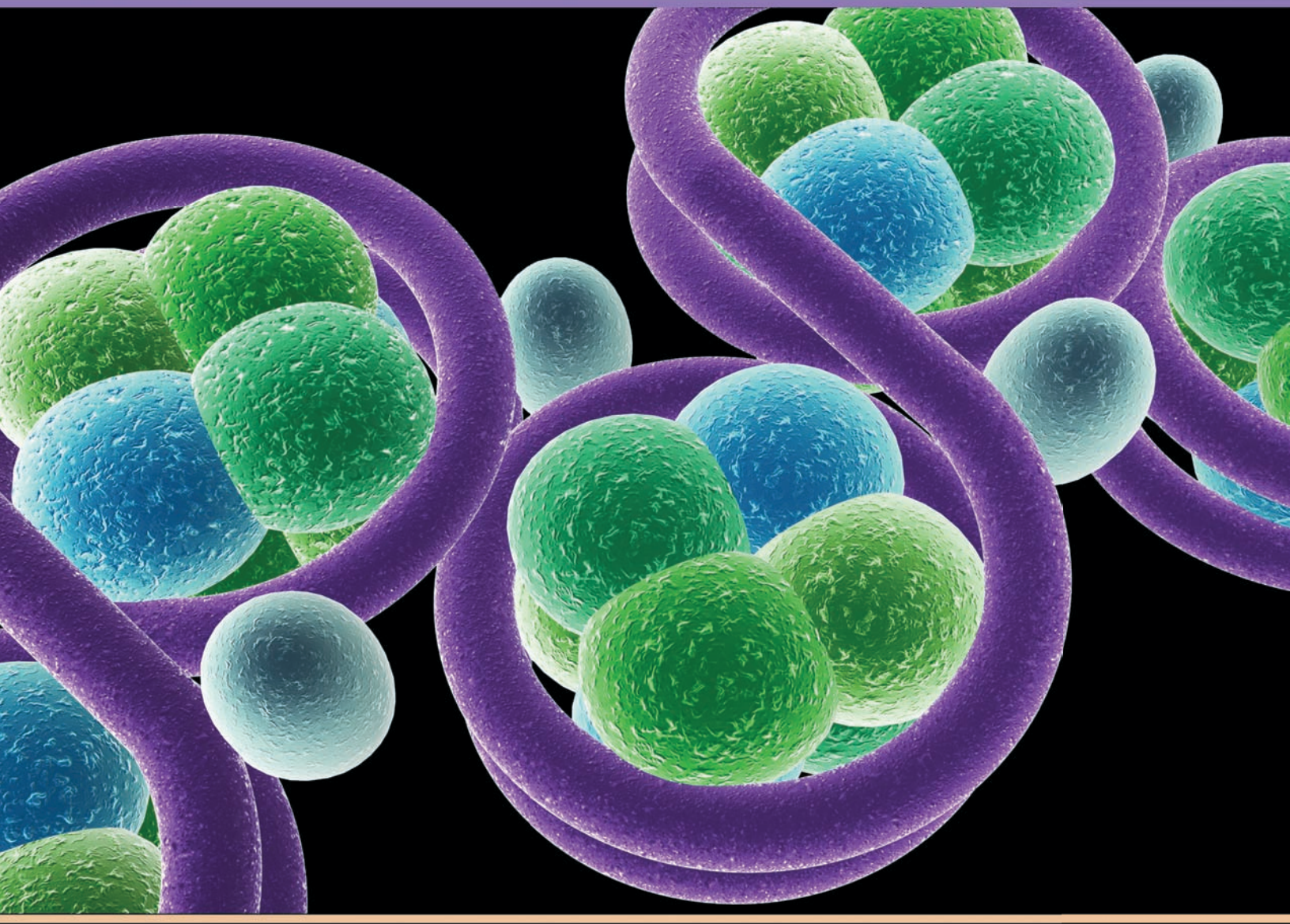
ACTIVE MOTIF®

MOTIFvations

The Newsletter of Active Motif May 2010 Volume 11 Number 2



FOCUS OF THIS ISSUE

Histones: Kits, ELISAs & Antibodies Designed to Unravel the Histone Code

NEW: Novel Peptide Array Simplifies Histone Modification Screening

The importance of antibodies as research tools in epigenetic and chromatin analysis requires careful screening to ensure accurate specificity against post-translational modifications, such as acetylation, methylation, phosphorylation and citrullination. To help overcome the obstacles of antibody characterization, Active Motif is pleased to introduce the MODified™ Histone Peptide Array, a novel peptide array that enables you to **screen 384 different histone modification combinations**, comprising 59 post-translational modifications, in a single experiment.

Better antibody characterization

With the increasing use of antibodies as a research tool in techniques such as ChIP, ChIP-seq, ChIP-chip and IF, it is critical to assess antibody specificity to ensure accurate data analysis. Active Motif's MODified Histone Peptide Array offers a better way to screen antibodies for cross reactivity. Each peptide array contains 384 different histone modification combinations, including up to four separate modifications on the same peptide. This extensive coverage of histone modifications will enable you to study not only individual sites, but also to determine the effects of neighboring modifications on antibody recognition and binding. MODified Histone Peptide Arrays screen 59 acetylation, methylation, phosphorylation and citrullination modifications on the N-terminal tails of histones H2A, H2B, H3 and H4.

MODified Histone Array advantages

- **Histone specific** – unique array panel specific for histones and histone modifications
- **Multiple combinations** – peptides contain up to four modification combinations each
- **Detects like a Western blot** – works with either ECL-based or colorimetric detection

Active Motif also offers antibodies for histones and histone modifications. For more information, please call or visit us at www.activemotif.com/hismods.

