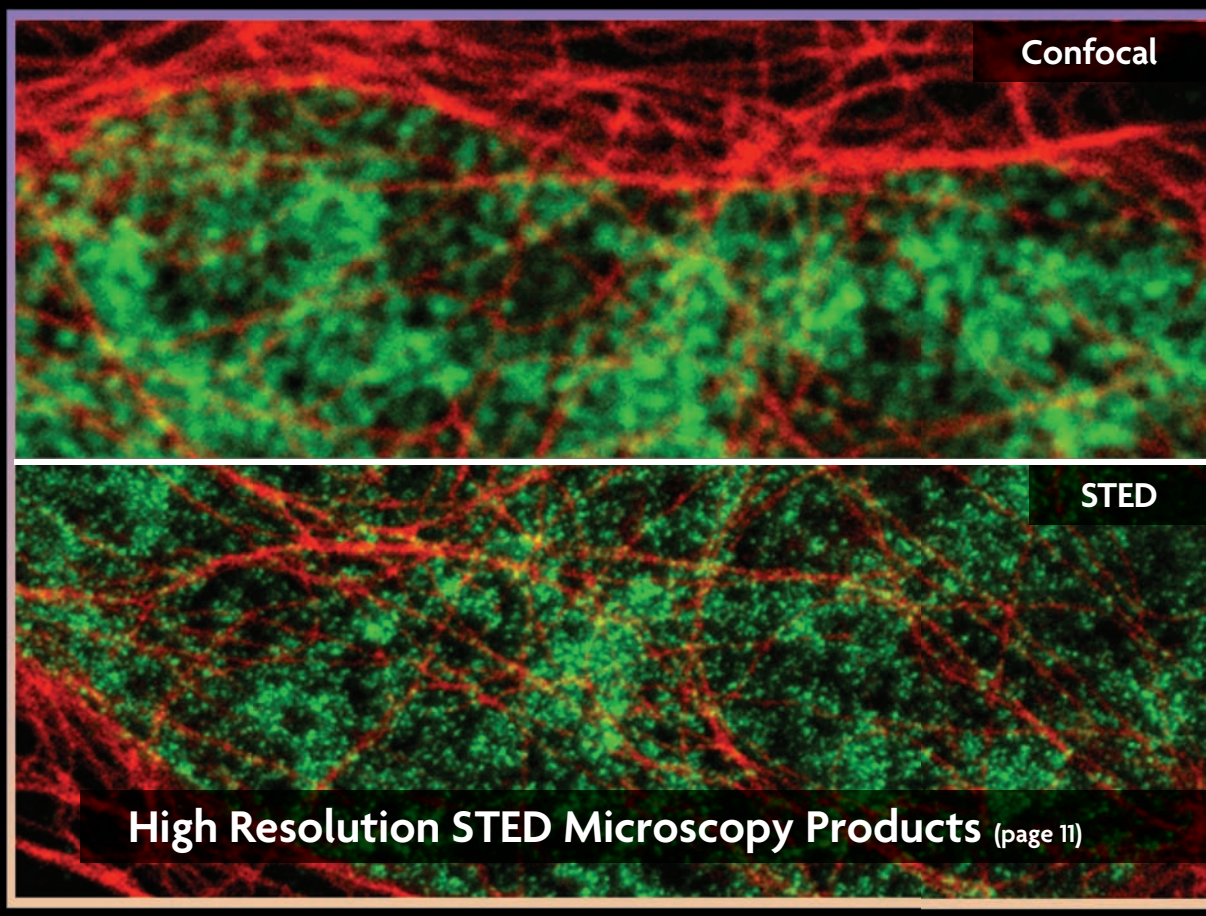
ACTIVE MOTIF®

MOTIFvations

The Newsletter of Active Motif October 2011 Volume 12 Number 2



High Resolution STED Microscopy Products (page 11)

IN THIS ISSUE

DNA Methylation, Sample Preparation & Antibodies

2 5-Hydroxymethylcytosine: A New Analysis in DNA Methylation

3 NEW: Biotin-based Enrichment of 5-Hydroxymethylcytosine

4 NEW: Enzymes for the Study of 5-Hydroxymethylcytosine

5 NEW: MeDIP Enrichment of 5-Methylcytosine or 5-Hydroxymethylcytosine

6 NEW: ChIP qPCR Primers and Controls Ensure Your Experiments Work Every Time

7 NEW: Multi-Sample Sonicator, Probe Sonicator and Cooled Sonication Platform

8 DNMT Assay to Analyze Changes in CpG Methylation

8 NEW: Dounce Homogenizer to Assist in Cell and Chromatin Preparations

9 NEW: Protease Inhibitor Cocktail for Better Sample Preparation

9 Nuclear Extraction Kit for Nuclear, Cytoplasmic or Whole-cell Lysates

10 Antibodies to Stem Cell Regulators to Further your Stem Cell Research

10 Highly Validated Antibodies and ELISAs for the Study of Nuclear Receptors

11 Antibodies and Reagents for High Resolution Confocal STED Microscopy

5-Hydroxymethylcytosine: A New Analysis in DNA Methylation

In mammals and other vertebrates, DNA methylation usually occurs at the C5 position of cytosines (5-mC), mostly within CpG dinucleotides. Normally, CpG islands, short CG-rich regions, are unmethylated; in cases where methylation does occur, the associated gene is silenced. Aberrant methylation of these CpG regions is often associated with disease.

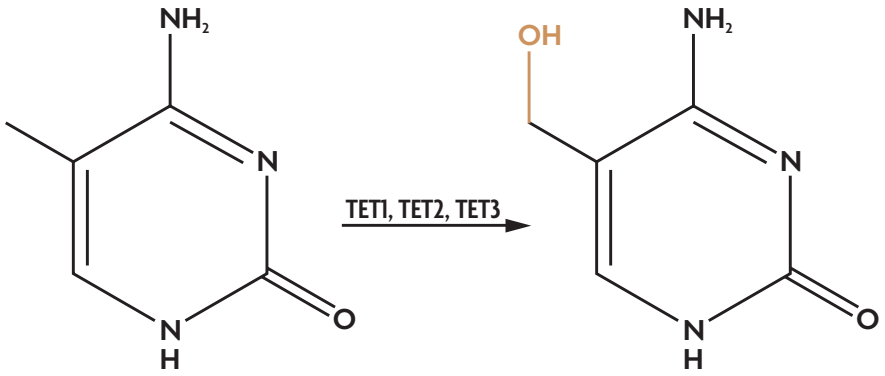


Figure 1: Schematic of the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC).
5-methylcytosine is converted to 5-hydroxymethylcytosine by the TET family of cytosine oxygenases.

