg	A-4003-050	$188
Anti-HDAC8	50 µg	A-4008-050	$188
Anti-Acetyl Histone H3-K9	25 µg	A-4022-025	$128
Anti-Acetyl Histone H3-K14	25 µg	A-4023-025	$128
Anti-Acetyl Histone H3-K18	25 µg	A-4024-025	$128
Anti-Acetyl Histone H3-K23	25 µg	A-4025-025	$128
Anti-Acetyl Histone H3-K56	25 µg	A-4026-025	$128
Anti-Acetyl Histone H4-K5	50 µg	A-4027-050	$139
Anti-Acetyl Histone H4-K8	50 µg	A-4028-050	$139
Anti-Acetyl Histone H4-K12	50 µg	A-4029-050	$139

Selected Epigenetic Antibodies	Size	Cat. No.	US Price
Anti-Acetyl Histone H4-K16	50 µg	A-4030-050	$139
Anti-Dimethyl Histone H3-K4	25 µg	A-4032-025	$128
Anti-Trimethyl Histone H3-K4	25 µg	A-4033-025	$128
Anti-Dimethyl Histone H3-K9	25 µg	A-4035-025	$128
Anti-Trimethyl Histone H3-K9	25 µg	A-4036-025	$128
Anti-Dimethyl Histone H3-K27	25 µg	A-4038-025	$128
Anti-Trimethyl Histone H3-K27	25 µg	A-4039-025	$128
Anti-Dimethyl Histone H3-K36	25 µg	A-4041-025	$128
Anti-Trimethyl Histone H3-K36	25 µg	A-4042-025	$128
Anti-Dimethyl Histone H3-K79	25 µg	A-4044-025	$128
Anti-Trimethyl Histone H3-K79	25 µg	A-4045-025	$128
Anti-Dimethyl Histone H4-K20	25 µg	A-4047-025	$128
Anti-Trimethyl Histone H4-K20	25 µg	A-4048-025	$128

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A BRIEF HISTORY

Epigentek was the first company to develop and market most of the important tools that are widely used today as epigenetic solutions.

As a pioneer and innovator, Epigentek has made the following important contributions to epigenetic field:

- First to develop a series of fast DNA bisulfite modification kits, reducing DNA bisulfite conversion processing time from 16 hours to just 2 hours. (2005)
- First to develop a microplate-based, high throughput, and rapid ChIP kit series. (2005)
- First to establish an epigenetic drug discovery service. (2006)
- First to develop a whole cell-based DNA bisulfite modification kit. (2006)
- First to develop a microplate-based, high throughput MeDIP kit series. (2006)
- First to develop an ELISA-based global DNA methylation quantification kit series. (2006)
- First to develop a non-radioactive DNA and histone methylase assay kit series. (2006)
- First to offer an complete line of kits for histone modification quantification at all sites/patterns. (2008)
- First to develop a rapid enzymatic DNA hydrolysis (single nucleoside preparation) kit. (2009)
- First to develop the next generation DNA bisulfite conversion method, from input DNA to converted DNA eluate in just 30 minutes. (2010)

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