ACTIVE MOTIF®

MOTIFvations

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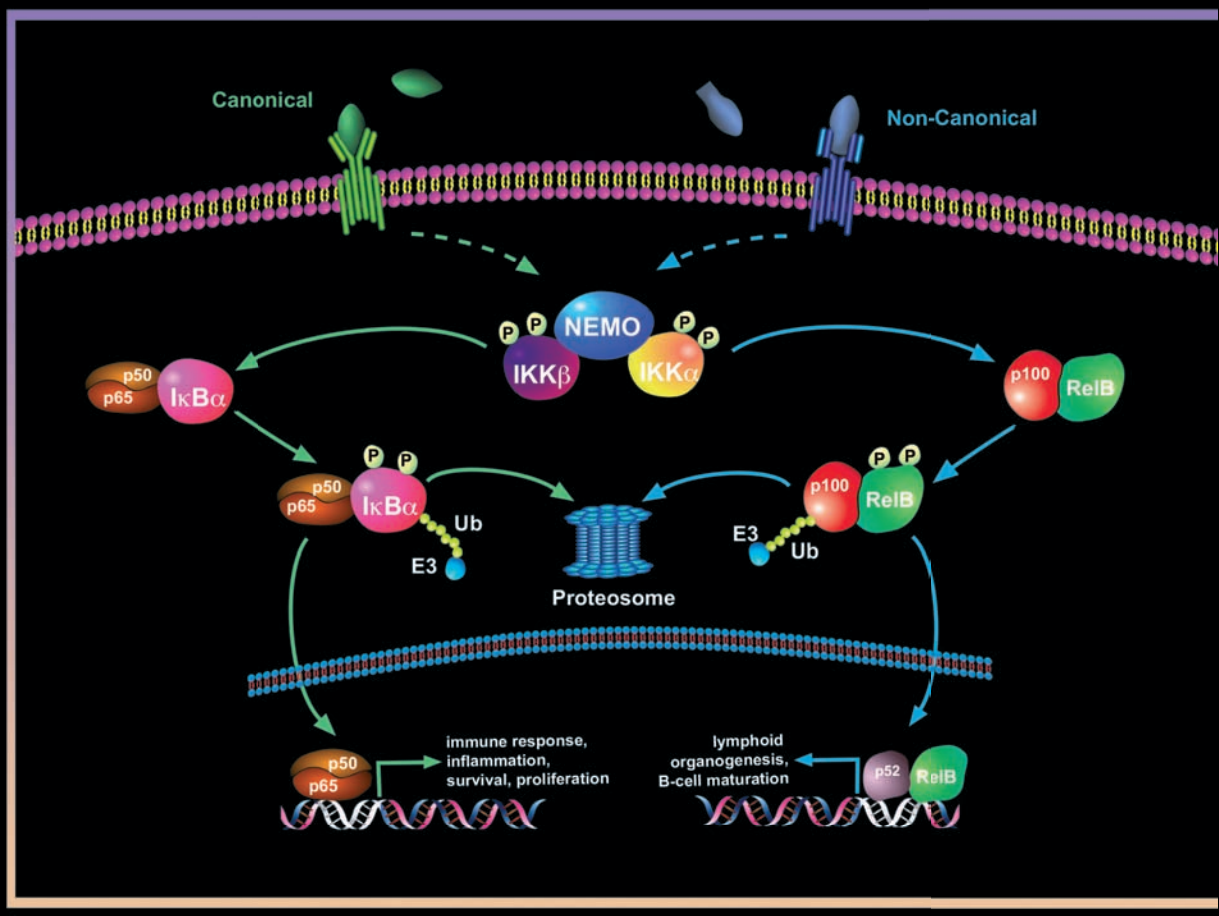

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Epigenetics: Influencing the Kinetics of NFκB Signal Transduction

The nuclear factor-κB (NFκB) signaling pathway is widely studied due to its involvement in the regulation of genes that control inflammation, immune response, cell survival, cell proliferation and human disease.

The NFκB Rel family of transcription factors consists of a set of evolutionarily conserved DNA-binding proteins that includes p65, p50, p52, RelB and c-Rel. Inactive NFκB is sequestered in the cytoplasm by the IκB family of inhibitory proteins. Activation of the NFκB signaling pathway induces phosphorylation of IκB proteins by IκB kinases (IKK). Depending on the type of stimulation, the classical (also referred to as 'canonical') or the 'non-canonical' NFκB signaling pathways can be activated (Figure 1). Phosphorylation by the IKK complex leads to ubiquitination and degradation of the inhibitor IκB molecules by the proteasome. The liberated NFκB complexes translocate to the nucleus where they bind DNA and regulate gene expression by binding to κB DNA-binding sites.

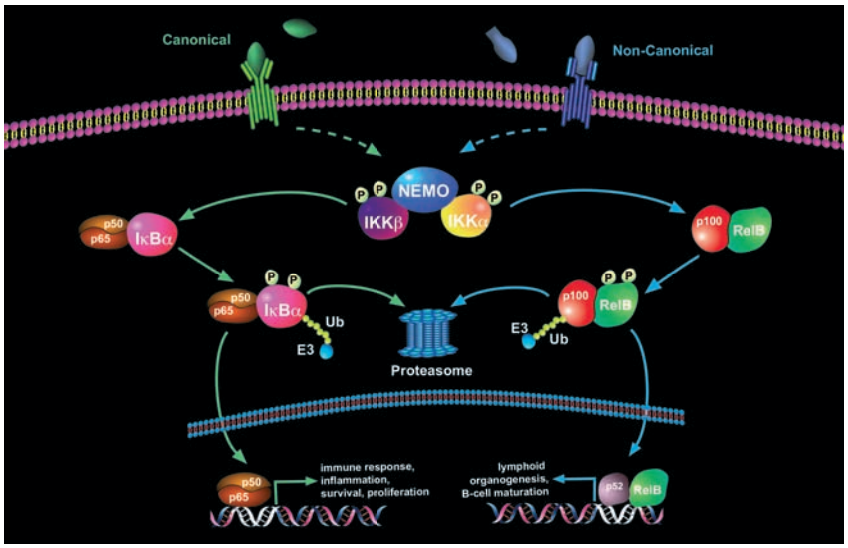

Figure 1: The NFκB signaling pathway.
Depiction of the NFκB canonical and non-canonical signaling pathways.