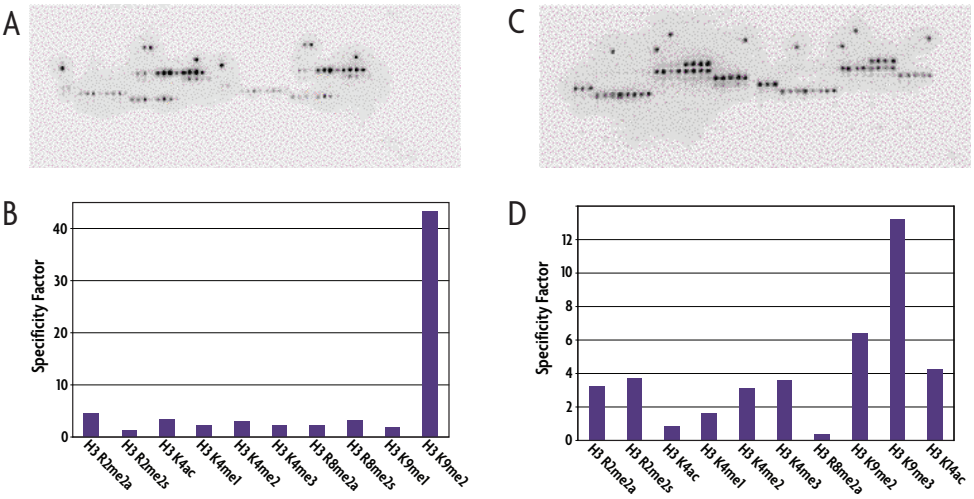


Figure 1: The MODified Histone Peptide Array was used to evaluate the specificity of two different histone antibodies. Active Motif's Histone H3 dimethyl Lys9 mAb (Catalog No. 39683, page 9) was compared with a Histone H3 trimethyl Lys9 mAb. Each antibody was used at the recommended Western blot dilution factor. An anti-mouse HRP secondary antibody was used at 1:2500 dilution, followed by ECL detection and image capture with CCD camera. Spot densitometry was used to analyze intensity. Results were compared with the provided reference grid to determine the associated peptide content and graphed as a fold intensity to represent the specificity factor. **A)** Camera image of Active Motif's H3K9me2 mAb at 1 µg/µl dilution. **B)** Results indicate that Active Motif's Histone H3 dimethyl Lys9 mAb has very little cross reactivity with other histone modifications. **C)** Camera image of H3K9me3 mAb at 1:1,000 dilution. **D)** Results indicate that the Histone H3 trimethyl Lys9 mAb antibody has a high degree of cross reactivity with other histone modifications.

How does it work?

To generate MODified Arrays, Active Motif synthesizes a series of 19mer histone H2A, H2B, H3 and H4 peptides, each of which may contain as many as four modifications. These are spotted in duplicate onto a glass slide, generating an array with a total of 384 unique histone modification combinations.

Following overnight blocking, the array is incubated with antibody for one hour, washed and then incubated for one hour with either a horseradish peroxidase or alkaline phosphatase conjugated secondary antibody. Developing solution is then added and the image captured using film or a CCD camera; no special equipment is needed. Software is used to analyze the spot intensity; results are compared with the provided reference file showing the grid pattern and associated peptide content (Figure 1). For more details, go to www.activemotif.com/modified.

Product	Format	Catalog No.
MODified™ Histone Peptide Array	1 array	13001
	5 arrays	13005

Get 10% off any single pack size MODified™ Histone Peptide Array.
Visit www.activemotif.com/promo for complete details.

NEW: Easy Analysis of Deubiquitinating Enzymes (DUBs)

Measuring the activity of deubiquitinating enzymes (DUBs) can provide great insight into ubiquitin-dependent metabolic pathways. The DUB-Detector™ Assay contains a fluorescent ubiquitin substrate that when processed by the cysteine protease class of DUBs releases a fluorescent signal that is proportional to the amount of enzymatic activity (Figure 1). This sensitive assay works with extracts or recombinant proteins and can be used to **determine the kinetics of the enzymatic reaction, or to screen for DUB inhibitors**.

Advantages of DUB-Detector

- Fluorescent assay can be detected with an excitation wavelength of 485 nm and an emission wavelength of 535 nm
- Includes both a positive control extract and a DUB inhibitor
- Fast procedure can be completed in less than 1 hour
- Great for either kinetic or end point analysis

Product	Format	Catalog No.
DUB-Detector™	1 x 96 rxns	40110

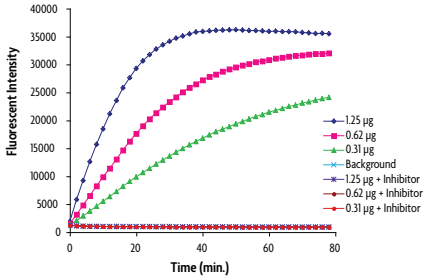


Figure 1: Kinetic analysis of HeLa nuclear extract. HeLa nuclear extracts were assayed at 0.313, 0.625 and 1.25 µg per reaction in the presence or absence of 1 µM Inhibitor for 20 minutes. Following the incubation, Fluorescent Substrate (100 nM) was added to each well and the fluorescent intensity was measured every two minutes. Data shown are the results from duplicates.

NEW: First Commercially Available Recombinant Acetylated Histones

Histone acetylation is a post-translational modification that affects the nucleosome structure and therefore the ability of transcription factors to access the DNA and regulate gene expression. To better support research on histone acetylation, Active Motif is releasing its **first acetylated recombinant histones**.* These histones can be used as substrates for *in vitro* assays or as positive controls for acetylation detection.

Another first for Active Motif

Active Motif was the first company to offer recombinant methylated histones with site and degree specificity. Now we have done it again with recombinant acetylated histones. Our patent pending technology enables us to acetylate the histone tail, without altering the native peptide bonds. Our acetylated recombinant histones are ideal for use as substrates for *in vitro* assays or as positive controls for acetylation detection.

For complete information on all of our recombinant histones, please visit www.activemotif.com/recombhis.

Product	Format
Recombinant Histone H3 acetyl Lys9 **NEW**	25 µg
Recombinant Histone H3 acetyl Lys14 **NEW**	25 µg
Recombinant Histone H2A	50 µg
Recombinant Histone H2B	50 µg
Recombinant Histone H3 (C110A)	50 µg
Recombinant Histone H3 Lys4 (mono-, di-, or trimethyl)	50 µg
Recombinant Histone H3 Lys9 (mono-, di-, or trimethyl)	50 µg
Recombinant Histone H3 Lys27 (mono-, di-, or trimethyl)	50 µg
Recombinant Histone H3 Lys36 (mono-, di-, or trimethyl)	50 µg
Recombinant Histone H3 Lys79 (mono-, di-, or trimethyl)	50 µg
Recombinant Histone H4	50 µg
Recombinant Histone H4 Lys20 (mono-, di-, trimethyl)	50 µg

*Patent Pending.

