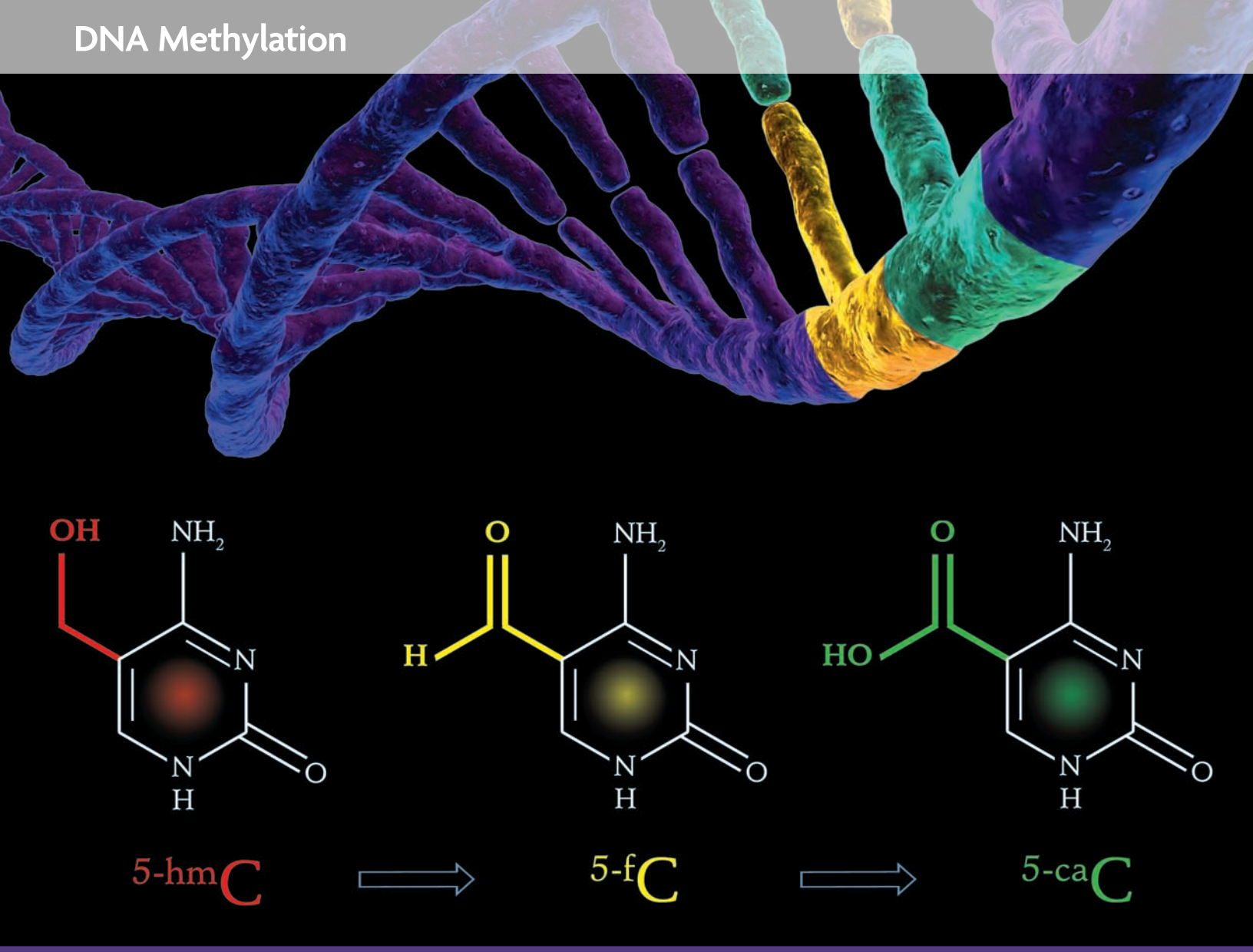


DNA Methylation

innovative technologies for DNA methylation analysis

5-hmC Analysis

Methylated DNA Enrichment

DNMT Assays

Bisulfite Conversion

Whole Genome Amplification

Methylated DNA Standards

DNA Methylation Services

DNA Methylation Antibodies

Active Motif offers an expansive line of products to provide researchers with the necessary tools for achieving success when analyzing DNA methylation.

Whether you are performing bisulfite conversion or evaluating the methylation status of CpG islands, Active Motif makes it simpler than ever to get faster and more reliable results.

ACTIVE MOTIF®

Enabling Epigenetics Research

DNA Methylation is a heritable epigenetic event that plays a pivotal role in gene regulation, especially during development and in carcinogenesis. Methylation occurs via the enzymatic transfer of methyl groups by DNA methyltransferase enzymes to the C-5 position of cytosine in CpG dinucleotides to produce 5-methylcytosine (5-mC). However, alternate forms of cytosine methylation have been identified, such as 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC). Methylation at the C-3 position (3-methylcytosine (3-mC)) has also been shown to occur. Due to the important regulatory roles of DNA methylation in gene silencing, imprinting and chromosomal inactivation, and because aberrant methylation has been linked to developmental defects and cancers, Active Motif has generated a variety of products to streamline DNA methylation analysis.

5-Hydroxymethylcytosine (5-hmC)

5-Hydroxymethylcytosine (5-hmC), derived from the oxidation of **5-Methylcytosine (5-mC)** by TET enzymes, is a novel epigenetic marker that is also an important regulator of development and carcinogenesis. 5-hmC is often associated with actively transcribed genes, supporting a complementary role to 5-mC or an intermediary function during demethylation. High levels of 5-hmC are observed in the central nervous system of higher organisms and are also linked to pluripotency of stem cells.

Hydroxymethyl Collector™ for fast, efficient enrichment of 5-hydroxymethylcytosine

The Hydroxymethyl Collector™ Kit was designed for the highly specific capture of DNA fragments that contain 5-hmC residues. The method takes advantage of an efficient chemical labeling procedure that enables the enriched samples to be collected as double-stranded DNA fragments. This makes it easy to prepare libraries for various downstream applications, including Next-Generation sequencing (Figure 1).

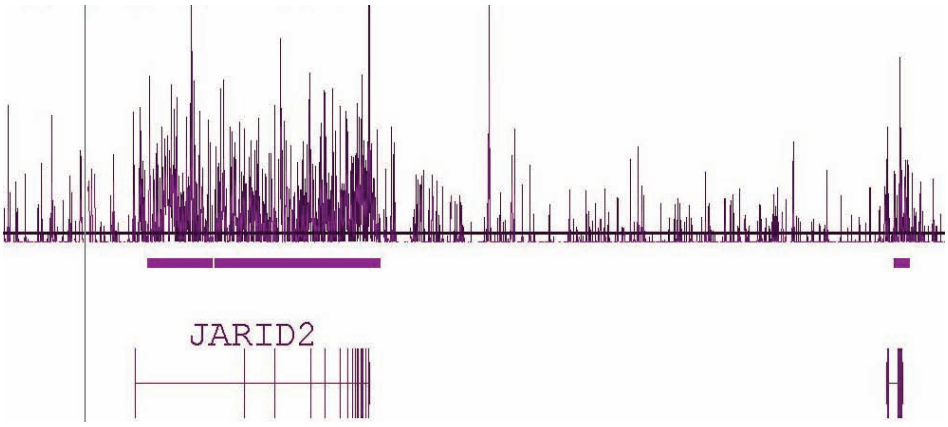


Figure 1: Human tiling array using DNA enriched with Hydroxymethyl Collector.
Human brain DNA that was enriched using the Hydroxymethyl Collector Kit was amplified by whole-genome amplification and hybridized to an Affymetrix Human Tiling 2.0R Array A containing chromosomes 1 and 6. This image shows a 1.2 million base pair view of chromosome 6 where there is a clear enrichment of 5-hmC across the entire length of the JARID2 gene.