Evidence is building that epigenetic modifications play a critical role in orchestrating NFκB-mediated transcriptional activation. There is a significant understanding of the contribution of the synergistic effects of various cofactors and signaling networks on the spatiotemporal coordination of NFκB-mediated transcriptional events at the cellular level. However, the fine-tuning and kinetics of the response to those signals at the genome level can best be explained by analyzing how histone modification patterns influence and are, in turn, influenced by the activity of NFκB. In particular, recent studies have focused on how epigenetic events influence the accessibility of the NFκB transcription factor/cofactor enhanceosome complex to target κB DNA-binding sites within the chromatin structure.

Active Motif offers a variety of products to analyze the complex dynamics of NFκB transcription factor binding and associated epigenetic events. To learn more about Active Motif's NFκB-related products, please give us a call or visit us on our website at www.activemotif.com/nfkb.

Non-radioactive Methods to Evaluate Transcription Factor Binding

To facilitate the study of DNA-binding activity, Active Motif offers its **TransAM™ Kits**, which are highly-sensitive, non-radioactive transcription factor ELISAs that enable detection of even the smallest changes in the levels of activated transcription factors. In addition, the 96-stripwell format allows for both high and low throughput screening of samples. Its sensitivity, flexibility and specificity make the TransAM method the most published alternative to electrophoretic mobility shift assays (EMSA). To view a complete list of available TransAM™ Kits, please visit us at www.activemotif.com/transam. Recombinant proteins are also available for use as standard curves in the TransAM NFκB p50 and TransAM NFκB p65 Kits.

Active Motif offers the **Gelshift™ Chemiluminescent EMSA Kit** as a simple, convenient, non-radioactive solution for assessing variant transcription factor binding to DNA targets. The non-radioactive format displays enhanced sensitivity when compared to traditional ^{32}P or digoxigenin EMSA methods.

Product	Format	Catalog No.
TransAM™ NFκB Family (p50, p52, p65, c-Rel & RelB)	2 x 96 rxns	43296
TransAM™ NFκB p50	1 x 96 rxns	41096
TransAM™ NFκB p52	1 x 96 rxns	48196
TransAM™ NFκB p65	1 x 96 rxns	40096
Gelshift™ Chemiluminescent EMSA	100 rxns	37341

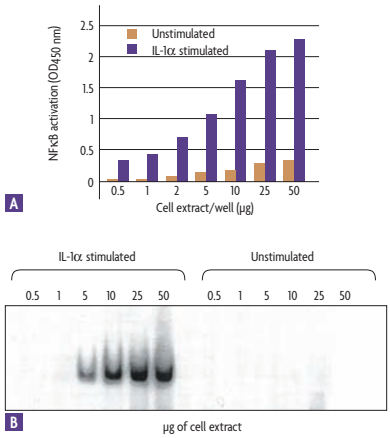

Figure 1: Comparison of non-radioactive TransAM Kits and radioactive gelshift.
Activated NFκB p50/DNA-binding results are shown for human fibroblast WI-38 cells that were stimulated with IL-1α for 30 minutes. Increasing amounts of whole-cell extracts were assayed using either TransAM NFκB p50 (A) or gelshift (B). The TransAM method is 10-fold more sensitive and provides quantitative data.

Additional Tools to Aid your NFκB Research (for Custom Services, see page 9)

Phospho-protein Detection

- FACE™ NFκB p65 Profiler (Ser468 & Ser536)
- FunctionELISA IκBα

Luciferase Reporter Assays

- RapidReporter® pRR-High-NFκB vector
- RapidReporter® pRR-High-NFκB Assay
- RapidReporter® *Gaussia* Luciferase Assay

In Vivo Fluorescent Labeling

- LigandLink™ pLL-1-NFκB p65

SUMOylation

- SUMOlink™ SUMO-1
- SUMOlink™ SUMO-2/3

Antibodies

- Highly characterized antibodies including: IκBα, IKKα, IKKβ, IKKγ, NFκB p50, NFκB p65, NFκB p100 and more

www.activemotif.com/abs

Extracts

- Variety of untreated and stimulated nuclear, cytoplasmic and whole-cell extracts to study NFκB signaling

www.activemotif.com/extracts

Recombinant Proteins

- NFκB-related recombinant proteins, including NFκB p50, NFκB p65, IKKβ and others

www.activemotif.com/proteins

To learn more about all of Active Motif's NFκB-related products, please visit www.activemotif.com/nfkb.

NEW: 5-Hydroxymethylcytosine Antibodies Enhance DNA Methylation Studies

Conventional methods for studying DNA methylation (enrichment by antibody or methylated-DNA binding protein, methylation sensitive enzyme digestion and bisulfite sequencing) cannot distinguish 5-hydroxymethylcytosine (5-hmC) from 5-methylcytosine (5-mC). To help **study this novel form of DNA methylation**, Active Motif offers two polyclonal antibodies and a new monoclonal antibody that are specific for the 5-hmC modification. Like all of our antibodies for epigenetics research, they are highly specific and have been validated for use in various practical applications, including methylated DNA immunoprecipitation (MeDIP), immunofluorescence and dot blot.