NEW: 5-Hydroxymethylcytosine – The Newest Epigenetic Mark, and the First Antibody for Its Study

DNA is packaged through its incorporation into chromatin, the wrapping of DNA around histone proteins, which facilitates higher-order organization of the genome. This packing of DNA into chromatin has many heritable effects upon DNA-dependent processes. 'Epigenetic' changes like histone modifications exist in addition to epigenetic events that occur on the DNA itself. CpG dinucleotide clusters, or 'islands', scattered throughout the genome (up to 50% of human gene promoters contain CpG islands) are subject to methylation of the 5th carbon position of the cytosine. DNA methylation participates in genome-wide processes such as the regulation of gene expression, genomic imprinting, silencing of repetitive elements and, when aberrant, contributes to cancer. DNA methylation can also recruit methyl-CpG binding proteins (MeCP2 and the MBD family) that are effectors of downstream regulatory events.

The enzymes that catalyze the addition of the methyl group to DNA are well characterized, but the process by which CpG methylation is reversed is not straightforward. It has recently been described that a series of enzymatic reactions leading to the deamination of 5-methylcytosine (5-mC) results in the removal of the methylated base, as the deaminated 5-mC leads to a DNA mismatch that is repaired by the nucleotide excision system.

In 2009, a second potential pathway for DNA demethylation was identified, coincident with the discovery of an alternative form of DNA CpG methylation. Kriaucionis and Heintz reported on the relative abundance of 5-hydroxymethylcytosine (5-hmC) in a specific cell type in the brain in which the genome is organized to a large extent into euchromatin. Another group identified the enzymatic pathway by which 5-mC is converted to 5-hmC (Tahiliani *et al.*) by the TET1 enzyme and related proteins (Figure 1). 5-hmC is replaced with cytosine by DNA repair proteins, effectively leading to demethylation of the CpG dinucleotide.

References
Huang *et al.* (2010) *PLoS One* 26: e8888.
Jin *et al.* (2010) *Nucl Acids Res* in press.
Kriaucionis and Heintz (2009) *Science* 324: 924-929.
Nestor *et al.* (2010) *Biotechniques* 48: 317-319
Tahiliani *et al.* (2009) *Science* 324: 930-935.

The identification of this novel DNA modification requires that new tools be created in order to study the role of this event. None of the traditional methods of DNA methylation analysis apply to 5-hmC. Bisulfite treatment of 5-hmC turns it into cytosine 5-methylenesulfonate (CMS), which makes it appear to be 5-mC in downstream analysis. Also, the typical enzymatic (methylation sensitive restriction enzymes) and enrichment (by antibody or methyl-binding protein) techniques don't work on 5-hmC.

New tool enables the study of 5-hmC

To facilitate the study of this new and potentially important regulatory event, Active Motif has developed the first antibody that recognizes 5-hmC, our 5-Hydroxymethylcytidine antibody. It specifically recognizes DNA that contains 5-hmC, but it does not recognize DNA containing 5-mC (Figure 2).

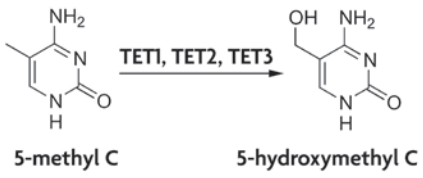


Figure 1: Conversion of 5-mC to 5-hmC.
The methyl group at position 5 of 5-methylcytosine is oxidized to 5-hydroxymethylcytosine by the TET family of iron-dependent oxygenases.

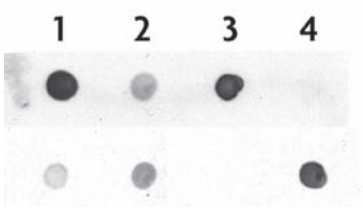


Figure 2: Dot blots using 5-hmC and 5-mC antibodies.
Top panel: 5-Hydroxymethylcytidine antibody (Catalog No. 39769; 1:10,000 dilution).
Bottom panel: 5-Methylcytidine antibody (1:1,000 dilution).

Lane 1: DNA derived from mouse embryonic stem cells (150 ng).
Lane 2: DNA derived from mouse spleen (600 ng).
Lane 3: A 27-base oligonucleotide containing 5-Hydroxymethylcytosine (1.2 ng).
Lane 4: A 24-base oligonucleotide containing 5-Methylcytosine (2000 ng).

Why do you need Active Motif's new 5-hmC antibody?

- It discriminates 5-hmC from 5-mC; only 5-hmC is recognized
- Enrichment by existing 5-mC antibodies doesn't work on 5-hmC
- Enzymatic approaches and bisulfite sequencing cannot differentiate 5-mC from 5-hmC in DNA
- Methyl-CpG DNA binding proteins do not recognize 5-hmC

To find out more about Active Motif's ground-breaking new 5-Hydroxymethylcytidine antibody, please visit us at www.activemotif.com/5hmc.

NEW: Improved Format for Methylated CpG Island Enrichment

Due to the scientific importance of CpG shores and regions of low-density methylation, the MethylCollector™ Ultra Kit* has been modified to include both low and high salt binding buffers. This improved format provides a better enrichment solution for **efficient capture of low-density CpG methylation**.

MethylCollector™ Ultra advantages

- Detection from 1 ng – 1 µg DNA
- Works on double-stranded DNA fragmented by sonication or enzymatic digestion
- MBD2b/MBD3L1 heterodimeric protein capture is more efficient than MeDIP or MBD2 alone
- Proteinase K elution step eliminates the need for time consuming salt gradient elution protocols
- Results in less than 3 hours
- Enriched DNA is suitable for use in endpoint or real time PCR, WGA, microarray analysis or sequencing