*Patent Pending.

How it works: β-glucosyltransferase enzyme is used to transfer a modified glucose moiety to 5-hmC that is then chemically labeled with a biotin conjugate for capture using streptavidin magnetic beads (Diagram 1, next page). This unique chemistry ensures no cross-reactivity with 5-mC, and it is not limited by the specific properties or consensus sequences that constrain traditional methods, such as glucosyl-sensitive restriction enzyme digestion. Furthermore, modification of 5-hmC occurs independent of sequence context, enabling the detection of both CpG and non-CpG methylated DNA. The use of streptavidin magnetic beads allows for quick and efficient recovery of samples, and the specificity of the streptavidin capture enables more stringent binding and wash conditions. The result is a reduction in background and increased sensitivity, allowing enrichment of DNA fragments containing as few as two 5-hmC residues.

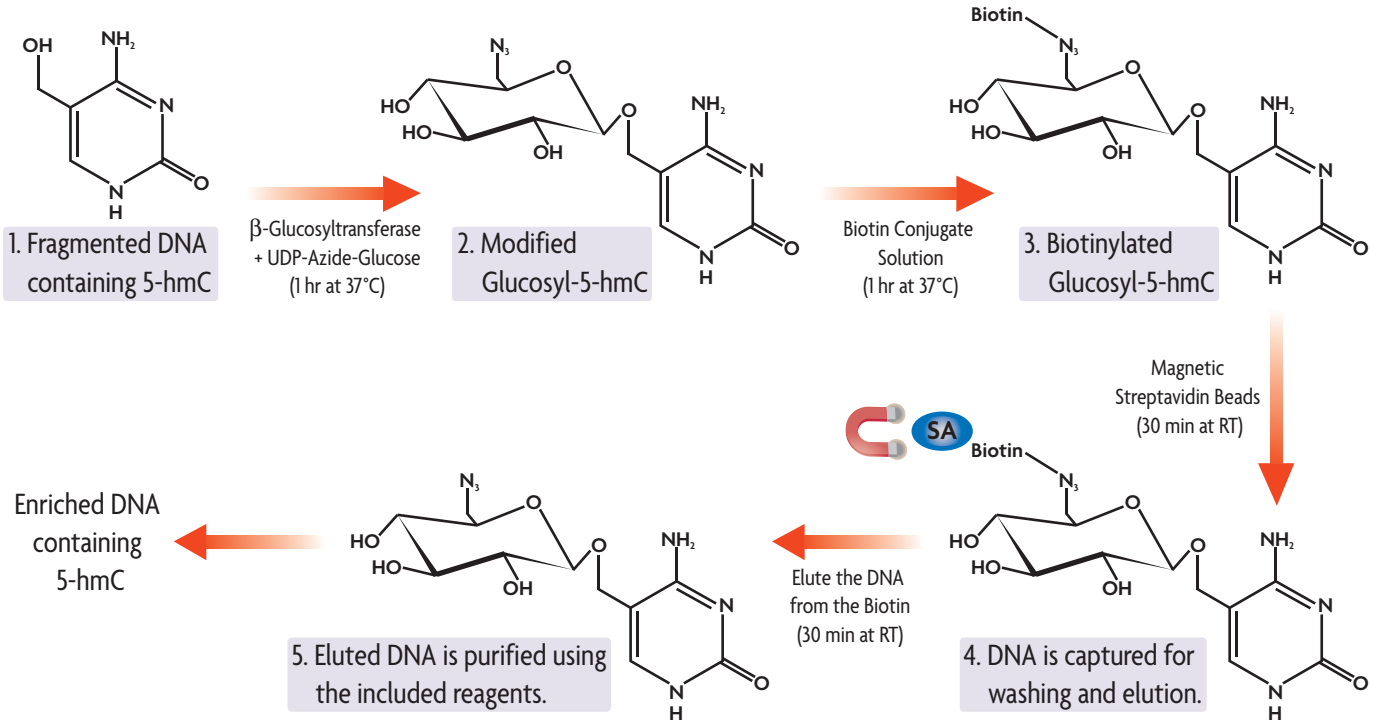


Diagram 1: Hydroxymethyl Collector method.
Fragmented dsDNA (100-500 bp) is combined with β-Glucosyltransferase enzyme in the presence of a UDP-Azide-Glucose donor. The enzyme adds this modified glucose onto the 5-hmC residues. A biotin conjugate is then attached and the complex is captured using streptavidin magnetic beads and a magnet. Elution Buffer is added, which releases the 5-hmC enriched DNA fragments from the biotin linker. Finally, the included purification reagents are utilized to clean up the DNA prior to its use in downstream applications.

Specific immunocapture of 5-hmC DNA using antibody-based enrichment

Active Motif's hMeDIP Kit was designed for enrichment of hydroxymethylated DNA. Using a highly selective 5-hmC antibody for specific immunocapture of hydroxymethylated genomic DNA fragments, the hydroxymethylated DNA immunoprecipitation, or hMeDIP, method allows genome-wide targeted enrichment of hydroxymethylated DNA sequences. The high level of specificity of the 5-hmC antibody used in our hMeDIP Kit offers several advantages, including the ability to efficiently immunoprecipitate both single-stranded and double-stranded DNA, to distinguish between 5-hmC and 5-mC, and to detect both CpG and non-CpG methylated DNA. Furthermore, the use of magnetic beads for capture ensures rapid processing of samples. The hMeDIP Kit can be run in parallel to our MeDIP Kit for comparative analysis of DNA methylation patterns. The hMeDIP Kit also includes unmethylated, 5-methylcytosine and 5-hydroxymethylcytosine control DNAs and PCR primers to ensure the specificity of the 5-hmC immunocapture (Figure 2).

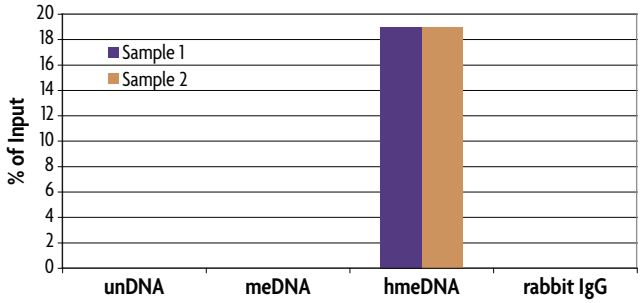


Figure 2: Specificity of the hMeDIP Kit.
Mse I digested human genomic DNA (500 ng) was spiked with 25 pg of either methylated (meDNA), hydroxymethylated (hmeDNA) or unmethylated APC DNA (unDNA). Samples were then processed using the hMeDIP Kit with purified 5-hydroxymethylcytosine pAb. Eluted DNA was purified and tested using real-time PCR with the included APC PCR primer mix. The 5-hydroxymethylcytosine antibody specifically enriched the IP sample containing the hydroxymethylated APC DNA, but did not enrich for methylated or unmethylated DNA. The APC locus analyzed in this experiment is not methylated in human genomic DNA and therefore should not amplify. This experiment was performed to detect the presence of the spiked control DNA only. Samples were assayed in duplicate.