The importance of DNA methylation

DNA methylation is an epigenetic event in which DNA methyltransferases (DNMTs) catalyze the reaction of a methyl group to the fifth carbon of cytosine in a CpG dinucleotide. This modification helps to regulate gene expression and is also involved in genomic imprinting, while aberrant DNA methylation is often associated with disease.

5-methylcytosine (5-mC) is a type of DNA methylation found in plants and vertebrates. A second type of DNA methylation exists, 5-hydroxymethylcytosine (5-hmC), which results from the enzymatic conversion of 5-methylcytosine into 5-hydroxymethylcytosine by the TET family of cytosine oxygenases (Figure 1). Elevated levels of 5-hydroxymethylcytosine have been observed in neurons and embryonic stem cells. It is possible that 5-hmC represents a pathway by which DNA is demethylated, as 5-hydroxymethylcytosine is recognized by the DNA mismatch repair system and replaced with unmethylated cytosine.

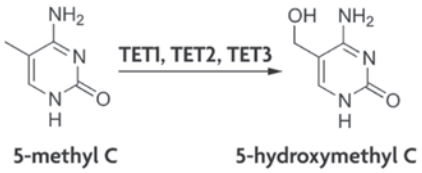

Figure 1: Schematic of conversion of 5-mC to 5-hmC.
5-methylcytosine is converted to 5-hydroxymethylcytosine by the TET family of enzymes.

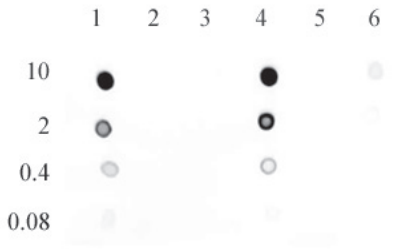

Figure 2: 5-Hydroxymethylcytidine monoclonal antibody tested by dot blot analysis.
DNA samples were spotted (indicated in ng on the left) and blotted with 5-Hydroxymethylcytidine monoclonal antibody (Clone 59.1) at a dilution of 0.2 µg/ml.
Lanes 1-3: double-stranded DNA.
Lanes 4-6: single-stranded DNA.
Lanes 1 & 4: DNA containing 5-hydroxymethylcytosine.
Lanes 2 & 5: DNA containing 5-methylcytosine.
Lanes 3 & 6: unmethylated DNA.

Methylated DNA Standard Kit

In addition to the antibodies to study DNA methylation, Active Motif offers our Methylated DNA Standard Kit. This kit includes three recombinant DNA standards derived from the APC gene regulatory region: unmethylated DNA, 5-methylcytosine methylated DNA and 5-hydroxymethylcytosine methylated DNA. This kit (which also includes PCR primers specific to the APC gene) can be used to provide controls for experiments studying the different types of DNA methylation (see Figure 1 on page 5).

Antibodies to study DNA methylation

Active Motif has three antibodies specific for the 5-hydroxymethylcytosine form of DNA methylation. Our newest release, the 5-Hydroxymethylcytidine mouse monoclonal antibody (Clone 59.1) has been validated for use in MeDIP and dot blot (Figure 2). Like our polyclonal antibodies, the new monoclonal does not require DNA to be denatured to its single-stranded form in order to work in a MeDIP experiment. This can be advantageous when analyzing the resulting DNA using high-throughput Next-Gen sequencing. We also offer the more traditional 5-Methylcytidine mouse monoclonal antibody to study 5-mC DNA methylation in a variety of applications.

Full line of DNA Methylation products

Active Motif offers a variety of antibodies, assay kits and services for the epigenetics and chromatin research community. To learn more about the different DNA methylation tools available, visit www.activemotif.com/dnamt. For a complete listing of DNA methylation antibodies, including application data, visit www.activemotif.com/dnamethabs.

Product	Format	Catalog No.
5-Hydroxymethylcytidine mAb (Clone 59.1)	100 µg	39999
5-Hydroxymethylcytidine antibody (rabbit IgG)	100 µg	39791
5-Hydroxymethylcytidine antibody (rabbit serum)	100 µl	39769
5-Methylcytidine antibody mAb (Clone 33D3)	50 µg	39649
Methylated DNA Standard Kit	3 x 2.5 µg	55008

NEW: hMeDIP Assay Simplifies the Study of 5-hmC DNA Methylation

Active Motif's new hMeDIP Kit helps simplify the analysis of 5-hydroxymethylcytosine methylation. 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 (5-hmC) antibody to **selectively enrich for DNA fragments with 5-hydroxymethylcytosine methylation** from the rest of the genomic DNA population. Some advantages of the hMeDIP Kit are that it not only differentiates between 5-hydroxymethylcytosine DNA and 5-methylcytosine DNA, but also that the fragmented genomic DNA used in the assay can be double-stranded DNA, which prevents the problems associated with linker bias when preparing the enriched DNA for downstream Next-Gen sequencing.

Why use hMeDIP over other DNA methylation enrichment techniques?

Unlike traditional methods used to study DNA methylation, such as enzymatic approaches & bisulfite conversion that cannot directly differentiate between 5-mC and 5-hmC DNA methylation, or methyl CpG binding protein enrichment which can only recognize and bind 5-mC methylation, hMeDIP is able to use the selective affinity of the immunocapture antibody to distinguish between 5-methylcytosine and 5-hydroxymethylcytosine DNA methylation.

Additionally, the hMeDIP technique is able to enrich for DNA fragments containing cytosine methylation regardless of the sequence context. While most DNA methylation in mammalian tissues occurs in a CpG context, studies have found that 15-20% of total cytosine methylation in embryonic stem (ES) cells occurs at sequences other than CpG.

These features make the hMeDIP Kit ideal for researchers interested in identification of total cytosine methylation, or in differentiating between 5-mC and 5-hmC methylation.