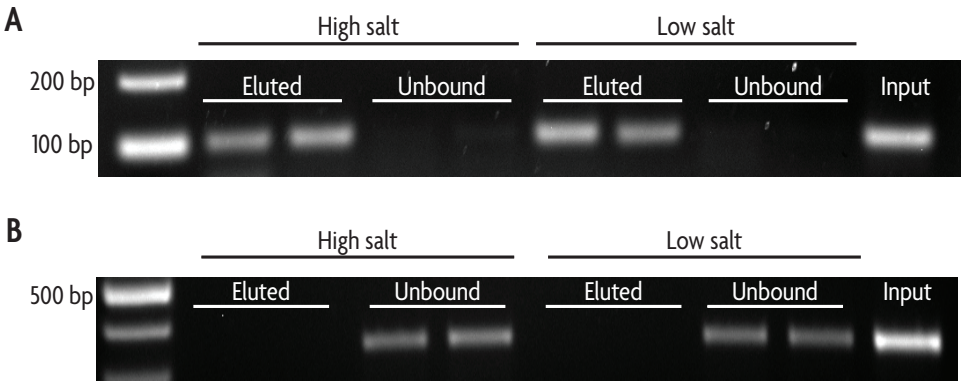


Figure 1: MethylCollector Ultra specifically enriches for CpG-methylated DNA.
MethylCollector Ultra was performed with 100 ng *Mse* I digested human, male genomic DNA using both high and low salt binding conditions. Eluted DNA was cleaned and analyzed in endpoint PCR for 32 cycles. **A)** The methylated NBR2 promoter, which contains 7 CpGs, is specifically enriched and eluted under both binding conditions. **B)** The unmethylated APC promoter, which contains 29 CpGs, remains in the unbound fraction for both binding conditions, illustrating the specificity of the MBD2b/MBD3L1 heterodimeric protein at enriching only DNA with methylated CpG dinucleotides.

Specifically Detect Unmethylated CpG Islands for Better Validation of Results

Active Motif's UnMethylCollector™ Kit** is the first commercially available kit for the **specific isolation and enrichment of unmethylated CpG dinucleotides**. Instead of relying on negative results from methyl-specific binding techniques to identify hypomethylated promoters, UnMethylCollector offers a specific, reliable technique that provides a positive readout, which is a much better method to obtain or validate your results.

How does UnMethylCollector™ work?

UnMethylCollector uses a His-tagged CXXC domain to specifically bind the unmethylated CpG islands of genomic DNA fragments, which have been prepared by sonication or enzymatic digestion. The protein-DNA complexes are captured with nickel-coated magnetic beads. Subsequent wash steps are performed using an optimized buffer and the included magnet to separate unmethylated fragments from the rest of the genomic DNA. The enriched, unmethylated CpG islands are then eluted in the presence of high salt.

Even more methylation research tools

For bisulfite conversion analysis, don't forget to check out Active Motif's MethylDetector™ Kit. And, our 5-Methylcytidine monoclonal antibody is great for MeDIP assays. For complete product listings and additional details on all of the DNA methylation assays and antibodies offered by Active Motif, please visit www.activemotif.com/dnamt.

Product	Format	Catalog No.
MethylCollector™ Ultra	30 rxns	55005
UnMethylCollector™	30 rxns	55004
MethylDetector™	50 rxns	55001
Fully Methylated Jurkat DNA	10 µg	55003
Jurkat genomic DNA	10 µg	55007
5-Methylcytidine monoclonal antibody	50 µg	39649

Get 15% off UnMethylCollector™ when you buy it with MethylCollector™ Ultra. Visit www.activemotif.com/promo for complete details.

**Patent Pending.

* MethylCollector Ultra & MethylCollector are covered under U.S. Patent No. 7,425,415.

Choose the ChIP Kit That is Best for Your Intended Application, From the Source that Developed Them All

The different ways in which ChIP is used have multiplied in recent years, making it critical to correctly match the specific kit with the intended application. Active Motif has a long history of innovation in kits for different ChIP applications, having simplified the protocol with magnetic beads, developed enzymatic shearing procedures, introduced a high-throughput ChIP kit, and a Re-ChIP kit for sequential ChIP assays on a single sample. Let us help you **make ChIP a simple and productive endeavor**.

The New Leader in ChIP

Active Motif was first to introduce ChIP kits employing magnetic beads, and we have only increased our efforts since. We offer ChIP-IT™ Express HT, which enables you to perform 96 ChIPs simultaneously, and Re-ChIP-IT™, which solves the complexities of performing sequential ChIP assays on a single sample; we offer your choice of enzymatic digestion or sonication for chromatin shearing, as well as the most complete line of ChIP-validated chromatin antibodies, which we design and produce ourselves in conjunction with our ChIP kits (see www.activemotif.com/chipabs). With others, ChIP is an interesting afterthought. For us, ChIP is our core identity.

Advantages of magnetic beads

The ChIP-IT Express Kit is our flagship ChIP product, which was created and refined by years of work at Active Motif. The result is a 1-day ChIP protocol that utilizes magnetic beads, which provide a number of advantages over traditional ChIP methods:

- **No pre-blocking needed** – magnetic beads are inert
- **Compatible with ChIP-Sequencing** – no salmon-sperm DNA is used in the protocol
- **No need for DNA purification** – our specialized ChIP Elution Buffer eliminates the need for expensive DNA purification columns
- **Perform ChIP with fewer cells** – routinely perform chromatin IP with 100,000 cells or fewer (Figure 1)
- **Optimized components, buffers & protocols** – Active Motif sweats all the details, so you don't have to

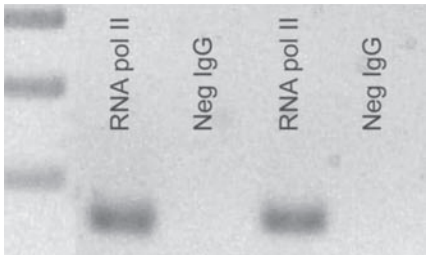


Figure 1: PCR of chromatin IP performed on 100,000 cells. Typically, ChIP requires 2 million cells per reaction. With ChIP-IT Express, it is possible to perform successful ChIP with 100,000 cells or less. In the gel above, ChIP was performed in duplicate on chromatin isolated from 100,000 HeLa cells using the Negative Control IgG, RNA pol II antibody and GAPDH PCR primers supplied in the ChIP-IT Control Kit – Human (Cat. No. 53010).

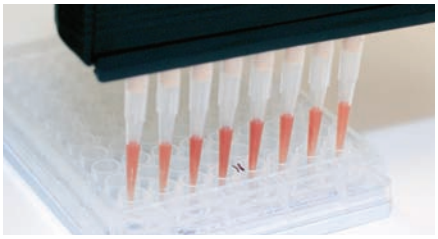


Figure 2: True high-throughput ChIP. With the efficient plate-based protocol of ChIP-IT Express HT, you can process up to 96 ChIP reactions at a time.

Which ChIP-IT Kit is right for your application?