In 2009, Kriaucionis and Heintz as well as Tahiliani *et al.* discovered that a novel form of DNA methylation, 5-hydroxymethylcytosine (5-hmC), exists in mammals, and is elevated in neurons and embryonic stem cells. 5-Hydroxymethylcytosine results from the enzymatic conversion of 5-methylcytosine into 5-hydroxymethylcytosine by the TET family of cytosine oxygenases (Figure 1). To date, little is understood about the functional relevance of 5-hmC in the mammalian genome.

One of the difficulties in studying 5-hmC is the fact that many of the traditional techniques employed to study DNA methylation, such as bisulfite conversion or certain methylation-sensitive restriction enzymes, do not distinguish between 5-mC and 5-hmC residues. Methyl CpG binding protein enrichment methods only serve to selectively bind 5-mC DNA methylation, but cannot be used to enrich for 5-hmC. In an effort to better analyze the location and function of 5-hydroxymethylcytosine, new tools and techniques will need to be employed.

Active Motif is pleased to offer antibodies, assays and enzymes for the direct analysis of 5-hydroxymethylcytosine. We were the first company to provide a 5-hydroxymethylcytosine antibody that could be used for the detection and immunoprecipitation of 5-hmC DNA. We have since expanded our product offering to include enzymes capable of distinguishing between 5-mC and 5-hmC residues, and affinity enrichment kits to selectively isolate 5-hydroxymethylcytosine DNA.

Additional product details are available within this newsletter. To learn more about all of the 5-hmC products available, please give us a call or visit us on our website at www.activemotif.com/hmc.

Save 10% on reagents to study 5-Hydroxymethylcytosine
For a limited time, get 10% off select products for 5-hmC.
For complete details and a list of eligible products,
please visit www.activemotif.com/promo.

NEW: Biotin-based Enrichment of 5-Hydroxymethylcytosine

To better interrogate the functional relevance of 5-hydroxymethylcytosine (5-hmC) DNA methylation, Active Motif has developed an assay kit that enables detection and affinity enrichment of DNA fragments containing the 5-hydroxymethylcytosine residue. The Hydroxymethyl Collector™ kit utilizes a β-glucosyltransferase enzyme to transfer a modified glucose moiety to 5-hydroxymethylcytosine residues in double-stranded DNA. This modified glucose is then used to chemically attach a biotin conjugate for capture and enrichment with magnetic streptavidin beads. The enriched DNA can then be analyzed by PCR, microarray or sequencing to better understand the role of 5-hydroxymethylcytosine in mammalian genomes.

How does Hydroxymethyl Collector™ isolate 5-hmC DNA?

First, genomic DNA is fragmented to a size range of 100-500 base pairs. The DNA is then incubated in the presence of a β-glucosyltransferase enzyme and a modified UDP-Glucose donor. The enzyme transfers the glucose to 5-hydroxymethylcytosine residues, creating glycosyl-5-hydroxymethylcytosine.

Click chemistry is used to introduce a biotin conjugate onto the modified glucose. Magnetic streptavidin beads and the included bar magnet are used to capture the biotinylated 5-hmC DNA fragments. The elution buffer releases the DNA fragments from the biotin conjugation, leaving you with DNA that is enriched for 5-hmC methylation.

Following purification, with the included purification reagents, the DNA is ready to use in PCR, microarray or sequencing.

What's in the box?

The Hydroxymethyl Collector Kit contains all the reagents needed to perform the glucosylation reaction, biotin conjugation, streptavidin capture, elution and purification of 5-hydroxymethylcytosine DNA. A positive control hydroxymethylated DNA standard and PCR primers are also included in the kit to confirm the success of the enrichment reactions.

References
Song *et al.* (2010) *Nature Biotechnology* 29: 68-72.

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 Affymetrix Human Tiling 2.0R Array A containing chromosomes 1 and 6. The 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.

What are the advantages?

- Covalent labeling of 5-hmC ensures accurate capture of DNA fragments containing hydroxymethylcytosine
- The biotin-streptavidin capture system enables the use of stringent binding and wash conditions
- The technique is sensitive enough to enrich DNA fragments containing as few as two 5-hmC residues
- Simple procedure can be completed in less than 4 hours
- Enriched DNA is suitable for use in analysis of individual loci by PCR, or whole-genome analysis by sequencing or microarray (Figures 1 & 2)

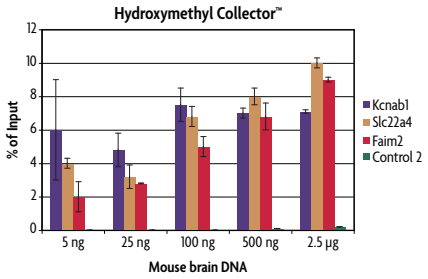


Figure 2: Real-time PCR analysis of the enrichment of the 5-hmC DNA from mouse brain.
Mouse brain DNA was assayed at various quantities in the Hydroxymethyl Collector Kit. Enriched DNA was analyzed by real-time PCR across multiple loci. The enriched DNA was quantified and plotted as a percentage of the starting material. This graph shows the Hydroxymethyl Collector Kit has a range of detection from 5 ng to 2.5 µg. The Mouse Negative Control Primer Set 2 (Cat. No. 71012) primer served as a negative control.

To learn more about all of Active Motif's 5-Hydroxymethylcytosine products, please call or visit us at www.activemotif.com/hmc.