Kit	Antibody	DNA Input	Input Range
hMeDIP	5-hmC purified rabbit pAb	double-stranded or single-stranded DNA	100 ng – 1 µg

Table 1: hMeDIP Kit sample requirements.

Included methylated DNA controls

The hMeDIP Kit also includes methylated DNA controls for use in determining the efficiency of the enrichment. The controls are 338 base pair DNA fragments that are completely unmethylated, 5-methylcytosine methylated or 5-hydroxymethylcytosine methylated. When these control DNAs are spiked into sample DNA the specificity of the capture antibody can be confirmed via real time PCR with the included PCR primers (Figure 1). The Methylated DNA Standards used as controls in the hMeDIP Kit can also be purchased separately (see page 4).

Visit www.activemotif.com/dnamt to learn more about the wide variety of DNA methylation analysis tools and assay kits offered by Active Motif.

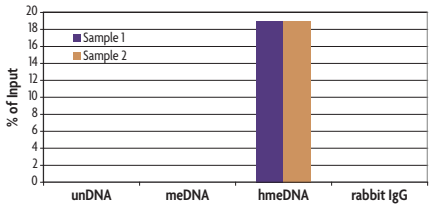

Figure 1: hMeDIP results using "spiked" control DNA. *Mse* I digested human genomic DNA (500 ng) was spiked with 25 pg of either methylated (meDNA), hydroxymethylated (hmeDNA) or unmethylated (unDNA) DNA controls. These samples were processed using the hMeDIP Kit with the 5-hmC antibody and compared to a negative control rabbit IgG antibody. Real time PCR with the included APC PCR primers was run and the recovered DNA was plotted as a percentage of the total input DNA (500 ng). The results show the hMeDIP Kit is specific for enrichment of 5-hydroxymethylcytosine DNA.

As part of its recent acquisition of Genpathway, Active Motif now offers a 5-Hydroxymethylcytosine MeDIP-Seq service. For details, please see page 9 or visit www.genpathway.com.

Product	Format	Catalog No.
hMeDIP	10 rxns	55010
MeDIP COMING SOON	10 rxns	55009

Complete Analysis of Histone Post-translational Modifications

Whether you are studying transcription factors such as NFκB or chromatin-modifying proteins, understanding the implications of histone post-translational modifications as they relate to gene regulation and chromatin context will be critical to determining how these modifications affect or contribute to disease. Active Motif's unique portfolio of histone technologies provides researchers with a complete solution for analysis. To either download or request a copy of our Histone Analysis Products brochure by mail, please visit www.activemotif.com/info.

Histone Purification Kits that Preserve Post-translational Modifications

Histone Purification Kit advantages

Active Motif's unique Histone Purification and Histone Purification Mini Kits enable the isolation of core histones from any cell culture or tissue sample while preserving their post-translational modifications (Figure 1). Unlike standard acid extraction techniques, it is possible to isolate core histones as either a single fraction or to further separate them into H2A/H2B and H3/H4 fractions.

Which kit is right for you?

- **Histone Purification** – can process samples of up to 2.5 mg which can be eluted as either a single fraction with all four histones or as separate H2A/H2B and H3/H4 fractions
- **Histone Purification Mini** – has a capacity of up to 0.5 mg per sample which is eluted as a single fraction containing H2A, H2B, H3 and H4

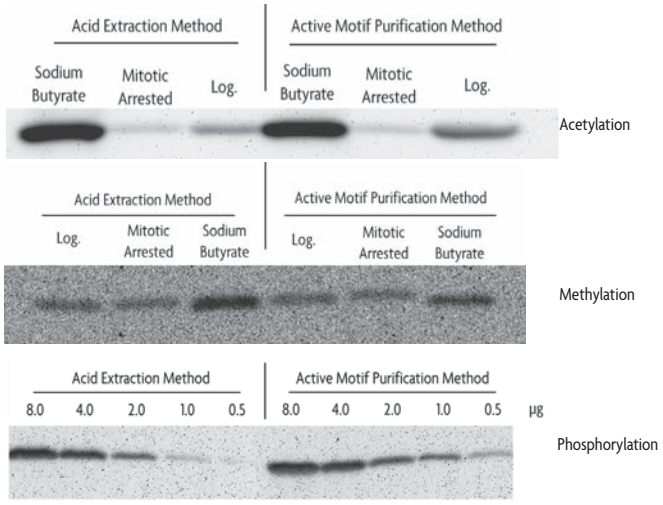

Figure 1: Active Motif's Histone Purification Kits preserve post-translational modifications.
Acetylation, methylation and phosphorylation states are preserved as well or better with the Histone Purification Kit than standard acid extraction methods.

Product	Format	Catalog No.	Price ($US)
Histone Purification Kit	10 rxns	40025	275
Histone Purification Mini Kit	20 rxns	40026	275

NEW: Recombinant Histone Proteins to Analyze Site-specific Modifications

Recombinant histone proteins with site-specific modifications

Active Motif continues to expand its line of recombinant methylated and acetylated histones. We have added 16 new recombinant proteins to our portfolio, including methylation of lysine 14, 18 and 23 on histone H3 and methylation of lysine 5 and 16 on histone H4. Additionally, we now offer recombinant histone H3 acetylated at lysine 23.

Why use recombinant histones?

These recombinant proteins more closely mimic "natural" histones than peptides, making them ideal substrates for functional assays and activity screens. The recombinant proteins are suitable for use with Active Motif's histone modifying enzymes and are even included in our Histone Modification ELISAs to generate standard curves for histone quantification.

New biotinylated recombinant H3

To expand the versatility of our recombinant histones, Active Motif now offers Recombinant Histone H3 biotinylated at the N-terminus.