5-Hydroxymethylcytosine-specific enzymes to distinguish between 5-mC and 5-hmC

Active Motif offers two different enzymes for use in distinguishing between the 5-mC and 5-hmC forms of DNA methylation, the PvuRts1I restriction enzyme and the β-Glucosyltransferase enzyme.

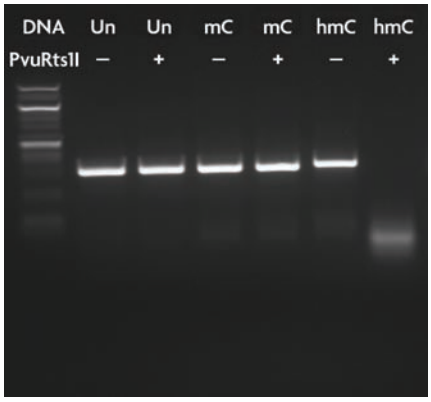


Figure 3: PvuRts1 I enzyme digestion of 5-hmC DNA. One µg of unmethylated (Un), 5-methylcytosine (mC) or 5-hydroxymethylcytosine (hmC) Methylated DNA Standard (Catalog No. 55008) was incubated in the absence or presence of 1 unit PvuRts1 I enzyme for 30 minutes at 22°C. Each reaction was run on a 2.5% agarose gel alongside a 1 kb DNA ladder.

The **PvuRts1I restriction enzyme** is the only enzyme to directly differentiate between the 5-mC and 5-hmC forms of DNA methylation. Therefore, it serves as a valuable tool for analyzing DNA methylation patterns within the genome. The enzyme is not only able to directly distinguish between 5-mC and 5-hmC, but is also able to cleave both glucosylated and non-glucosylated 5-hmC DNA, thus eliminating the need to glucosylate 5-hmC residues prior to digestion analysis (Figure 3).

The **β-Glucosyltransferase enzyme** labels 5-hmC DNA by transferring a glucose moiety from UDP-Glucose to the 5-hmC residue in double-stranded DNA to create glucosyl-5-hmC DNA (Diagram 2). The 5-hmC DNA can be quantified directly by radioactive labeling of 5-hmC with a [^{14}C] UDP-Glucose donor. Alternatively, 5-hmC DNA methylation patterns can be analyzed using glucosyl-sensitive restriction enzyme digestion.

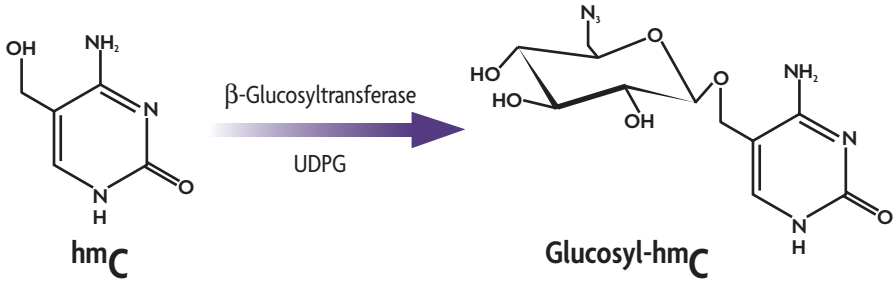


Diagram 2: Depiction of the β-Glucosyltransferase enzyme reaction.

Recombinant Tet1 for 5-hmC conversion assays

The **Tet1 protein** is a member of the TET family of cytosine oxygenases that convert 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC). For researchers interested in studying the mechanism of how 5-hmC is generated, Active Motif offers a recombinant Tet1 protein for use in 5-hmC conversion assays (Figure 4).

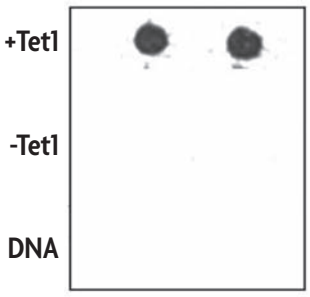


Figure 4: Recombinant Tet1 activity assay. Double-stranded DNA containing 5-methylcytosine was incubated with 5 µg of recombinant Tet1 enzyme (+Tet1) or without Tet1 enzyme (-Tet1). These samples and an unmethylated DNA control (DNA) were then spotted onto a nylon membrane and incubated with 5-Hydroxymethylcytosine antibody (Catalog No. 39769) to detect the conversion of 5-methylcytosine into 5-hydroxymethylcytosine.

Ordering information for 5-hmC products

For more information and complete product details, please call or visit us at www.activemotif.com/dnamt.

Product	Format	Cat. No.
Hydroxymethyl Collector™	25 rxns	55013
hMeDIP	10 rxns	55010
PvuRts1I restriction enzyme	50 units	55011
β-Glucosyltransferase enzyme	500 units	55012
Recombinant Tet1 protein, active	25 µg	31363

Active Motif also offers custom services (page 9) and antibodies (page 10) for the analysis of 5-hydroxymethylcytosine. For a complete up-to-date list of all of our available products to study 5-hmC, please call or visit us at www.activemotif.com/hmc.

5-Methylcytosine (5-mC)

5-Methylcytosine (5-mC), also known as the "5th base," is formed when DNA methyltransferases catalyze the transfer of a methyl group from S-adenosyl-L-methionine to cytosine. 5-mC is found in CpG-rich regions, and its function is associated with transcriptional repression, particularly as it relates to genomic imprinting, repression of transposable elements and gene silencing.

MethylCollector Ultra™ for rapid isolation of CpG methylated DNA

The MethylCollector™ Ultra Kit is a rapid magnetic assay for targeted enrichment of CpG methylated DNA from genomic DNA fragments that have been prepared by sonication or enzymatic digestion. Our magnetic bead based protocol enables CpG enrichment to be completed in less than 3 hours, much faster than antibody immunoprecipitation, which typically requires overnight incubation. MethylCollector Ultra is based on the Methylated CpG Island Recovery Assay (MIRA) which uses a MBD2b/MBD3L1 protein complex that binds with high affinity to methylated CpG dinucleotides (Diagram 3). This methodology provides better enrichment than assays that utilize methyl-binding protein (MBD) alone, enabling recovery of DNA fragments containing as few as 5 methylated CpGs, or as little as 1 ng of DNA (an equivalent of ~200 cells).