Table 1 (on page 7) gives a brief overview of the different features of our various ChIP-IT Express and ChIP-IT Kits.

ChIP-IT Express HT: High-throughput ChIP is now a reality

Performing multiple ChIP experiments simultaneously using traditional methods only compounds the difficulty of the assay. That is why Active Motif developed ChIP-IT Express HT as the solution to this problem. Now, it is possible to perform the time-saving ChIP-IT Express protocol in a 96-well format (Figure 2), with everything optimized to the level you have come to expect from Active Motif. ChIP-IT Express HT is compatible with our enzymatic or sonication-based shearing kits for chromatin preparation, as well as with our ChIP-IT Control Kits.

Re-ChIP-IT for Sequential ChIP experiments from one sample

Deciphering the Histone Code often requires showing that two marks or associated proteins occur at the same site in the genome. Sequential ChIP (or Re-ChIP) involves sequential chromatin immunoprecipitations using different antibodies in series on the same sample. Sequential chromatin IP was technically challenging and difficult, until now. Active Motif's Re-ChIP-IT Kit has been optimized for this technique, making it easy to perform sequential ChIP, so you can more easily localize two different proteins or histone modifications to the same genetic locus (Figure 3, next page).

What next? If your DNA sample is scarce, amplify it

If you started with a low cell number, chances are the chromatin recovered after ChIP is limiting, constraining the experiments you can do next. To help, Active Motif offers its GenoMatrix™ Whole Genome Amplification Kit, which makes it possible to amplify all recovered DNA fragments. The kit uses a new approach that virtually eliminates representational bias, so the amplified DNA pool contains the same sequence representation as the starting material (Figure 4). For more information, please visit www.activemotif.com/wga.

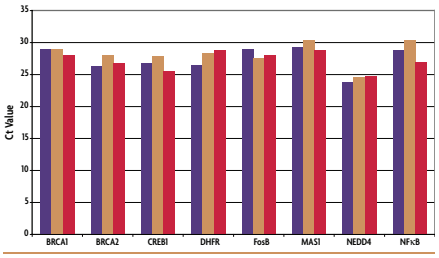


Figure 4: Representative and quantitative DNA amplification without sequence bias.
Quantitative real-time PCR was carried out on DNA samples using primers indicated below each data set and the Ct value for each sample plotted. The DNA was derived from an original DNA sample amplified using the GenoMatrix Whole Genome Amplification Kit (red bars), amplified with a competitor's whole genome amplification kit (copper bars) or not subjected to whole genome amplification (purple bars).

Choose from the widest variety of ChIP kits and accessories available

Active Motif is committed to offering comprehensive solutions for ChIP, enabling you to do your experiments with the confidence that our products have been optimized and improved over many years. In addition to our kits, we offer useful reagents like Ready-to-ChIP Chromatin and species-specific ChIP-IT Control Kits. Let our experience help you work through the challenges of ChIP, which will free up your time and energy for designing your experiments. For more complete information, please give us a call or visit us on the web at www.activemotif.com/chip.

IP Method	Shearing Method: Sonication	Shearing Method: Enzymatic
Magnetic	**ChIP-IT™ Express** • Includes protein G magnetic beads, which reduce background, take less time and enable the use of less material **Shearing:** Sonication **Controls:** Offered separately	**ChIP-IT™ Express Enzymatic** • Includes protein G magnetic beads, which reduce background, take less time and enable the use of less material **Shearing:** Enzymatic **Controls:** Offered separately
Centrifugation	**ChIP-IT™** • Includes protein G agarose beads for standard centrifugation in the IP and wash steps **Shearing:** Sonication **Controls:** Included (human)	**ChIP-IT™ Enzymatic** • Includes protein G agarose beads for standard centrifugation in the IP and wash steps **Shearing:** Enzymatic **Controls:** Included (human)

Table 1: Brief overview of the various shearing and IP/washing options offered in the ChIP-IT Express and ChIP-IT Kits.

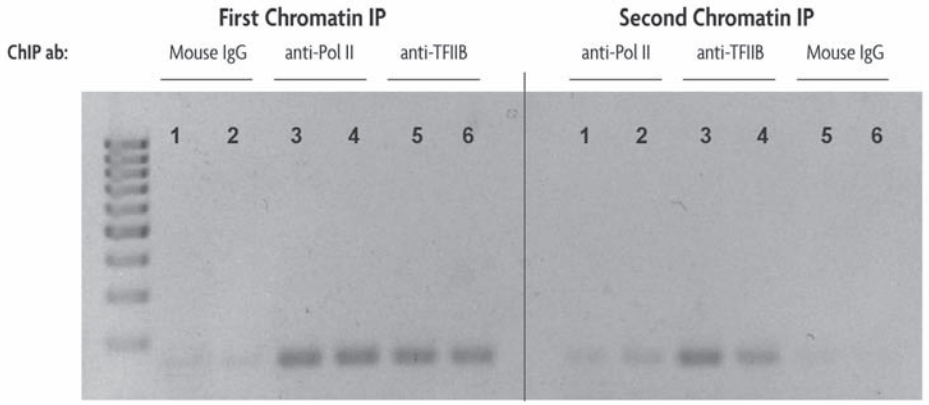


Figure 3: Sequential chromatin immunoprecipitation using Re-ChIP-IT.
The lane numbers are the same in each panel to indicate that the DNA is from the same chromatin sample. The left panel shows the results of PCR performed on an aliquot of DNA removed from the experiment after the first ChIP step; the right panel shows PCR results on DNA from chromatin samples after both ChIP steps. For example, chromatin samples subjected to first ChIP using Mouse IgG as a negative control (lanes 1 and 2 in the left panel) were then subjected to a second ChIP with an RNA Pol II antibody (lanes 1 and 2 in the right panel). Chromatin samples in which Mouse IgG was used as either the first antibody (lanes 1 and 2) or second antibody (lanes 5 and 6) show little amplification of GAPDH DNA in either the left (first ChIP) or right panel (first and second ChIP). Chromatin samples in which the first antibody used was anti-RNA Pol II and the second antibody was anti-TFIIB (lanes 3 and 4) show good amplification of GAPDH DNA after the second ChIP (right panel) indicating co-localization of RNA Pol II and TFIIB at the same region of the GAPDH promoter.