Product	Format	Catalog No.	Price ($US)
Hydroxymethyl Collector™	25 rxns	55013	375

NEW: Enzymes for the Study of 5-Hydroxymethylcytosine

Active Motif offers enzymes to study not only the mechanism of the conversion of 5-methylcytosine (5-mC) into 5-hydroxymethylcytosine (5-hmC), but also enzymes that enable differentiation between these two forms of DNA methylation. These enzymes help to overcome some of the technical limitations that previously existed in the analysis of 5-hydroxymethylcytosine.

β-Glucosyltransferase enzyme for 5-hmC modification

For researchers looking to study overall changes in 5-hydroxymethylcytosine, Active Motif offers separately the same β-Glucosyltransferase enzyme included in the Hydroxymethyl Collector™ Kit (see page 3). This enzyme can modify the 5-hmC residue with the addition of a glucose moiety. Following glucosylation, the 5-hmC residues will be protected from glucosyl-sensitive restriction enzymes, such as MspI or GlaI. Alternatively, if the β-Glucosyltransferase enzyme is used with a radiolabeled UDP-glucose donor, it enables direct labeling of hydroxymethylated residues.

The β-Glucosyltransferase enzyme is provided with a 10X reaction buffer, a UDP-Glucose (uridine diphosphoglucose) donor and 1 M DTT. One unit of enzyme is defined as the amount of enzyme required to fully protect 0.5 µg of a 5-hmC DNA standard in 1 hour at 37°C in a total reaction volume of 50 µl from degradation by 4 units of a glucosyl-sensitive restriction enzyme, as shown in Figure 1. The 5-hmC methylated DNA standard tested is a 338 base pair oligonucleotide containing 122 hydroxymethylcytosine residues. For more details on Active Motif's Methylated DNA Standards (Catalog No. 55008), please visit our website at www.activemotif.com/hmc.

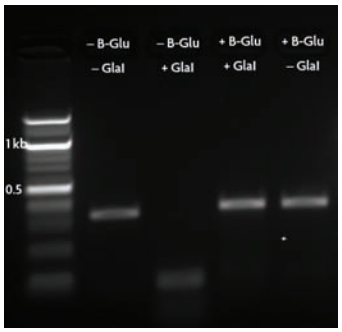


Figure 1: Protection of 5-hmC by glucosylation. A 5-hydroxymethylated DNA Standard (0.5 µg) was incubated in the absence or presence of 1 unit of β-Glucosyltransferase for 1 hour at 37°C. Following column purification, reactions were treated with 4 units of glucosyl-sensitive restriction enzyme, GlaI.

Tet1 for 5-hmC conversion assays

Active Motif offers a recombinant Tet1 protein for use in 5-hmC conversion assays. Tet1 is a member of the TET family of cytosine oxygenases that convert 5-methylcytosine into 5-hydroxymethylcytosine. This enzyme can be used to convert 5-mC containing DNA into 5-hmC containing DNA (Figure 2). To learn more, please visit our website.

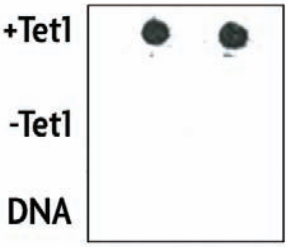


Figure 2: Recombinant Tet1 activity assay. Double-stranded DNA containing 5-methylcytosine was incubated with 5 µg of recombinant Tet1 enzyme (+Tet1) or without Tet1 enzyme (-Tet1). These samples and an unmethylated DNA control (DNA) were then spotted onto a nylon membrane and incubated with 5-Hydroxymethylcytosine antibody (Catalog No. 39769) to detect the conversion of 5-methylcytosine into 5-hydroxymethylcytosine.

PvuRts1I enzyme for direct digestion of hydroxymethylated DNA

While glucosyl-sensitive restriction enzymes offer a method to enzymatically differentiate between 5-mC and 5-hmC, they require the prior addition of a glucose moiety to the 5-hydroxymethylcytosine residue. Active Motif's novel new PvuRts1I restriction enzyme, however, is able to bypass this intermediary step and directly differentiate between 5-mC and 5-hmC residues. PvuRts1I directly cleaves hydroxymethylated DNA in its non-glucosylated form, but will not digest 5-methylcytosine or unmethylated cytosine residues (Figure 3). The enzyme also cleaves glucosylated-5-hmC DNA, but at a lower efficiency. To learn more about this unique enzyme's digestion properties, please visit our website and download the data sheet.

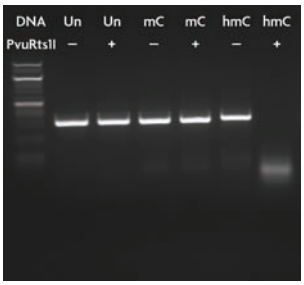


Figure 3: PvuRts1I enzyme digests 5-hmC DNA. One µg of unmethylated (Un), 5-methylcytosine (mC) or 5-hydroxymethylcytosine (hmC) Methylated DNA Standard (Catalog No. 55008) was incubated in the absence or presence of 1 unit PvuRts1I restriction enzyme for 30 minutes at 22°C. Each reaction was run on a 2.5% agarose gel alongside a 1 kb DNA ladder. Only hydroxymethylated DNA was digested in the presence of the PvuRts1I restriction enzyme.

Product	Format	Catalog No.	Price ($US)
β-Glucosyltransferase enzyme	500 Units	55012	275
PvuRts1I restriction enzyme	50 Units	55011	225
Recombinant Tet1 protein, active	25 µg	31363	320

NEW: MeDIP Enrichment of 5-Methylcytosine or 5-Hydroxymethylcytosine

Active Motif now has two kits available for the enrichment of methylated DNA via antibody immunoprecipitation. Methylated DNA immunoprecipitation (MeDIP) is an immunocapture technique in which an antibody specific for methylated cytosines is used to immunoprecipitate methylated genomic DNA fragments. The **hMeDIP Assay** uses a highly specific, purified 5-hydroxymethylcytidine antibody to selectively enrich for DNA fragments containing 5-hydroxymethylcytosine methylation from the rest of the genomic DNA population, while the **MeDIP Assay** uses a monoclonal 5-methylcytidine antibody to selectively enrich for traditional 5-methycytosine DNA methylation.