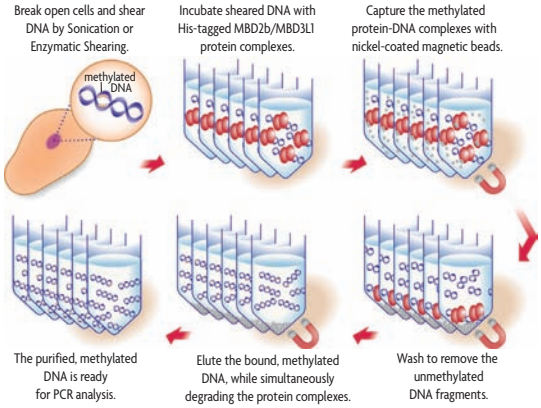


Diagram 3: Flow chart of the MethylCollector Ultra process.
In MethylCollector Ultra, genomic DNA is sheared by either sonication or enzymatic digestion, then incubated with the recombinant His-MBD2b/MBD3L1 protein complex. Magnetic beads capture the protein-DNA complexes. Optimized buffers ensure that fragments with little or no methylation are removed. Methylated DNA is then eluted from the beads. Following clean up, the eluted DNA is ready for use in various downstream applications.

Advantages

- **Improved efficiency** – high-affinity binding provides greater enrichment than other MBD capture methods
- **Faster procedure** – magnetic protocol can be completed in less than 3 hours
- **Uses minimal sample material** – works with as little as 1 ng (~200 cells) of fragmented DNA
- **Controls ensure success** – includes positive control DNA and PCR primers
- **Versatility** – eluted DNA is suitable for use in various downstream applications such as endpoint or real-time PCR, bisulfite conversion, or microarray analysis

*Technology covered under U.S. Patent No. 7,425,415.

Higher enrichment capability than other methods

A direct comparison of Active Motif's MethylCollector Ultra Kit versus a competitor's MBD-Biotin capture method illustrates the specificity of MethylCollector Ultra. Enriched methylated DNA was analyzed by qPCR using primers for two different promoters (Figure 5), methylated SNRPN promoter (red), and unmethylated FOXD2 (blue). The MethylCollector Ultra Kit shows early amplification of the SNRPN locus at 28 cycles, while the unmethylated FOXD2 amplifies at 36 cycles. This validates the increased enrichment capability of the MBD2b/MBD3L capture protein and the specificity of the assay for CpG methylated DNA. Competitor MM's technique showed no enrichment of methylated DNA, as both methylated and unmethylated DNA amplified at 28 cycles.

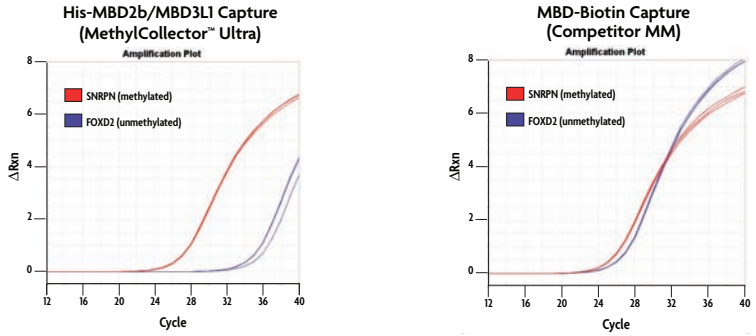


Figure 5: Real-time PCR analysis reveals the specificity of MethylCollector Ultra vs competing technologies.
100 ng of human, male genomic DNA was digested with *Mse* I and tested using MethylCollector Ultra and competitor MM's kit. Eluted DNA was analyzed using PCR primers for both methylated, SNRPN (red), and unmethylated, FOXD2 (blue), promoters. Only MethylCollector Ultra showed specific enrichment for CpG methylated DNA as shown by the clear separation in amplification cycles.

UnMethylCollector™ for positive identification of unmethylated CpGs

UnMethylCollector™ is the first commercially available kit for the specific isolation and enrichment of unmethylated CpG dinucleotides. UnMethylCollector utilizes the specificity of the CXXC binding domain towards unmethylated CpGs to capture and enrich for DNA fragments that lack methylation. This makes it possible to identify hypomethylated promoters and to study the effects of compounds that inhibit methylation. Instead of relying on negative data from methyl-specific binding techniques to identify unmethylated promoters, UnMethylCollector offers a targeted, reliable technique that provides positive identification of unmethylated CpG regions (Figure 6).

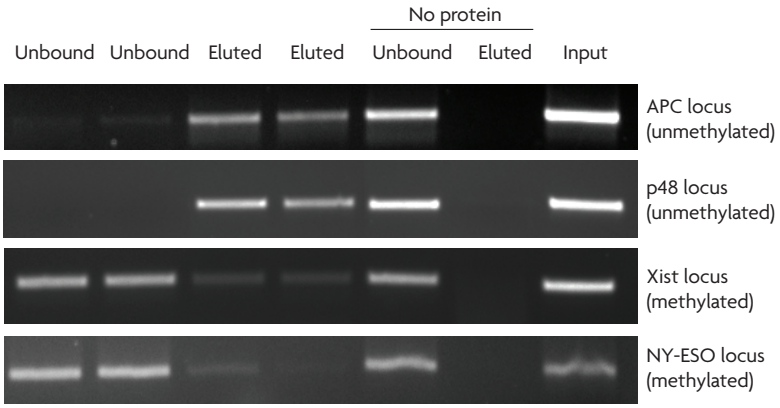


Figure 6: Endpoint PCR results confirm the specificity of UnMethylCollector across multiple loci. The human, male genomic DNA (200 ng) provided in UnMethylCollector was tested. Both unbound and eluted fractions were collected and analyzed in PCR for 36 cycles across multiple loci. The two unmethylated promoters (APC and p48) showed strong bands in the eluted fractions, while the methylated promoters (Xist and NY-ESO) were primarily found in the unbound fraction.

Optimized reagents ensure specific isolation

Active Motif's UnMethylCollector Kit uses a recombinant His-CXXC protein to specifically bind unmethylated DNA fragments containing as few as one CpG dinucleotide. The kit provides two binding buffers, a low-salt buffer for use with samples containing less than 5 CpGs per fragment and a higher salt buffer for efficient binding of fragments with more than 5 CpGs. Nickel-coated magnetic beads capture the protein-DNA complexes and the unmethylated DNA is eluted from the beads. Following clean up, the eluted DNA is ready for use.

A side-by-side comparison of fractions obtained from both MethylCollector and MethylCollector Ultra Kits illustrate the specificity of each technique at binding and enriching for the appropriate methylation status across multiple loci (Figure 7).

Additionally, DNA collected from UnMethylCollector was bisulfite treated using Active Motif's MethylDetector™ Kit and analyzed by sequencing. Of the 8 clones sequenced for the unmethylated APC locus, only one clone contained a single methylated CpG of the 19 CpG sites within the sequenced region (Figure 9, next page).