Product	Format	Catalog No.
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Express HT	96 rxns	53018
Re-ChIP-IT™	25 rxns	53016
GenoMatrix™ Whole Genome Amplification Kit	50 rxns	58001

Simple, Effective Monitoring of Phosphorylation Using In-cell Detection

Active Motif's Fast Activated Cell-based ELISA (FACE™) Kits are a simple, sensitive, cell-based method to **detect protein phosphorylation directly in the cell**, without the trouble of making cell extracts or running gels.

FACE advantages

- **Cell based** – it stays on the plate
- **Accurate** – combination of two antibody readouts reliably gives relative phosphorylation state
- **Fast** – 5 hour protocol

In-cell ELISAs: Cell Signaling made easy

Active Motif's In-cell ELISAs require just 2 hours of hands-on time. Cells are grown in 96-well culture plates, stimulated with an agonist, fixed in formaldehyde, and probed separately with an antibody to the protein in question and a phospho-specific antibody to a site on the same protein. Detection is with a secondary antibody and your choice of colorimetric or chemiluminescent readout. Comparing the two signals readily gives you a relative phosphorylation readout (Figure 1), and your results are easily normalized to cell number using the provided Crystal Violet Dye.

A variety of kits to choose from

FACE Kits are available for over 20 different targets (see web address below). The Suspension Cell FACE module was designed to work with all FACE Kits; it improves results when working with suspension cells by providing 96-well filter plates that make it easier to perform washing & liquid handling steps. And, with FACE Maker Kits, you can use your own primary and secondary antibodies to detect any target or modification state of interest.

Simplify your phospho-assays today!

For complete information on the FACE product line, please give us a call or visit us at www.activemotif.com/face.

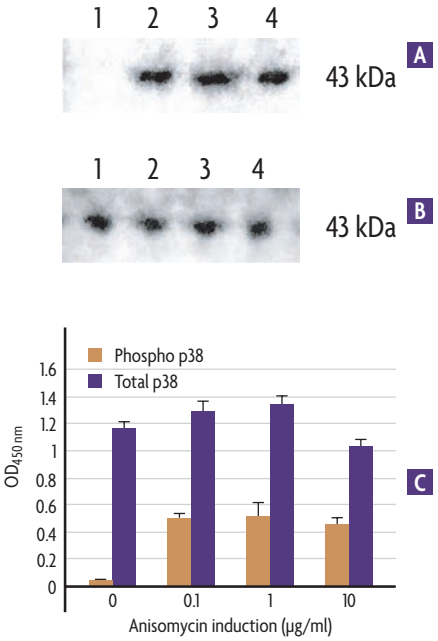


Figure 1: Phospho and total p38 MAPK assays. Macrophage 4/4 cells were grown in 10 cm dishes to 80% confluency, serum-starved for 16 hours and stimulated with anisomycin for 15 minutes. Cell lysates were made and Western blots performed using phospho- (A) and total-p38 antibodies (B). For FACE, 4/4 cells were grown in 96-well plates, stimulated as above, fixed and then assayed in triplicate using the FACE p38 Kit (C). Data were corrected for cell number through use of the kit's Crystal Violet Dye. Western blot data provided courtesy of Dr. Henri H. Versteeg and Dr. Maikel P. Peppelenbosch.

Detect Active Transcription Factors with TransAM™ Assays

TransAM™ Kits are a simple and efficient, non-radioactive alternative to gelshift assays that can **detect small changes in transcription factor activity**. TransAM Family Kits enable you to measure the activity of entire families of transcription factors in a single experiment. In addition to being the most cited transcription factor DNA-binding assay in literature references, Active Motif also offers the largest selection of transcription factor targets.

TransAM advantages

- Detects only active transcription factor-DNA binding events
- Isoform specific detection
- Works on all sample types including cell lines, primary isolates and tissues
- Quantitative results in less than 3 hours

To see the complete list of all 48 TransAM Kits, visit www.activemotif.com/transam.

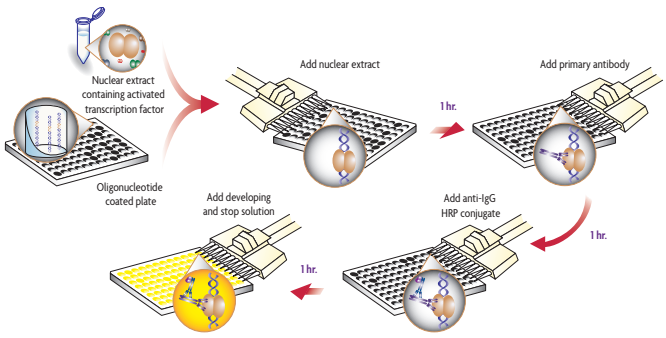


Figure 1: Flow chart of the TransAM process. Activated transcription factor in the sample binds to the immobilized oligo, containing the transcription factor consensus-binding site, in each well. Incubation with the supplied primary and secondary antibodies and addition of developing solutions provides a specific, quantitative readout of activated transcription factor.

Antibodies for the Study of Polycomb Group Proteins

The organization of DNA into chromatin has broad effects on genome based processes, especially gene expression and chromosome & chromatin structure. Open chromatin structure is a hallmark of transcriptionally active regions of the genome (euchromatin), whereas silenced, inactive regions of the genome are found in a more compact chromatin architecture (heterochromatin). Some regions of the genome that contain developmentally regulated genes can switch from one state to the other (facultative heterochromatin).

The Polycomb group proteins are important players in regulating facultative heterochromatin and the expression state of genes found there. In doing so, they help regulate cell fate and lineage decisions. Polycomb proteins are found primarily in two complexes: PRC2, which is recruited to Polycomb regulated regions through interactions with DNA binding proteins, and PRC1, involved in the spreading and maintenance of the repressed state. For example, PRC2 contains the EZH2 histone methyltransferase, specific for lysine 27 of histone H3. Polycomb group proteins function in the maintenance of cellular identity in hematopoietic stem cells, embryonic stem cells and many different tissue types in vertebrate development. Additionally, they play a role in cancer, as EZH2 levels are elevated in prostate cancer (see page 11).

Active Motif offers a variety of well-characterized antibodies to assist in the study of Polycomb proteins. For more information, please visit us at www.activemotif.com/polycombabs.