How does the MeDIP Assay work?

Active Motif's new MeDIP Assay utilizes a highly specific monoclonal antibody that recognizes 5-methylcytosine residues on single-stranded DNA to immunoprecipitate and enrich for methylated DNA fragments. The 5-methylcytidine antibody is added in the presence of a bridging antibody and protein G magnetic beads. Following an overnight incubation, the included magnet is used to collect the beads for washing and elution.

For added convenience, the kit also includes a negative control mouse IgG antibody, positive control *Mse*I digested human genomic DNA and real time PCR primers that can be used to verify the efficiency of the MeDIP enrichment.

How does the hMeDIP Assay differ?

One of the ways the hMeDIP Assay differs from the MeDIP Assay is that it does not require the use of single-stranded DNA fragments. The 5-hydroxymethylcytidine antibody used in the hMeDIP kit is capable of recognizing both double-stranded and single-stranded 5-hmC methylated fragments. This is advantageous because the eluted DNA will be double-stranded, which can bypass problems associated with linker bias when preparing enriched DNA for downstream Next-Gen sequencing.

Another difference is the hMeDIP Kit uses a polyclonal antibody, therefore, it does not require the assistance of a bridging antibody for complete capture by the protein G magnetic beads. Finally, the hMeDIP kit includes three methylated DNA standards (unmethylated, 5-methylcytosine and 5-hydroxymethylcytosine) for use as spike controls in the IP reaction to determine efficiency.

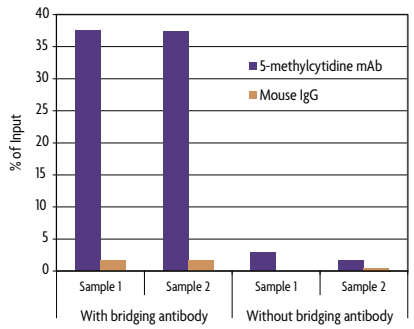


Figure 1: Real time PCR results using the MeDIP Assay. *Mse* I digested human genomic DNA (500 ng) was processed in duplicate using the MeDIP Assay. Reactions contained either the 5-methylcytidine monoclonal antibody or the negative control mouse IgG in the presence or absence of bridging antibody. Eluted DNA was purified and tested in real time PCR with the included ZC3H13 PCR primer mix. The methylated ZC3H13 locus is specifically enriched in the IP reactions using the 5-methylcytodine antibody and bridging antibody, while reactions that lacked bridging antibody, or contained the negative control mouse IgG showed no enrichment.

Antibodies for methylation analysis

For researches interested in detection of methylated DNA instead of enrichment by MeDIP and hMeDIP Assay Kits, Active Motif also offers the antibodies separately. For 5-hydroxymethylcytosine detection, researchers have the option of the purified polyclonal antibody that is used in the hMeDIP kit, the unpurified polyclonal whole rabbit serum or our exclusive monoclonal antibody, Clone 59.1. For 5-methylcytosine detection, both the monoclonal 5-methylcytidine and Bridging antibodies that are included in the MeDIP Kit are available separately.

5-Hydroxymethylcytosine Services

In addition to our reagents and assays, Active Motif also offers Epigenetic Services for the analysis of 5-hydroxymethylcytosine. Our service offerings include hMeDIP-seq and hMeDIP-chip for genome-wide analysis of hydroxymethylation. This enables you to take advantage of Active Motif's expertise without having to be an expert yourself. Visit www.activemotif.com/services to learn about our Epigenetic Services.

Product	Format	Catalog No.	Price ($US)
MeDIP	10 rxns	55009	350
hMeDIP	10 rxns	55010	350
5-Hydroxymethylcytidine antibody mAb (Clone 59.1)	100 µg	39999	330
5-Hydroxymethylcytidine antibody pAb	100 µl	39769	330
5-Hydroxymethylcytidine antibody pAb (IgG)	100 µg	39791	330
5-Methylcytidine antibody mAb (Clone 33D3)	50 µg	39649	330
Bridging Antibody for Mouse IgG	500 µg	53017	75

NEW: ChIP qPCR Primers and Controls Ensure Your Experiments Work Every Time

The number of variables in ChIP experiments makes them extremely complex, from fixation to cell type to sonication to antibody incubation, enrichment and analysis. To make your ChIP experiments more successful, we have developed a line of accessory kits and reagents designed to make it easier for you to troubleshoot your ChIP experiments and to validate your results.

ChIP Control qPCR Primer Sets

The proper analysis tools are crucial for carrying out successful chromatin IP. The correct positive and negative control PCR primers are essential to determine the success or failure of your experiment. Active Motif offers a large variety of species-specific qPCR primer sets for use as positive and negative controls for many of the more common ChIP targets, including histone modifications, transcription factors and methylated DNA. Use of our human, mouse, rat, Zebrafish, *Drosophila* and yeast primer sets will save you the time and effort required to design, synthesize and test your own species- and gene-specific control primers. Our qPCR primer sets are used regularly by our Epigenetics Services division and so have been rigorously tested and validated to work for you. When used in combination with our ChIP-validated antibodies and Ready-to-ChIP Chromatin, you have all the tools you need for successful chromatin IP. To see the many different primer sets available, go to www.activemotif.com/chipprimers.

Control qPCR Primer advantages

- Available for many popular ChIP targets
- Both positive and negative controls offered for a variety of species
- Tested on multiple cell lines
- Supplied in solution and ready to use

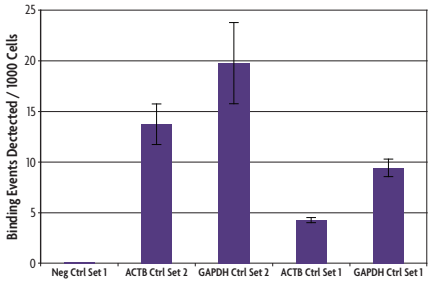


Figure 1: ChIP of RNA pol II CTD phospho Ser5 mAb. ChIP was performed using 10 µg of chromatin from human LP1 cells and 10 µg RNA pol II CTD phospho Ser5 antibody (Catalog No. 61085). ChIP DNA was used in qPCR with Active Motif's human control qPCR primer sets, as indicated. Data are presented as Binding Events Detected per 1000 Cells.