To see an up-to-date list of the more than 40 recombinant histone proteins currently available, please visit us at www.activemotif.com/recombhis.

NEW: Histone ELISAs for Sensitive Detection of Acetylated Histones

Quantify your histone modifications

The Histone Modification ELISA Kits offer a quick and easy way to screen and quantify histone modifications within your sample. The kits are designed as sandwich ELISAs that utilize a histone H3 capture antibody and a detecting antibody that is specific for the modified residue of interest.

Active Motif currently offers more than ten different Histone ELISAs for methylation and phosphorylation marks on histone H3. Now, Active Motif has expanded its line of ELISAs to include assays to detect acetylation of histone H3 lysine 9 and lysine 14. Each kit also includes a positive control recombinant protein that can be used to generate a standard curve for histone quantification (Figure 1). The Total Histone H3 ELISA is available to measure total histone levels or to normalize quantities of modified histones from the sample.

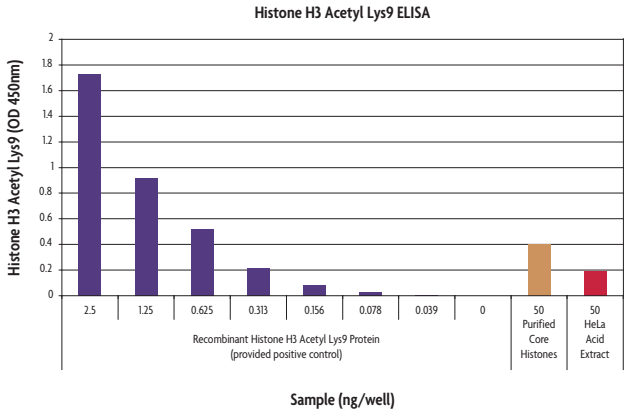

Figure 1: Detection of histone H3 lysine 9 acetylation in purified core histones and acid extracted histones. The Histone H3 acetyl Lys9 ELISA was used to quantitate the amount of histone H3 lysine 9 acetylation present in HeLa core histones made using Active Motif's Histone Purification Mini Kit (Cat. No. 40026, page 6) and HeLa acid extracts. The Recombinant Histone H3 acetyl Lys 9 protein that is provided in the kit was assayed from 0.039 - 2.5 ng/well as a standard curve. Data was quantified against the linear range of the standard curve. Data shown are from wells assayed in duplicate.

Product	Format	Catalog No.
Histone H3 acetyl Lys9 ELISA	1 x 96 rxns	53114
Histone H3 acetyl Lys14 ELISA	1 x 96 rxns	53115
Total Histone H3 ELISA	1 x 96 rxns	53110

To see a complete list of the over 10 Histone Modification ELISAs available, including methylation and phosphorylation ELISAs, please visit www.activemotif.com/hiselisa.

Screen for Histone Interactions Using the MODified™ Histone Peptide Array

Unique array to screen interactions

The MODified™ Histone Peptide Array enables analysis of antibody, protein and enzyme interactions with histones and their post-translational modifications. The array contains 384 different combinations of acetylation, phosphorylation, methylation and citrullination modifications on the N-terminal tails of histones H2A, H2B, H3 and H4.

To learn more about the MODified Histone Peptide array, or to download the free Array Analyse software, please visit www.activemotif.com/modified.

MODified Array advantages

- **Histone specific** – unique array panel tests for specific histone modifications
- **Study neighboring effects** – each peptide contains up to four modification combinations, enabling the analysis of the effects of neighboring modifications
- **Detects like a Western blot** – fast and easy to use; works with either ECL-based or colorimetric detection
- **Free software for analysis** – measures spot intensity and generates Excel-compatible files for analysis

Product	Format	Catalog No.
MODified™ Histone Peptide Array	1 array	13001
	5 arrays	13005
MODified™ Array Labeling Kit	5 rxns	13006

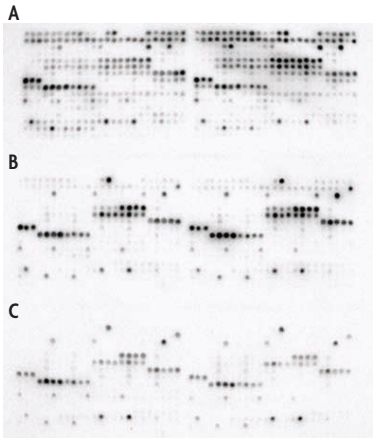

Figure 1: Detection of G9a histone methyltransferase activity using the MODified Histone Peptide Array. MODified Histone Peptide Arrays were treated with A) 25 µM G9a methyltransferase, B) 25 µM G9a mutant methyltransferase or C) a no enzyme control overnight in the presence of 1 mM AdoMet. The arrays were then labeled with a Histone H3 dimethyl Lys9 antibody. Novel methylation sites were observed on the array treated with wild-type G9a histone methyltransferase, showing the activity of G9a enzyme on the peptide substrate.

NEW: Obtain Analysis-ready DNA with the Chromatin IP DNA Purification Kit

Chromatin Immunoprecipitation (ChIP) is a powerful, well-established technique for studying interactions between chromatin-associated proteins and specific regions of the genome. While the use of ChIP in combination with genome-wide analysis techniques can yield a tremendous amount of information regarding the distribution of transcription factors and histone modifications, most downstream analysis techniques require DNA that has been purified away from the components and contaminants present in your eluted ChIP sample. Active Motif's Chromatin IP DNA Purification Kit enables you to **quickly and easily clean up your ChIP DNA samples** to make them ready for analysis, eliminating the need for labor intensive and time consuming phenol/chloroform extraction.

What is the Chromatin IP DNA Purification Kit process?