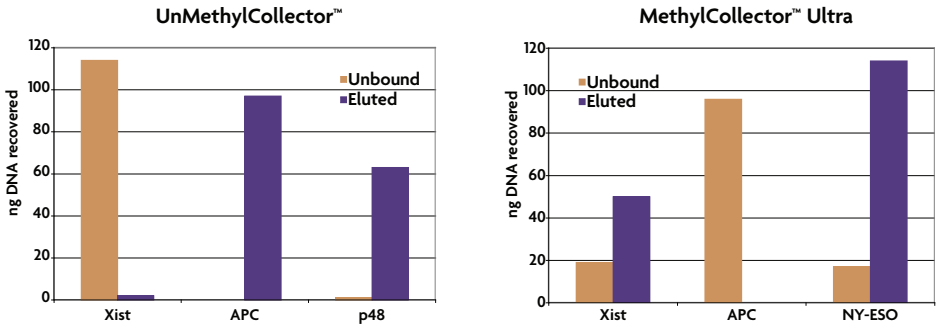


Figure 7: Direct comparison of UnMethylCollector and MethylCollector Ultra illustrates specificity. Both UnMethylCollector and MethylCollector Ultra were run according to the protocols using either 200 ng or 100 ng, respectively, of the provided *Mse* I digested human, male genomic DNA. Real-time PCR analysis was run across multiple loci on both the unbound and eluted fractions. UnMethylCollector clearly captures the unmethylated loci (APC and p48) while MethylCollector Ultra enriches for methylated promoters (Xist and NY-ESO).

APC – contains 29 CpGs and is unmethylated in healthy tissues
p48 – contains 22 CpGs and is unmethylated in healthy tissues
Xist – contains 8 CpGs and is methylated in males (it is unmethylated in females)
NY-ESO – contains 6 CpGs and is methylated in human DNA

UnMethylCollector™ advantages

- **Sensitivity** – detects unmethylated CpGs from 10 ng - 1 µg of DNA fragmented by sonication or enzymatic digestion
- **Faster procedure** – magnetic protocol can be completed in less than 3 hours
- **Controls ensure success** – includes positive control DNA and PCR primers
- **Versatility** – eluted DNA is suitable for use in various downstream applications such as PCR, sequencing, or amplification and labeling for microarray analysis

*Patent Pending

MeDIP for highly selective enrichment of methylated DNA

In addition to its hMeDIP Kit, Active Motif also offers a MeDIP Kit for selective enrichment of single-stranded DNA fragments containing 5-mC from genomic DNA. The MeDIP Kit utilizes a highly specific monoclonal 5-mC capture antibody for methylated DNA immunoprecipitation (MeDIP). The antibody is able to distinguish between 5-mC and 5-hmC, making this approach more selective than conventional bisulfite conversion or enzymatic methods. A one-step immunoprecipitation and the use of Protein G magnetic beads reduces the protocol time to ensure rapid recovery of methylated DNA. The MeDIP Kit also includes a bridging antibody to optimize enrichment, as well as human genomic DNA and PCR primers for use as controls to confirm the efficiency of the immunocapture. (Figure 8).

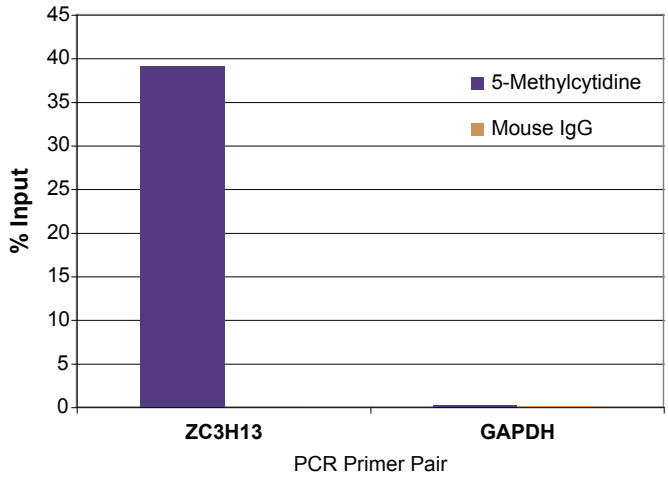


Figure 8: Real-time PCR results of the control DNA with ZC3H13 and GAPDH PCR primer sets.
Mse I digested human genomic DNA (500 ng) was processed using the MeDIP Kit with the 5-methylcytosine mAb or negative control mouse IgG. Eluted DNA was column purified with Active Motif's Chromatin IP DNA Purification Kit (Catalog No. 58002) and tested using real-time PCR with the included ZC3H13 PCR primer mix and a negative control GAPDH PCR primer mix. The 5-methylcytosine antibody specifically enriched methylated DNA with the ZC3H13 locus but did not enrich for DNA with either the GAPDH locus or the mouse IgG. Data shown are the results from samples assayed in duplicate.

Bisulfite conversion made simpler with MethylDetector™

Active Motif's MethylDetector™ sodium bisulfite conversion kit simplifies the analysis of DNA methylation. It comes complete with optimized reagents for performing DNA conversion with bisulfite, plus time-saving DNA purification columns. It also includes positive control PCR primers that enable you to validate the success of the conversion procedure before spending extra time and money on sequencing, or other analysis methods (Figure 10). This reproducible assay generates 99% conversion efficiency of unmethylated cytosines to uracils.

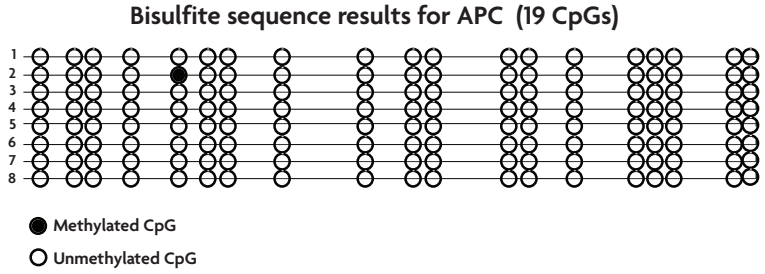


Figure 9: MethylDetector confirms the specificity of the UnMethylCollector™ Kit for unmethylated CpGs.
UnMethylCollector was used to enrich for unmethylated DNA fragments. This DNA was bisulfite treated using the MethylDetector Kit. Converted DNA was amplified by PCR. The gel extracted PCR product was cloned and 8 colonies were selected for sequencing using the unmethylated APC promoter region. Only one clone contained a single methylated CpG of the 19 CpG sites within the sequenced region. This validates that UnMethylCollector specifically enriches unmethylated DNA fragments.

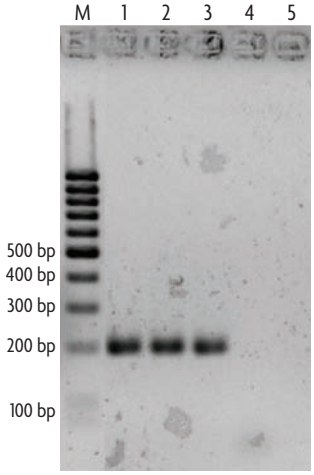


Figure 10: Agarose gel analysis of PCR products generated with MethylDetector.
Three separate DNA conversions (lanes 1-3) were performed using the MethylDetector Kit. Converted DNA was compared to an unconverted DNA control (lane 4) and to a no DNA control (lane 5). The presence of a PCR product using the conversion-specific PCR primers included in the kit verifies the conversion efficiency and reproducibility of the MethylDetector assay.

DNMT Activity / Inhibition Assay

Active Motif's DNMT Activity / Inhibition Assay is a rapid, non-radioactive method for screening the DNA methyltransferase (DNMT) activity of recombinant DNMT enzymes or nuclear extract samples. The 96-stripwell format also allows for both high and low throughput screening. Using the affinity of methyl-CpG-binding domain (MBD) protein toward methylated DNA, the assay targets DNMT activity from your sample to catalyze the transfer of methyl groups to a universal CpG-enriched DNA substrate that is immobilized on the plate. DNMT activity can then easily be quantified by spectrophotometry (Figure 11).