ChIP-IT™ Control Kits

Chromatin immunoprecipitation is a DNA enrichment technique, not purification, so ChIPs are unavoidably contaminated with non-specific chromatin. This often leads to false PCR products that can make data difficult to interpret and complicate the validation of antibodies and primer sets. To solve these problems, we offer species-specific ChIP-IT™ Control Kits that provide positive and negative control antibodies, a positive control PCR primer set and a PCR buffer/DNA loading dye that makes your PCR reactions gel-ready.

Ready-to-ChIP Chromatin

For your convenience, Active Motif offers its Ready-to-ChIP chromatin from a number of ENCODE cell lines that have been optimally sheared by sonication and validated in ChIP for inclusion as a positive control or test sample. As a result, you can more easily validate your own antibodies and primer sets. The chromatin can be used with all of the ChIP-IT Express Kits and controls, so you can be certain that the only variable in validating a new antibody for ChIP is the antibody itself.

ChIP-IT Express Shearing Kits

Designed to work specifically with our ChIP-IT Express Kits, the sonication and enzymatic shearing kits give you the same shearing components as those found in ChIP-IT Express Kits, but in greater quantities to allow you to optimize your shearing conditions before proceeding with ChIP experiments.

For more information about our accessories and industry-leading ChIP kits, visit www.activemotif.com/chip.

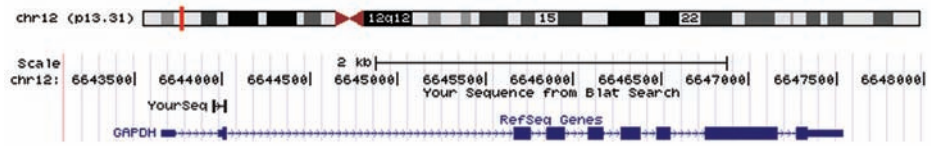


Figure 2: Genomic Localization of Human Positive Control Primer Set GAPDH-2. Image representing the relative location of the Human Positive Control Primer Set GAPDH-2 primer set amplicon within the genome, as generated by the UCSC Genome Browser. The top image represents the location on chromosome 12, the bottom image represents the amplicon relative to the GAPDH gene.

NEW: Multi-Sample Sonicator, Probe Sonicator and Cooled Sonication Platform

Sonication is frequently used to prepare sheared chromatin for ChIP, as well as for standard cell disruption, DNA/RNA shearing and other homogenization applications. Active Motif is pleased to introduce its EpiShear™ line of sonication products, which will save you time and effort while ensuring that you obtain more reproducible results.

Programmable generators – why not choose complete control?

Both the EpiShear™ Multi-Sample Sonicator and the EpiShear™ Probe Sonicator are controlled by microprocessor units that offer both programmable and manual operation. Each has a keypad and digital display that make it easy to program the amplitude and duration of On and Off pulse cycles. Pulse intensity to be set from 20-100%, enabling you to choose exact parameters for your cell type. The digital display shows the total elapsed time, and provides real-time energy monitoring of both wattage and joules. These features make it easier to obtain more reproducible results when you perform subsequent sonications.

Process up to 8 samples at once

The Multi-Sample Sonicator is a high-intensity cup horn sonicator. Its 750 watts of power enable continuous operation to shear even the most problematic samples, like T cells. The unit can process up to eight 1.5 ml vial samples at once, and comes with a sound enclosure and a thermoelectric chiller (Figure 1). For more complete information, please visit us at www.activemotif.com/cuphorn.

Sonicate large or small samples

While the EpiShear™ Probe Sonicator is a compact, economical unit (Figure 2), it is still a fully programmable unit with all the features that will help ensure your reproducible results. It is supplied with a 1/8" microtip probe that enables you to shear one 500 µl to 15 ml sample at a time. Other size probes are available that expand its range from 200 µl to 50 ml. For more complete information, please visit www.activemotif.com/probe.

Figure 1: The EpiShear Multi-Sample Sonicator / Chiller.
The EpiShear Multi-Sample Sonicator / Chiller includes a powerful 750-watt generator with a keypad and digital display that make it easy to program and monitor sonication. The unit's cup horn sonicator is housed in a compact sound enclosure to reduce sonication noise. The cup horn can process up to 8 samples simultaneously, which rotate continuously to ensure all samples are processed equally. It is mounted in an acrylic bath that is filled with water. The bath and enclosure are plumbed with quick-connect hoses that attach to a thermoelectric chiller, which keeps the samples at 2-4°C during sonication.

Figure 2: The EpiShear Probe Sonicator and the EpiShear Cooled Sonication Platform.
The EpiShear Probe Sonicator is a compact 120-watt unit that can process small or large samples. The EpiShear Cooled Sonication Platform, sold separately, precisely positions the probe every sonication, greatly enhancing sample-to-sample reproducibility. The tube coolers, available in 3 sizes, eliminate the need to rest the sample in an ice bath between pulses.

Make any probe sonicator easier to use

The EpiShear Cooled Sonication Platform, affectionately known as "Big Jack", greatly increases reproducibility by enabling you to precisely position the depth of the probe in your sample every time. It can be used in a sound enclosure or on a base. Coolers that fit microfuge, 15 ml and 50 ml tubes keep your sample cold during sonication, so you don't have shuttle it to and from an ice bucket. See www.activemotif.com/platform.

DNMT Assay to Analyze Changes in CpG Methylation

DNA methylation usually occurs at the fifth carbon of cytosine, within the context of a CpG dinucleotide. Modifications at these sequences by DNA methyltransferase enzymes (DNMTs) can have profound effects on transcription. Active Motif's DNMT Activity / Inhibition Assay is a fast, user-friendly assay to simplify the measurement of DNA methyltransferase activity or the effects of inhibitor compounds without the need for radioisotopes.