Once your ChIP experiments are complete, the DNA purification procedure can be started immediately. The entire procedure takes only five to ten minutes, depending upon the number of samples to purify. The Chromatin IP DNA Purification Kit is compatible with samples from all of Active Motif ChIP-IT™ Kits, or from any standard chromatin IP kit or procedure, whether they use mechanical or enzymatic shearing of chromatin, agarose or paramagnetic bead enrichment. It can also be used to purify DNA from other Active Motif kits, including the new hMeDIP Kit, MethylCollector™ Ultra and UnMethylCollector™. The new Chromatin IP DNA Purification Kit is designed for use with a microcentrifuge for sample processing, but can also be used with a vacuum manifold.

Because binding of DNA to the silica matrix of the DNA purification column is pH-dependant, the kit's Binding Buffer contains a convenient pH indicator dye. This makes it possible for you to see the pH of your samples before applying them to the column, helping ensure successful purification of your ChIP DNA (Figure 1). After binding, the DNA on the column is washed, then eluted using the included Elution Buffer.

DNA recovery and yield

After elution of the DNA, you now have samples of high quality and purity, suitable for a number of downstream analysis techniques, including PCR (end-point or quantitative), Southern blotting, microarray analysis or Next-Gen sequencing (Figure 2). Depending upon the number of cell equivalents of chromatin used in each ChIP reaction, the amount of recovered DNA will be from 100 ng to 1 µg. DNA can be successfully recovered from ChIP experiments starting with as few as 10,000 cells. DNA fragments below 50 base pairs in length are not recovered efficiently, but this shouldn't be a problem as the recommended average size for chromatin fragments in a typical ChIP reaction is 500 base pairs.

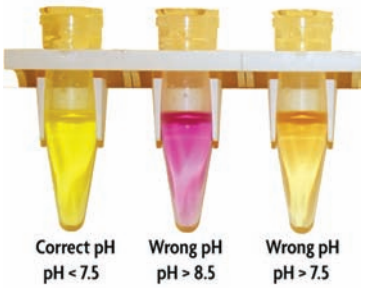

Figure 1: DNA Binding Buffer pH indicator dye. The DNA Purification Binding Buffer has a pH indicator dye so the pH of the solution can be easily determined. The DNA should only be applied to the column if the solution is bright yellow (left), indicating a pH under 7.5. DNA will not bind to the column if the pH is higher than 7.5. (NaOAc is included to reduce sample pH, if needed.)

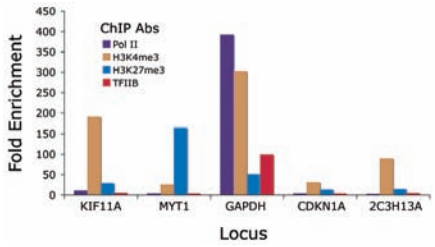

Figure 2: Quantitative PCR performed on ChIP DNA. Quantitative PCR was performed using DNA purified with the Chromatin IP DNA Purification Kit after ChIP using the indicated antibodies (ChIP Abs). Chromatin IP experiments were performed using the ChIP-IT Express Kit (Catalog No. 53008) and Ready-to-ChIP HeLa Chromatin (Catalog No. 53015, 7.5 x 10^5 cell equivalents per ChIP). Quantitative PCR was carried out using primers specific for the indicated gene (Locus). The normalized data represent fold enrichment over ChIP experiments carried out with control IgG.

Chromatin IP DNA Purification Kit advantages

- Optimized for use with epigenetics applications
- Compatible with all of Active Motif's ChIP-IT Kits, as well as with kits from other manufacturers
- Purify DNA ChIP'd using agarose or paramagnetic bead methods
- Convenient pH indicator to ensure proper binding of your samples
- Can also be used to purify DNA from our hMeDIP, MethylCollector and UnMethylCollector Kits

To find out more about our new Chromatin IP DNA Purification Kit, please visit us at www.activemotif.com/dnapure.

Product	Format	Catalog No.
Chromatin IP DNA Purification Kit	50 rxns	58002

NEW: Active Motif Now Offers Genome-wide Analysis Services for Epigenetics

As part of its recent acquisition of Genpathway, genome-wide analysis services are now offered by Active Motif. This is the perfect combination of products and know-how. The leader in kits and reagents for the study of chromatin biology and epigenetics now offers services and expertise to facilitate your research into transcription factor distribution and function, histone modifications and DNA methylation.

- **FactorPath**™ – discovery, identification and quantitation of transcription factor and cofactor binding sites across the genome
- **TranscriptionPath**™ – discovery and identification of actively transcribed genes at the DNA level to detect genome-wide changes in gene expression within minutes of treatment
- **MethylPath**™ – discovery, identification and quantitation of methylated DNA regions; also includes our new 5-hmC MeDIP-Seq and bisulfite sequencing services
- **HistonePath**™ – map histone modifications and/or enzymes that regulate histone modifications across the genome
- **Bisulfite Sequencing** – determine the locations and methylation status of cytosines

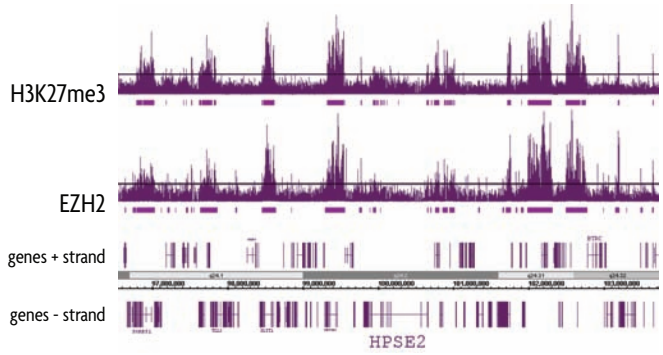

Figure 1: ChIP-Seq data for histone H3 lysine 27 methylation and EZH2.
Chromatin IP was performed using antibodies specific for H3K27me3 and EZH2, the H3K27 methyltransferase, followed by high-throughput sequencing of the ChIP DNA. The data are presented in an 8 million base pair window and shows nearly identical genomic localization for histone H3 lysine 27 methylation and the enzyme responsible for depositing the mark. Shown is a region of chromosome 10q surrounding the HPSE2 gene.