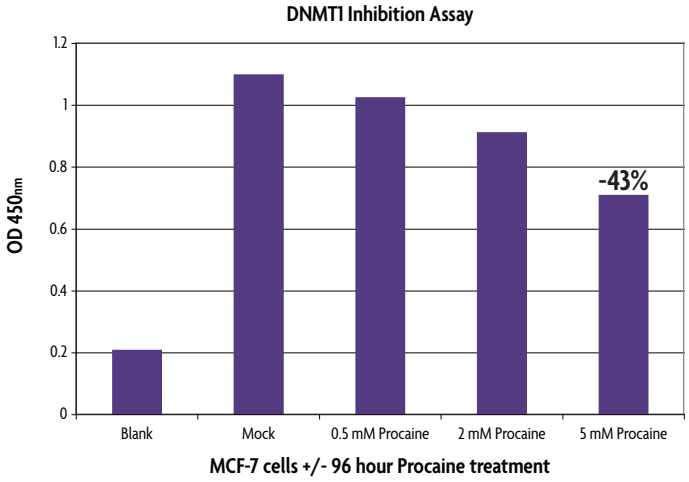


Figure 11: DNMT inhibition in MCF-7 cells with procaine treatment.
The DNMT Activity / Inhibition Assay was used to screen for DNMT inhibition in MCF-7 cells that were either untreated or treated with procaine for 96 hours. Nuclear extracts were prepared using Active Motif's Nuclear Extract Kit (Catalog No. 40010). Five µg of each treatment condition were tested in the assay with a 1.5 hour incubation time and a 3 minute developing time. The 5 mM procaine treatment showed a 43% inhibition of DNMT activity as compared to the mock treated sample. Data shown are the results from wells assayed in duplicate.

Advantages

- **Non-radioactive** – colorimetric assay is easily quantified by spectrophotometry on a microplate reader at 450 nm
- **Sensitive** – unique methyl CpG binding domain (MBD) protein approach enhances the sensitivity of detection from either purified DNMT proteins or nuclear extracts
- **Fast** – assay can be completed in less than 3 hours
- **Less effort required** – kit is compatible with multi-channel pipettors, which greatly streamlines the wash steps
- **Flexible** – stripwell plate format enables screening in low or high throughput

DNA Standards for analyzing different types of methylation

To help ensure the accuracy of your results, Active Motif offers the Methylated DNA Standard Kit for use as a control when performing DNA methylation analysis. The kit provides 3 recombinant DNA standards for unmethylated DNA, 5-mC methylated DNA, and 5-hmC methylated DNA (Figure 12).

Because the DNA standards are derived from the APC gene promoter, the kit also includes an APC primer mix for amplification in both endpoint and real-time PCR.

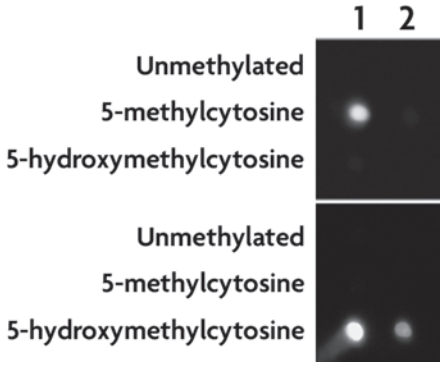


Figure 12: Dot blot analysis of Methylated DNA Standard Kit samples.
Single- and double-stranded DNA (50 ng) derived from each of the three different Methylated DNA Standards were spotted onto positively charged nylon membrane, then probed with 5-mC or 5-hmC antibody.
Top panel: 5-Methylcytosine antibody (Clone 33D3) (Cat. No. 39649, 1:1,000 dilution), detects only ssDNA.
Bottom panel: 5-Hydroxymethylcytosine antibody (Cat. No. 39769, 1:5,000 dilution).
Lane 1: Single-stranded DNA.
Lane 2: Double-stranded DNA.

GenoMatrix™ Whole Genome Amplification Kit

The GenoMatrix™ Whole Genome Amplification Kit provides all the reagents and buffers needed to quickly and easily perform up to 500-fold amplification on any type of genomic DNA, while maintaining the sequence representation of the starting material. The methodology has been optimized to work with as little as 10 ng of sample DNA obtained from MethylCollector™ or UnMethylCollector™ Kits, allowing for seamless transition into applications requiring a large quantity of DNA.

Genome-wide analysis services for DNA methylation

As part of its recent acquisition of Genpathway, Active Motif now offers custom services for the analysis of DNA methylation on a genome-wide scale. The epigenetic services available include:

- **MethylPath™** – discovery, identification, validation and quantitation of methylated DNA regions
- **Bisulfite Sequencing** – determination of the locations and methylation status of individual CpG dinucleotides
- **5-hydroxymethylcytosine MeDIP-Seq and MeDIP-chip** – analysis of 5-hmC methylation patterns on a genome-wide scale using our proprietary 5-hmC monoclonal antibody (Figure 13).

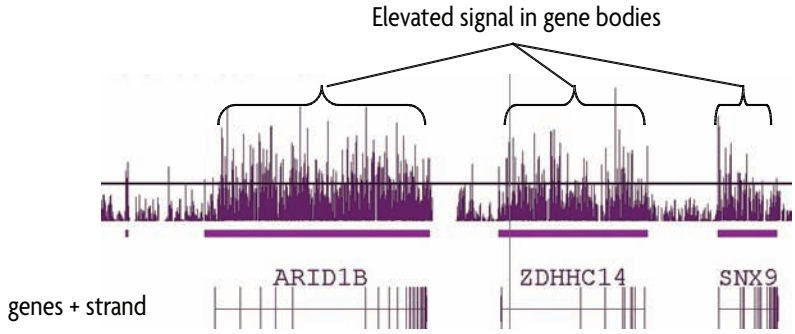


Figure 13: 5-hMeDIP-chip performed on human brain DNA.
Human brain DNA (2 µg) was immunoprecipitated with 10 µg of 5-Hydroxymethylcytosine antibody (Clone 59.1, Catalog No. 39999). Following hMeDIP, the DNA was amplified, labeled and hybridized to an Affymetrix Human Tiling 2.0R Array. Shown is a region from chromosome 6q containing the ARID1B, ZDHHC14 and SNX9 genes. The results show that 5-hydroxymethylcytosine is enriched primarily in the coding region of genes, rather than the promoter or regulatory regions.

For more information and complete details, please visit us at www.activemotif.com/services.

Choose the DNA methylation kits that best suit your specific research needs

Active Motif's extensive portfolio of kits for studying DNA methylation enables you to select from a variety of enrichment options, including whether to enrich for non-CpG or CpG methylated DNA, if ssDNA or dsDNA is enriched, as well as the capture method used. This allows you to choose the features you need in order to achieve the results you want in your DNA methylation research.