Non-radioactive DNMT Assay

The DNMT Activity / Inhibition Assay provides all the reagents needed to study DNA methyltransferase activity from recombinant DNMT enzymes or nuclear extract samples. The sensitive ELISA-based method is unique in that it utilizes a methyl-CpG binding domain (MBD) protein to detect methyltransferase activity, instead of a radioactively labeled methyl-donor. MBD proteins are capable of binding methylated DNA with a high affinity, which increases the sensitivity of the assay and enables detection from as little as 0.5 ng purified DNMT enzyme or 0.5 µg nuclear extract. In addition to screening for DNMT activity, the assay can also be used to screen for changes in CpG methylation following treatment by DNA methylation inhibitors (Figure 1).

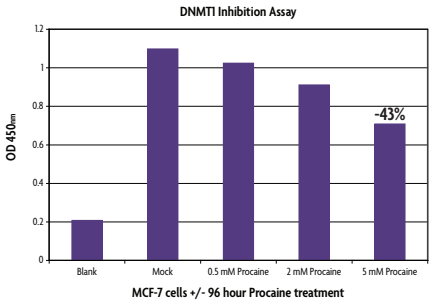


Figure 1: DNMT inhibition of MCF-7 cells with procaine. The DNMT Activity / Inhibition Assay was used to screen for DNMT inhibition in MCF-7 cells that were either untreated or treated with procaine for 96 hours. Nuclear extracts were prepared using Active Motif's Nuclear Extract Kit (see page 9) and 5 µg of each condition was assayed with a 1.5 hour incubation time and a 3 minute developing time. The 5 mM procaine treatment showed 43% inhibition of DNMT activity as compared to the mock treated samples.

DNMT Activity Assay advantages

- **Non-radioactive** – colorimetric assay is easily quantified on a microplate reader at 450 nm
- **Sensitive** – unique MBD protein approach enhances the sensitivity of detection from either purified proteins or nuclear extracts
- **Fast** – assay can be completed in less than 3 hours
- **Flexible** – stripwell plate allows screening of 1 to 96 samples
- **Versatile** – works with DNMT1, DNMT3a & DNMT3b proteins

To see all the available methylation kits, visit www.activemotif.com/dnamt.

Product	Format	Catalog No.	Price ($US)
DNMT Activity / Inhibition Assay	1 x 96 rxns	55006	495
Recombinant DNMT1 protein, active	10 µg	31335	320

NEW: Dounce Homogenizer to Assist in Cell and Chromatin Preparations

Obtain more complete cell lysis

Whether you are analyzing cell lysates by Western blot, or preparing chromatin for a chromatin immunoprecipitation experiment, the ability to obtain good results relies on accurate cell lysis. For cell and tissue samples that seem to be resistant to lysis under normal detergent conditions, the use of a dounce homogenizer can greatly improve lysis, thereby improving the recovery yields of protein or chromatin material.

Active Motif's new Dounce Homogenizer is ideal for preparation of cell lysates or chromatin. The dounce is available in two sizes, 1 ml for small sample volumes and 15 ml for larger sample preparations. Each glass dounce is supplied with two pestles of different sizes. One pestle fits tightly within the shaft of the dounce for maximum friction and cell disruption. The other pestle has a looser fit and is perfect to obtain a homogenous sample. Because the dounce homogenizer is made of glass, it can easily be cleaned and sterilized between uses.

Figure 1: Dounce homogenizer with two pestles.

Product	Format	Catalog No.	Price ($US)
Dounce Homogenizer	1 ml	40401	175
	15 ml	40415	225

NEW: Protease Inhibitor Cocktail for Better Sample Preparation

Broad-spectrum Protease Inhibitors

The same Protease Inhibitor Cocktail you have trusted in Active Motif's Nuclear Extract Kit (see details below) is now available separately. The cocktail is designed to prevent proteolytic degradation during lysis and extraction procedures from mammalian cells or tissues.

The Protease Inhibitor Cocktail contains a mixture of several proteases to offer a broad-spectrum of protection. The cocktail has inhibitors targeting cysteine, serine and aspartic acid proteases, as well as inhibitors for aminopeptidases. In addition to being ready-to-use, the Protease Inhibitor Cocktail is also supplied EDTA-free. The cocktail is packaged as a 100X liquid solution that has been stabilized in DMSO for improved accuracy, solubility and ease of use in comparison to traditional tablets. Select from either 1 ml (2 x 500 µl) or 5 ml pack sizes to suit your research needs.

Protease Inhibitor Cocktail advantages

- Broad-spectrum inhibition
- Optimized for use with mammalian cell and tissue extraction
- Convenient and stable liquid format for ease of use
- Supplied as a ready-to-use 100X stock that is EDTA-free

To see the complete Protease Inhibitor Cocktail formulation, visit our website at www.activemotif.com/pic.

Product	Format	Catalog No.	Price ($US)
Protease Inhibitor Cocktail	1 ml	37490	95
	5 ml	37491	295

Nuclear Extraction Kit for Nuclear, Cytoplasmic or Whole-cell Lysates

Active Motif's Nuclear Extract Kit is ideal for researchers interested in isolating high-quality nuclear, cytoplasmic or whole-cell lysates from mammalian cell or tissue samples. The optimized protocols and reagents simplify the extraction procedure so you obtain high yields every time.

How does the Nuclear Extract Kit generate such highly specific cell fractions?

Each Nuclear Extract Kit provides reagents for 100 or 400 extractions from 8.8 x 10^6 cells, which corresponds to HeLa cells grown to confluence in a 100 mm tissue culture dish. First, the cells are collected in ice-cold PBS in the presence of Phosphatase Inhibitors to limit further protein modifications, such as proteolysis, dephosphorylation, *etc*. Then, the cells are resuspended in Hypotonic Buffer to swell the cell membrane and make it fragile. The addition of detergent causes leakage of the cytoplasmic proteins into the supernatant, where they can be collected for further analysis. The remaining nuclear pellet is lysed and the nuclear proteins are solubilized in Lysis Buffer supplemented with our broad-spectrum Protease Inhibitor Cocktail.