In addition to the services listed to the left, we also offer customized assay development and specific services to optimize ChIP-based procedures, including antibody qualification and data analysis. To learn more about our genome-wide analysis services for chromatin biology, transcription, histones and DNA methylation, please visit our website at www.activemotif.com/services or email us at services@activemotif.com.

NEW: 5-Hydroxymethylcytosine MeDIP-Seq and MeDIP-chip Services

Due to the importance of DNA methylation in biology and disease, Active Motif now offers 5-hydroxymethylcytosine MeDIP-Seq and MeDIP-chip as custom services. Just provide us with frozen biological samples or purified DNA and we will perform the hMeDIP-Seq or hMeDIP-chip experiments, then provide you with, and help you interpret, the data.

Our hMeDIP genome-wide analysis services allow you to study 5-hmC methylation on a genome-wide scale utilizing Active Motif's expertise and research tools without having to be an expert in the techniques yourself. The advantage Active Motif has over other companies is that our proprietary monoclonal antibody recognizing 5-hydroxymethylcytosine methylation does not require that the DNA be made single-stranded before immunoprecipitation. The ability to use double-stranded DNA eliminates linker bias in the downstream processing steps prior to Next-Gen sequencing.

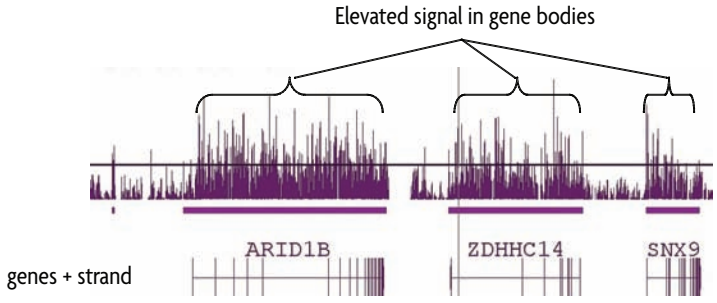

Figure 1: 5-hMeDIP-chip performed on human brain DNA.
Human brain DNA (2 µg) was immunoprecipitated with 10 µg of 5-Hydroxymethylcytidine antibody (Clone 59.1, Catalog No. 39999). Following hMeDIP, the DNA was amplified, labeled and hybridized to an Affymetrix Human Tiling 2.0R Array. Shown is a region 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.

Study Transcriptional Regulation with ChIP-validated Transcription Factor Abs

Gene expression depends on the recognition of specific promoter sequences by transcriptional regulatory proteins, which in turn recruit other proteins, such as RNA polymerase, in order to effect transcription. In the human genome, there are at least 2,600 genes that encode proteins involved in transcriptional regulation. These fall into three main categories: sequence specific DNA binding transcription factors (proteins that directly bind specific DNA regulatory sequences), general transcription factors (those associated with RNA polymerase holoenzyme complex) and transcriptional co-regulators (proteins recruited by the DNA binding factors).

Because one of the most important techniques used to study the function and genomic localization of these proteins is chromatin immunoprecipitation (ChIP), Active Motif offers an extensive line of ChIP-validated antibodies to important regulatory factors (Figure 1).

As part of its acquisition of Genpathway, Active Motif now offers FactorPath™ genome-wide transcription factor binding identification and analysis as a custom service. See page 9 for more details, or visit us at www.activemotif.com/factorpath.

To find out more about our ChIP-validated antibodies to transcriptional regulators, please call or visit us at www.activemotif.com/tfchipabs.

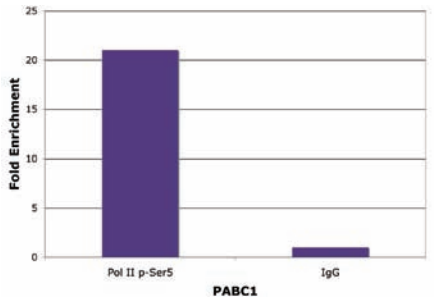

Figure 1: Quantitative PCR performed on RNA pol II CTD phospho Ser5 antibody ChIP'd DNA
Chromatin IP was performed using the ChIP-IT™ Express Kit (Catalog No. 53008) and HeLa chromatin (1.5 x 10^6 cell equivalents per ChIP) with 10 µg RNA pol II CTD phospho Ser5 antibody (Catalog No. 39749) or the equivalent amount of rabbit IgG as a negative control. Real time, quantitative PCR (RT-qPCR) was performed on DNA purified from each of the ChIP reactions using a primer pair specific for the PABPC1 gene. Data are presented as fold enrichment of the ChIP antibody signal versus the negative control IgG (arbitrarily assigned a value of 1) using the ddCT method.

Explore Chromatin Biology and Epigenetics using Antibodies Validated for use in Immunofluorescence

Visualizing a protein in its native cellular environment using immunofluorescence microscopy requires the use of very high-quality antibodies. They must be specific, of high titer and able to recognize native (non-denatured) protein. To assist you in your study of chromatin biology, epigenetics and transcription, Active Motif offers antibodies validated for use in immunofluorescence to a wide range of protein targets including:

- Chromatin-modifying proteins
- Histones and histone modifications
- Transcription factors
- Nuclear receptors
- Regulators of cell structure and function

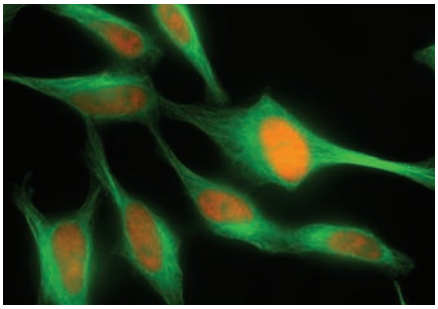

Figure 1: HDAC2 visualized by immunofluorescence.
HeLa cells were stained with HDAC2 monoclonal antibody (Clone 3F3, Catalog No. 39533) at a 1:1000 dilution. Red: HDAC2 mAb (Clone 3F3) Green: alpha-Tubulin mouse monoclonal (Clone 5-B-1-2, Catalog No. 39527) conjugated to Chromeo™ 488.