	Methylation at: CpG	Methylation at: non-CpG	DNA Enriched: ssDNA	DNA Enriched: dsDNA	Magnetic Beads	Capture Method
Hydroxymethyl Collector™	✓	✓	–	✓	✓	Chemical
MethylCollector™ Ultra	✓	–	–	✓	✓	Protein
UnMethylCollector™	✓	–	–	✓	✓	Protein
hMeDIP	✓	✓	✓	✓	✓	Antibody
MeDIP	✓	✓	✓	–	✓	Antibody

Ordering information for DNA methylation products

For more information and a complete list of our DNA methylation products, please call or visit us at www.activemotif.com/dnamt.

Product	Format	Cat. No.
MethylCollector™ Ultra	30 rxns	55005
UnMethylCollector™	30 rxns	55004
MeDIP	10 rxns	55009
MethylDetector™	50 rxns	55001
DNMT Inhibition / Activity Assay	1 x 96 rxns	55006
Methylated DNA Standard Kit	3 x 2.5 µg	55008
GenoMatrix™ Whole Genome Amplification Kit	50 rxns	58001

5-formylcytosine (5-fC) and 5-carboxylcytosine (5-caC)

5-formylcytosine (5-fC) and **5-carboxylcytosine (5-caC)** are novel DNA modifications found to exist in many vertebrate cell types, including embryonic stem cells. The TET family of cytosine oxygenase enzymes, which convert 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC), further oxidize 5-hmC into 5-fC and 5-caC. 5-fC and 5-caC appear in the paternal pronucleus after fertilization (Figure 15A), concomitant with the disappearance of 5-methylcytosine (5-mC). The levels of 5-fC and 5-caC are gradually diluted out by DNA replication, rather than being enzymatically removed (Figure 14). While this pathway represents a mechanism by which DNA methylation (5-mC) is removed, these novel modifications may also be serving unique functions in pre-implantation development.

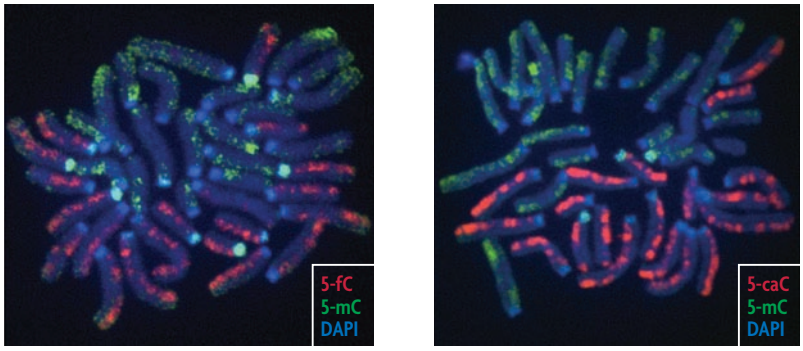


Figure 14: Replication-dependent loss of 5-fC and 5-caC revealed in a 2-cell metaphase embryo.
Shown are representative immunofluorescent images of mitotic chromosome spreads that have been co-stained with Active Motif's 5-Formylcytosine (5-fC) or 5-Carboxylcytosine (5-caC) antibodies (red, Catalog Nos. 61223 and 61225, respectively), a 5-methylcytosine (5-mC) antibody (green) and DAPI (blue) at the two-cell stage of mouse preimplantation development. The 5-fC and 5-caC antibodies were used at a 1:2000 dilution. The images reveal that at the two-cell stage, only one of the two sister chromatids is enriched for 5-fC and 5-caC, consistent with findings that 5-fC and 5-caC levels are diminished by half in blastomeres with each round of DNA replication (Inoue *et al.* (2011)*).

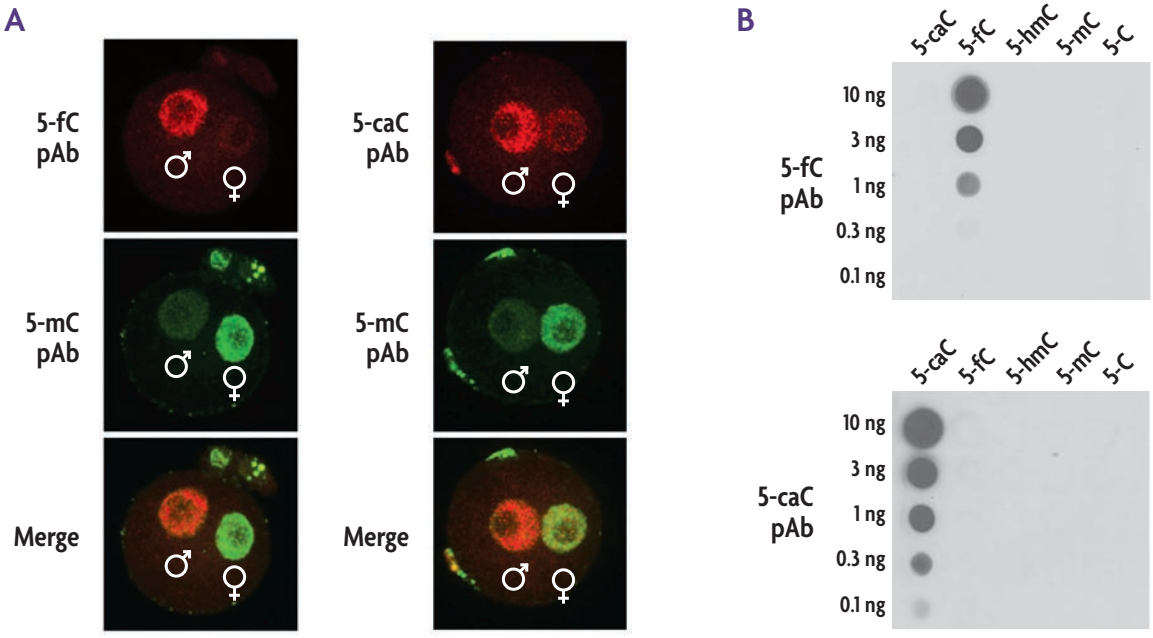


Figure 15: Characterization of 5-fC and 5-caC antibodies by whole mount staining and dot blot.
(A) Shown are representative whole mount confocal images of fertilized oocytes co-stained with Active Motif's 5-Formylcytosine (5-fC) and 5-Carboxylcytosine (5-caC) antibodies (red, Catalog Nos. 61223 and 61225, respectively), and a 5-methylcytosine (5-mC) antibody (green). The 5-fC antibody was used at a 1:4000 dilution and the 5-caC antibody was used at a 1:2000 dilution (Inoue *et al.**). **(B)** Dot blot analysis was used to confirm the specificity of the 5-fC antibody for 5-formylcytosine and the 5-caC antibody for 5-carboxylcytosine. Varying amounts of single-stranded DNA oligonucleotides corresponding to the immunogen and related sequences were spotted onto nitrocellulose and probed with the 5-fC antibody (upper image, 1:5,000 dilution) and the 5-caC antibody (lower image, 1:2000 dilution). Lane 1: oligomer containing 5-carboxylcytosine. Lane 2: oligomer containing 5-formylcytosine. Lane 3: oligomer containing 5-hydroxymethylcytosine. Lane 4: oligomer containing 5-methylcytosine. Lane 5: oligomer containing unmodified cytosine.

* Images were kindly provided by the laboratory of Yi Zhang, HHMI Investigator at the University of North Carolina at Chapel Hill. The shown images reference experimental data that is described in detail in Inoue *et al.* (2011) *Cell Research* 21: 1670-1676.