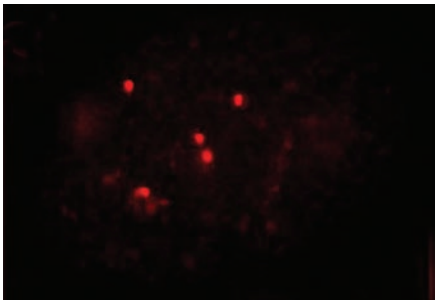


Figure 1: Ring1B staining in U2OS cells.
Human USOS osteosarcoma cells were stained with Ring1B monoclonal antibody (Catalog No. 39663) and visualized by indirect immunofluorescence.

ChIP-validated Histone Monoclonal Antibodies Ensure Your Success

Eliminate the lot-to-lot variability often found with polyclonals by using Active Motif's ChIP-validated histone modification monoclonal antibodies.

Manufacturing a ChIP-validated polyclonal antibody to a histone or histone modification is time consuming and labor intensive. And, once the first batch has been used up, you must start over again from scratch, with no guarantee of success, or even that the second batch of antibody will perform similarly to the first. These potentially inconsistent batch-to-batch process issues can cause problems when conducting studies that require a consistent methodological approach, such that all your data are comparable. When performing a technique as painstaking and tricky as chromatin immunoprecipitation, consistency is paramount.

To eliminate the batch-to-batch inconsistencies of polyclonals, Active Motif offers a range of ChIP-validated monoclonals. So, you can be sure you are getting the same antibody every time, no matter when you need it. For more, visit www.activemotif.com/hismodmono.

Figure 1. Chromatin IP using Histone H3 dimethyl Lys9 monoclonal antibody (Clone MABI 0307).
Chromatin IP was performed using the ChIP-IT Express Kit (Catalog No. 53008) and 100 µl of Ready-to-ChIP HeLa Chromatin (Catalog No. 53015) per ChIP. DNA was then purified from the immunoprecipitated chromatin and a region of the human HOXD13 promoter was amplified by PCR. **Lane 1:** ChIP using 10 µg of Histone H3 dimethyl Lys9 mAb. **Lane 2:** PCR input control. **Lane 3:** ChIP using negative control mouse IgG.

Product	Format	Catalog No.
Histone H2B ubiquityl Lys120 antibody (Clone 56)	100 µg	39623
Histone H3 antibody (Clone MABI 0301)	100 µg	39763
Histone H3 monomethyl Lys4 antibody (Clone MABI 0302)	100 µg	39635
Histone H3 dimethyl Lys4 antibody (Clone MABI 0303)	100 µg	39679
Histone H3 monomethyl Lys9 antibody (Clone MABI 0306)	100 µg	39681
Histone H3 dimethyl Lys9 antibody (Clone MABI 0307)	100 µg	39683
Histone H3 acetyl Lys27 antibody (Clone MABI 0309)	100 µg	39685
Histone H3 trimethyl Lys27 antibody	200 µl	39535
Histone H4 trimethyl Lys20 antibody (Clone MABI 6F8-D9)	100 µg	3967

NEW: Screen Histone PTMs with a Fast, Sensitive ELISA

Post-translational modifications (PTMs) on histone tails have been shown to correlate very closely with the nuclear processes of transcription, chromosome packaging and DNA damage repair. Due to the importance of these post-translational modifications, Active Motif has expanded its line of Histone Modification ELISAs. Kits are now available for the detection of lysine methylation at K4, K9 and K27, or serine phosphorylation at S10 and S28 on histone H3.

How do the Histone ELISAs work?

The Histone Modification ELISA Kits provide a simple, sensitive method for detecting changes in specific histone modifications, such as methylation or phosphorylation, from purified core histones or histones isolated by acid extraction. These kits are sandwich ELISAs that utilize a capture antibody against histone H3 and a detecting antibody specific to the modification of interest. An HRP-conjugated secondary antibody and developing solutions provide a sensitive colorimetric readout (Figure 1).

Each Histone Modification ELISA is also tested against other modifications to ensure specificity (Figure 2). Validated modification-specific controls are included in each kit.

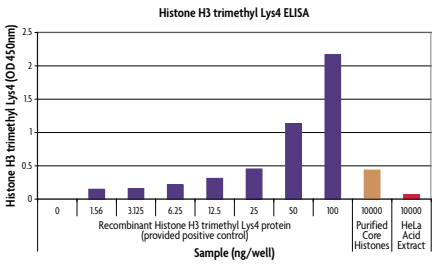


Figure 1: Histone H3 trimethyl Lys4 sensitivity. The Histone H3 trimethyl Lys4 ELISA was used to assay 10 µg HeLa core histones purified using Active Motif's Histone Purification Mini Kit (Catalog No. 40026) and 10 µg HeLa acid extract. The Recombinant Histone H3 trimethyl Lys4 protein provided in the kit was assayed from 1.5-100 ng/well as a reference standard curve.

For more information about our Histone Modification ELISAs, please call or visit www.activemotif.com/hiselisa.

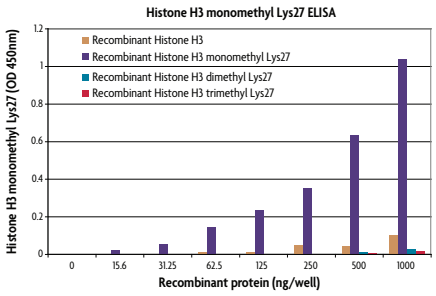


Figure 2: Histone H3 monomethyl Lys27 specificity. Recombinant Histone H3, mono-, di- and trimethyl Lys27 proteins were assayed from 15 ng - 1 µg per well using the Histone H3 monomethyl Lys27 ELISA. The results show low background with other modifications, demonstrating the specificity of the assay.

Histone Modification ELISA advantages

- **Sensitive** – works with purified core histones or acid extracted samples
- **Fast** – assay can be completed in less than 3 hours
- **Flexible** – 96-stripwell plate allows screening in low or high throughput
- **Controls ensure success** – modification specific controls included
- **Colorimetric assay** – easily quantified by spectrophotometry on a microplate reader at 450 nm

Validated controls also available

For further analysis of histone post-translational modifications, site- and degree-specific methylated proteins that are provided for use as a reference standard curve in each histone methylation ELISA are also available separately (page 3). Each recombinant histone is verified by high-resolution mass spectrometry, dot blot and immunoblot to confirm the methylation reaction is over 99% complete and the recombinant histone is specific for the target of interest.