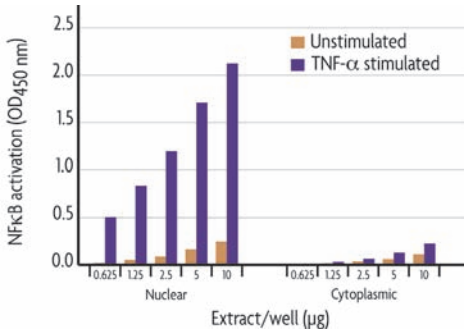


Figure 1: Specific extraction of cytoplasmic and nuclear extracts as tested in TransAM® NFκB p50 Kit. Nuclear and cytoplasmic extracts were prepared using the Nuclear Extract Kit from HeLa cells that were unstimulated or stimulated with TNF-α for 30 minutes. The extracts were assayed using TransAM NFκB p50 at the concentrations shown. Because activated NFκB translocates to the nucleus, only nuclear extracts from stimulated cells should contain activated NFκB.

For whole-cell lysate preparation, the cells are collected in PBS containing Phosphatase Inhibitors and then lysed in the Lysis Buffer containing Protease Inhibitor Cocktail.

Following protein quantification, the cytoplasmic, nuclear or whole-cell extracts can be used in a variety of downstream applications, including Western blotting, EMSA, transcription factor-DNA binding assays, such as Active Motif's TransAM® Assay Kits, or they are ready for use in Active Motif's DNMT Activity / Inhibition Assay (see page 8).

Product	Format	Catalog No.	Price ($US)
Nuclear Extract Kit	100 rxns	40010	205
	400 rxns	40410	585

Antibodies to Stem Cell Regulators to Further your Stem Cell Research

The biology of stem cells involves a wide variety of proteins found in the nucleus that are required to maintain cell pluripotency (the ability to differentiate into any cell type) and self-renewal. Many of these proteins are associated with chromatin and serve to keep large regions of the genome in an open, accessible configuration. In fact, chromatin biology and epigenetics are integral components of stem cell biology.

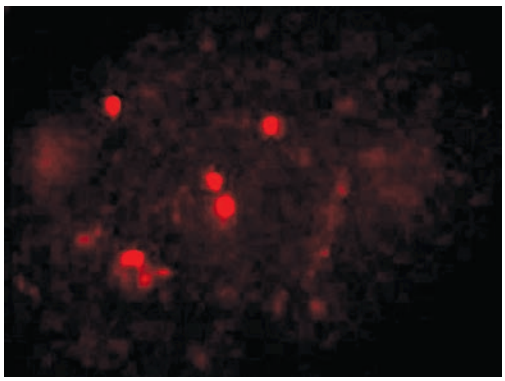


Figure 1: Ring1B staining in U2OS cells.
Human U2OS cells were stained with Ring1B monoclonal antibody (Catalog No. 39663) and visualized by indirect immunofluorescence.

To assist you in your study of stem cells and the role of chromatin regulation, Active Motif offers a wide range of antibodies to important stem cell proteins, including:

- Transcription factors
- Chromatin modifying proteins
- Histones and histone modifications
- Polycomb proteins

For more information on our antibodies to regulators of stem cell function, visit www.activemotif.com/stemcellabs.

Highly Validated Antibodies and ELISAs for the Study of Nuclear Receptors

Nuclear receptors are important regulators of endocrine activity, mediating responses to changing hormone levels. Most nuclear receptors bind steroid hormone ligands. But, there are some (orphan receptors) that do not, or for which the ligand has not yet been identified. Because nuclear receptors can bind DNA directly and regulate gene expression, they are also transcription factors, many of which recruit co-activators and co-repressors that possess histone-modifying activity. Active Motif offers a number of highly validated antibodies for the study of nuclear receptors and steroid hormone activity.

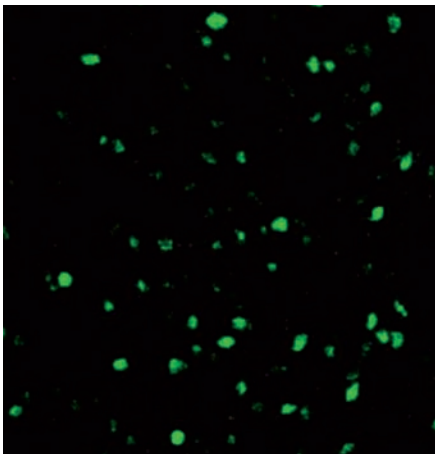


Figure 1: Immunofluorescence staining of Progesterone receptor.
Mouse hypothalamic section stained with Progesterone receptor antibody (Catalog No. 61023).

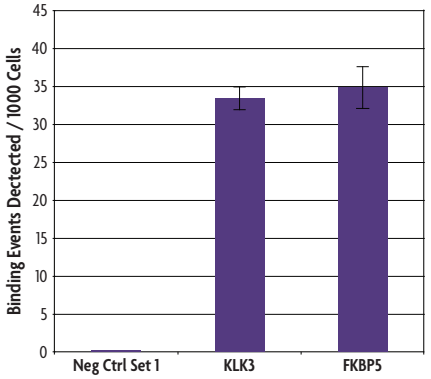


Figure 2: Androgen receptor analyzed by chromatin IP.
ChIP was performed using 30 µg of VCAP60 cell chromatin and 10 µl of Androgen receptor antibody (Catalog No. 39781). ChIP DNA was used in qPCR with the Human Negative Control Primer Set 1 (Catalog No. 71001) and primer pairs specific for the KLK3 and FKBP5 genes. Data are presented as Binding Events Detected per 1000 Cells.

For more information on Active Motif's antibodies to nuclear receptors, visit www.activemotif.com/nrabs.

Active Motif also offers sandwich ELISAs that enable you to quantitatively measure the levels of the Androgen, Estrogen and Progesterone nuclear receptors. For more complete information, please visit www.activemotif.com/nrelisa.