Antibodies for DNA methylation research

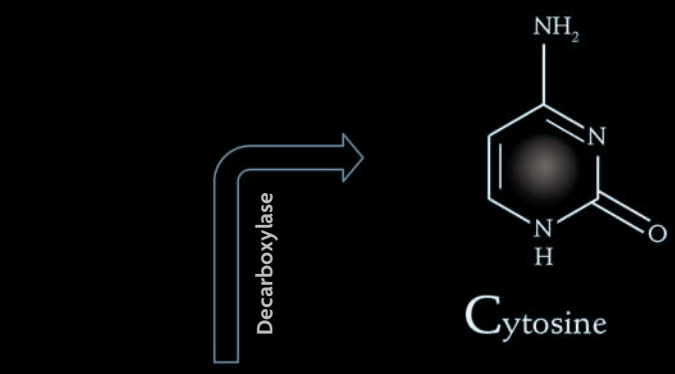
For your convenience, Active Motif offers a variety of DNA methylation-related antibodies. Active Motif is committed to providing the highest quality antibodies for studying the biology of the nucleus. Each antibody we make is rigorously tested. Many of the DNA methylation antibodies have been validated for use in ChIP and immunofluorescence (IF).

The table below is a partial list. For more information, and to see a complete list of DNA methylation antibodies, please visit our website at www.activemotif.com/methylabs.

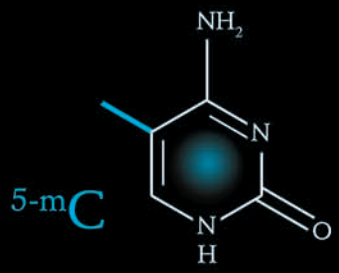
Target	Product	Reactivity	Application	Format	Catalog No.
3-mC	3-Methylcytosine polyclonal antibody (Serum)	Human, Mouse, Wide Range	DB	100 µl 10 µl	61179 61180
	3-Methylcytosine polyclonal antibody (IgG)	Human, Mouse, Wide Range	DB	100 µg 10 µg	61111 61112
5-caC	5-Carboxylcytosine polyclonal antibody (Serum)	Mouse, Not Species Specific	DB, IF	100 µl 10 µl	61225 61226
	5-Carboxylcytosine polyclonal antibody (IgG)	Not Species Specific	DB	100 µg 10 µg	61229 61230
5-fC	5-Formylcytosine polyclonal antibody (Serum)	Mouse, Not Species Specific	DB, IF	100 µl 10 µl	61223 61224
	5-Formylcytosine polyclonal antibody (IgG)	Not Species Specific	DB	100 µg 10 µg	61227 61228
5-hmC	5-Hydroxymethylcytosine monoclonal antibody	Human, Mouse, Wide Range	DB, MeDIP	100 µg 10 µg	39999 40000
	5-Hydroxymethylcytosine polyclonal antibody (Serum)	Human, Mouse, Wide Range	DB, IF, IHC, MeDIP	100 µl 10 µl	39769 39770
	5-Hydroxymethylcytosine polyclonal antibody (IgG)	Human, Mouse, Wide Range	DB, MeDIP	100 µg 10 µg	39791 39792
5-mC	5-Methylcytosine monoclonal antibody	Not Species Specific	FC, IHC, IP, MeDIP	50 µg	39649
CGBP	CGBP polyclonal antibody	Human, Mouse	WB	200 µl	39203
ELP3	ELP3 polyclonal antibody	Human	WB	100 µl 10 µl	39949 39950
DNMT1	DNMT1 monoclonal antibody	Human, Mouse	ChIP, IHC, IP, WB	100 µg	39204
DNMT2	DNMT2 polyclonal antibody	Human, Mouse	WB	100 µg	39205
DNMT3A	DNMT3A monoclonal antibody	Human, Mouse	ChIP, IF, IHC, WB	100 µg	39206
DNMT3B	DNMT3B monoclonal antibody	Human, Mouse	ChIP, IF, IP, WB	100 µg	39207
DNMT3L	DNMT3L polyclonal antibody	Human	WB	100 µl 10 µl	39907 39908
HP1	HP1α monoclonal antibody	Mouse	ChIP, ELISA, ICC, IF, IHC	100 µg 10 µg	39977 39978
MBD1	MBD1 monoclonal antibody	Human	WB	100 µg	39215
MBD2	MBD2 polyclonal antibody	Human	WB	200 µl 10 µl	39547 39548
MBD3	MBD3 monoclonal antibody	Human	WB	100 µg	39216
MBD4	MBD4 polyclonal antibody	Human	WB	100 µg	39217
MeCP2	MeCP2 polyclonal antibody	Human, Mouse	WB	200 µl 10 µl	39188 39189
Uhrf1	Uhrf1 polyclonal antibody	Human	WB	200 µl 10 µl	39625 39626

ChIP = Chromatin Immunoprecipitation; **DB** = Dot Blot; **FC** = Flow Cytometry; **IF** = Immunofluorescence; **ICC** = Immunocytochemistry; **IHC** = Immunohistochemistry; **IP** = Immunoprecipitation; **MeDIP** = Methylated DNA Immunoprecipitation; **WB** = Western Blot

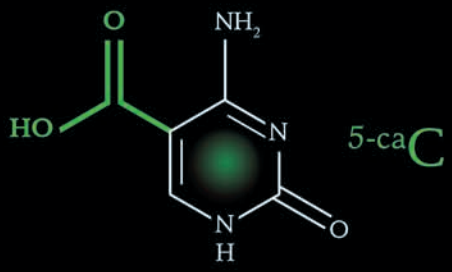
- CGBP Antibody
- Methylated DNA Standard Kit



- DNMT Activity/Inhibition Assay
- DNMT Antibodies
- HP1 Antibodies
- MBD Antibodies
- MeCP2 Antibodies
- Uhrf1 Antibody



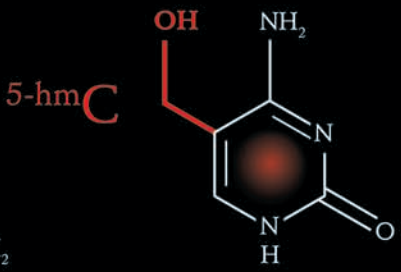
- MethylCollector Ultra™
- UnMethylCollector™
- MeDIP
- MethylDetector™
- 5-mC Antibodies
- Methylated DNA Standard Kit



- 5-caC Antibody

Tet

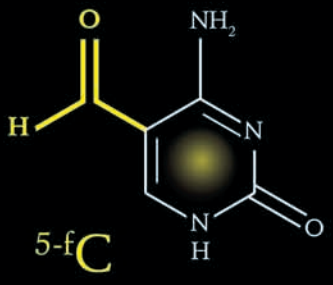
- Recombinant Tet1 Protein



- Hydroxymethyl Collector™
- hMeDIP
- PvuRts1I
- β-Glucosyltransferase
- 5-hmC Antibodies
- Methylated DNA Standard Kit

Tet

- Recombinant Tet1 Protein



Tet

- 5-fC Antibody

ACTIVE MOTIF®

Enabling Epigenetics Research