For researchers looking to study epigenetic events using acid extracts, Active Motif offers untreated and treated HeLa acid extracts, such as the paclitaxel treated HeLa acid extracts included in the histone phosphorylation ELISAs.

To learn more about our recombinant histones, acid extracts and associated histone modification antibodies, please visit us at www.activemotif.com.

Product	Format	Catalog No.
Histone H3 monomethyl Lys4 ELISA **NEW**	1 x 96 rxns	53101
Histone H3 dimethyl Lys4 ELISA **NEW**	1 x 96 rxns	53112
Histone H3 trimethyl Lys4 ELISA **NEW**	1 x 96 rxns	53113
Histone H3 dimethyl Lys9 ELISA	1 x 96 rxns	53108
Histone H3 trimethyl Lys9 ELISA	1 x 96 rxns	53109
Histone H3 monomethyl Lys27 ELISA **NEW**	1 x 96 rxns	53104
Histone H3 trimethyl Lys27 ELISA	1 x 96 rxns	53106
Histone H3 phospho Ser10 ELISA	1 x 96 rxns	53111
Histone H3 phospho Ser28 ELISA	1 x 96 rxns	53100
Total Histone H3 ELISA	1 x 96 rxns	53110
Histone Purification Mini Kit	20 rxns	40026

Using Histone and Histone Modifications as Biomarkers for Cancer

In eukaryotes, DNA is organized into chromatin through the wrapping of DNA around histone octamers to form the nucleosome. These histone proteins are subject to a variety of post-translational modifications (phosphorylation, acetylation, methylation...) that are involved in genome-dependent processes like transcription, chromosome organization and the like. These modifications, while important regulators of chromatin-mediated processes, can also be indicators of diseases such as cancer.

Recently, researchers at the University of California at Los Angeles identified particular histone modifications as potential biomarkers for the clinical outcome of pancreatic adenocarcinoma. By examining tissue samples from patients with pancreatic cancer, it was determined that low levels of either histone H3 lysine 4 dimethyl (H3K4me2), histone H3 lysine 9 dimethyl (H3K9me2), or histone H3 lysine 18 acetyl (H3K18ac) were associated with poor survival. Low levels of H3K4me2 and H3K18ac combined were the most significant indicator of clinical outcome. Says senior researcher Dr. David Dawson, "Using a specially devised test and algorithm, we were able to discriminate two groups of pancreatic cancer patients who were more or less likely to have longer disease-free remissions and overall survival."

Indeed, many examples of connections between histone modifications and cancer exist already (Table 1). Specific patterns of histone levels or histone modifications have been correlated with particular cancers. Additionally, increased expression of the histone H3 lysine 27 methyltransferase EZH2 is observed in prostate cancer. It is possible, and likely, that soon many more histone modifications will be identified as markers for other types of cancer.

Histone research simplified

To facilitate your research into histone modifications and their role in diseases like cancer, Active Motif has a variety of high-quality tools at your disposal.

Disease	Connection	Reference
Breast cancer progression	Histone H2A.Z levels	Hua *et al.* (2008) *Mol Syst Biol.* 4: 188. Svotelis *et al.* (2010) *Cell Cycle* 9: 364-370.
Chronic Lymphocytic Leukemia	Histone H2A levels	Su *et al.* (2009) *Proteomics* 9 :1197-1206.
Lung cancer recurrence	Histone macroH2A levels	Sporn *et al.* (2009) *Oncogene* 28: 3423-3428.
Non small cell lung cancer	Histone acetylation and methylation	Barlesi *et al.* (2007) *J. Clinic. Oncol.* 25: 4358-4364.
Pancreatic cancer	H3 acetylation and methylation	Manuyakorn *et al.* (2010) *J. Clin. Oncol.* 28: 1358-1365.*
Prostate cancer	H3 and H4 acetylation and methylation	Seligson *et al.* (2005).
Prostate cancer	Histone methyltransferase EZH2	Varambally *et al.* (2002).
Skin and other cancers	H4 acetylation and methylation	Fraga *et al.* (2005) *Nature Genet.* 37: 391-400.
Squamous cell carcinoma	H3 and H4 acetylation and methylation	I *et al.* (2010) *Cancer Epidem Biomark Prev.* 19:566-573.

*Data in this paper were generated in part using Active Motif's Histone H3 acetyl Lys18 pAb (Catalog No. 39693).

Table 1: Connections that have been shown between histone modifications and cancer types.

We offer a variety of antibodies to histones and histone modifications validated in applications like ChIP (page 9), Westerns and IF. Active Motif is the only company to offer histone purification kits that enrich histones out of human cells and PBMCs. We also offer a broad range of ELISAs to measure levels of histone modifications from acid extracts or purified histones (page 10). And, we offer a fluorescence polarization binding assay that enables you to identify proteins that interact with histone H3 methylated at either Lys9 or Lys27 (Figure 1). Please visit the links below to learn more.

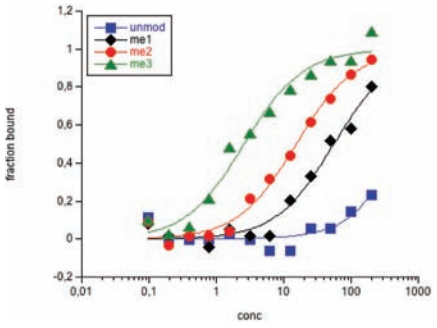


Figure 1: Binding of HP1 to histone H3 Methyl-Lys9. One µM of fluorescent histone H3 Lys 9 peptides, which are either unmodified or mono-, di- or trimethylated at K9, were added to a 96-well plate containing a serial dilution of HP1 protein (x axis) starting at a concentration of 200 µM. The plate was scanned on a Tecan Infinite F200 using 485 nm excitation and 535 nm emission filters with polarizers. A gain of 80 was used; 100 reads were taken of each well, then averaged.

Product Line	Web Address
Histone Modification FP Binding Assays	www.activemotif.com/hilite
Histone Modification ELISAs	www.activemotif.com/hiselisa
Histone Purification Kits	www.activemotif.com/histonepur
Histone and Histone Modification Antibodies	www.activemotif.com/histoneabs
Recombinant Histone Modifying Enzymes	www.activemotif.com/hismodenz
Recombinant Modified Histone Proteins	www.activemotif.com/recombhis