Antibodies and Reagents for High Resolution Confocal STED Microscopy

The resolution attainable in fluorescent microscopy is limited by the diffraction of light, which was shown by Ernst Abbe in 1873. The *Abbe Limit* restricts the ability of the observer to visually resolve objects that are separated by less than ~200 nm. Recently, several super-resolution techniques have been developed to overcome this limitation, providing the ability to image structures as small as 20 nm. These improvements in microscopy enable scientists to decipher the nanostructure of the cell in details that could not be visualized before.

One of these novel techniques is STED (**ST**imulated **E**mission **D**epletion), which can achieve up to 12-fold higher resolution than classical confocal microscopy. Active Motif is proud to help scientists take advantage of this powerful technology by providing IF-tested primary antibodies, and high-quality conjugated secondaries & sample preparation reagents that are certified by Leica Microsystems for use with its STED microscopes. For more complete information on STED, please visit www.activemotif.com/sted.

Optimized sample preparation for STED and classical confocal microscopy

Proper sample preparation is among the most significant factors for obtaining high-quality images in both STED and classical confocal microscopy. To help ensure that you consistently achieve the best results possible, Active Motif's scientists developed the Chromeo™ STED Immunofluorescence System and the MAX Stain™ Immunofluorescence Tools. These sample preparation reagents were designed to increase the intensity and specificity of your fluorescent labeling.

Customized conjugation protocols ensure the highest quality secondaries

The quality of any fluorescent secondary antibody depends on the spectral properties of the dye, the quality of the secondary antibody, and the dye-to-protein ratio & purity of the conjugate. To control all variables possible, Active Motif produces all its conjugates using protocols that have been optimized for each dye/antibody combination. This ensures the quality of the conjugates, and makes the fluorescent secondaries brighter while lowering the fluorescent background. For more information, visit www.activemotif.com/secondary.

Product	Format	Catalog No.	Price ($US)
Chromeo™ 488 Goat anti-Mouse IgG	1 mg	15031	135
Chromeo™ 488 Goat anti-Rabbit IgG	1 mg	15041	135
Chromeo™ 505 Goat anti-Mouse IgG	1 mg	15030	135
Chromeo™ 505 Goat anti-Rabbit IgG	1 mg	15040	135
Chromeo™ 494 Goat anti-Mouse IgG	1 mg	15032	135
Chromeo™ 494 Goat anti-Rabbit IgG	1 mg	15042	135
ATTO 647N (STED) Goat anti-Mouse IgG	250 µl	15038	260
ATTO 647N (STED) Goat anti-Rabbit IgG	250 µl	15048	260
ATTO 655 (STED) Goat anti-Mouse IgG	250 µl	15039	260
ATTO 655 (STED) Goat anti-Rabbit IgG	250 µl	15049	260
Chromeo™ STED Immunofluorescence System	1 kit	15260	230
MAXpack™ Immunostaining Media Kit	1 kit	15251	295

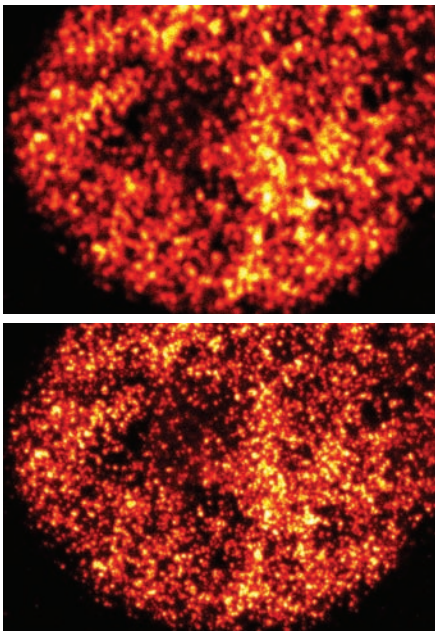


Figure 1 Active Motif's primary and fluorescent secondary antibodies in CW STED microscopy. HeLa cells were stained with alpha Tubulin mouse mAb (Clone 5-B-1-2) (Catalog No. 39527) and Chromeo 505 Goat anti-mouse IgG (Catalog No. 15030). The confocal image (top) and the STED image (bottom) are courtesy of Leica Microsystems, Germany.

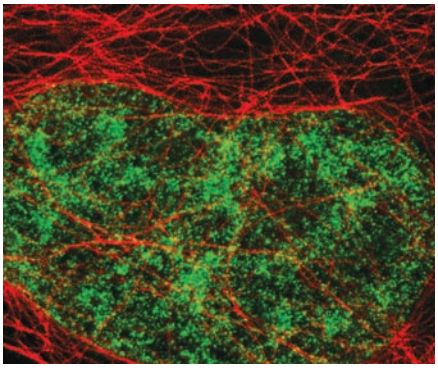


Figure 2: Active Motif's primary and fluorescent secondary antibodies in TCS STED microscopy. HeLa cells were stained with alpha Tubulin mouse mAb (Clone 5-B-1-2) (Cat. No. 39527) and Chromeo 494 Goat anti-mouse IgG (Cat. No. 15032). Histone H3 was stained with Histone H3 trimethyl Lys4 rabbit polyclonal antibody (Cat. No. 39159) and the ATTO 647N (STED) Goat anti-rabbit IgG (Cat. No. 15048) secondary antibody. The STED image is courtesy of Leica Microsystems, Germany.

ACTIVE MOTIF®

Enabling Epigenetics Research

Visit us at **www.activemotif.com**

Active Motif develops validated antibodies and assays that enable the discovery and characterization of key epigenetic processes.

Our products are developed in-house and supported by scientists with expertise in chromatin biology. For a complete product listing please visit www.activemotif.com.

Superior Antibodies & Kits for EPIGENETICS RESEARCH

CHROMATIN ANALYSIS
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HISTONE MODIFICATION
Antibodies & ELISAs, Arrays, HAT/HDAC assays, Histone purification and Recombinant Histones

DNA METHYLATION
Methylated DNA enrichment, bisulfite conversion, DNMT assays, whole genome amplification, hMeDIP, MeDIP, 5-hmC & 5-mC antibodies