Active Motif offers a wide range of primary antibodies validated for use in immunofluorescence. For more information, visit www.activemotif.com/ifabs.

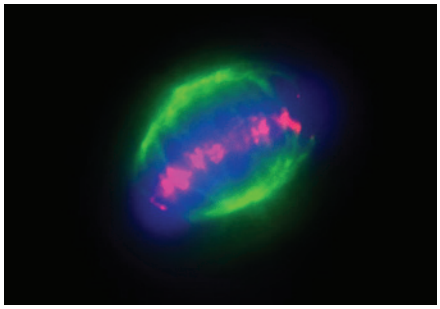

Figure 2: Aurora B visualized by immunofluorescence.
HeLa cells were stained with Aurora B polyclonal antibody (Catalog No. 39261) at a dilution of 1:200 and alpha-Tubulin monoclonal antibody (Clone 5-B-1-2, Catalog No. 39527) used at a dilution of 1:500. Red: Aurora B. Green: alpha-Tubulin. Blue: DAPI.

To find out more about our high-quality fluorescent secondary antibodies, visit www.activemotif.com/secondary.

Reagents for High-quality Fluorescence Microscopy

To produce superior results with our primary antibodies in immunofluorescence (IF) experiments, Active Motif has developed a variety of high-quality reagents including Chromeo™ secondary antibody conjugates and MAX Stain™ Immunofluorescence Tools. These optimized reagents can be used in a variety of fluorescence microscopy applications, even in novel high-resolution fluorescence microscopy (*e.g.* **ST**imulated **E**mission **D**epletion, or STED). We use these reagents in our daily work to develop novel assays and to evaluate our antibodies in a consistent and reliable manner.

Fluorescent secondary antibodies

Active Motif offers goat anti-mouse and goat anti-rabbit secondary antibodies that are conjugated to a number of high-quality dyes, including Chromeo™ fluorescent dyes (Table 1). Our optimized conjugation method, coupled with subsequent purification of the conjugate, makes our fluorescent secondaries brighter than other commercially available conjugates and lowers the background in many applications. Active Motif's antibody conjugates have been tested in applications such as flow cytometry and widefield, confocal and high-resolution fluorescence microscopy.

Fluorescent Dye	Absorption	Emission	Replaces
Chromeo™ 488	498 nm	524 nm	FITC, Alexa 488*
Chromeo™ 494	489 nm	624 nm	unique
Chromeo™ 505	514 nm	530 nm	Oregon Green derivatives
Chromeo™ 546	550 nm	567 nm	Cy3, Alexa 546*
Chromeo™ 642	647 nm	666 nm	Cy5, Alexa 647*

Table 1: Fluorescent properties of dyes when conjugated to secondary antibodies.

* Alexa Fluor dyes are a registered trademark of Life Technologies™.

MAX Stain™ Immunofluorescence Tools

MAX Stain Immunofluorescence Tools eliminate the challenge of getting consistent high-quality fluorescent images by providing a complete set of optimized reagents. The MAXblock and MAXwash reagents use non-mammalian agents to effectively block non-specific antibody binding. The MAXpack Immunostaining Media Kit includes one each of the MAXblock, MAXbind and MAXwash reagents.

- **MAXblock™ Blocking Medium** – effectively blocks non-specific antibody binding without reducing signal intensity or specificity
- **MAXbind™ Staining Medium** – increases the antibody binding specificity and intensity of your IF experiments
- **MAXwash™ Washing Medium** – eliminates non-specific binding of primary and secondary antibodies

Product	Format	Catalog No.
MAXblock™ Blocking Medium	150 ml	15252
MAXbind™ Staining Medium	250 ml	15253
MAXwash™ Washing Medium	1000 ml	15254
MAXpack™ Immunostaining Media Kit	1 kit	15251

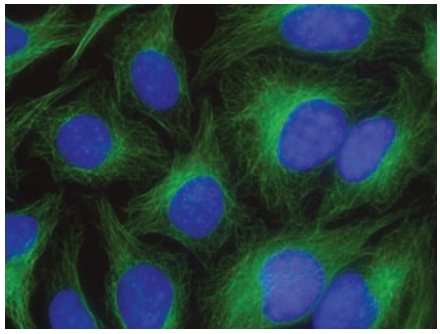

Figure 1: Chromeo fluorescent secondaries and MAX Stain Tools provide bright, high-quality images. HeLa cells were stained with alpha Tubulin mouse mAb (Catalog No. 39527) and Chromeo 488 Goat anti-mouse IgG (Catalog No. 15031). The nuclei have been counterstained with DAPI. In all steps of slide preparation, MAX Stain Immunofluorescence Tools have been used.

Why use Chromeo™ conjugated secondary antibodies?

- **Brightness** – high fluorescent intensity improves sensitivity
- **Limited photobleaching** – enables multiple exposures and increased exposure times
- **Flexibility** – conjugates work under multiple fixation conditions
- **Specificity** – low fluorescent background

Fluorescent Chromeo™ Dyes

In addition to the secondary antibody conjugates, Chromeo™ Dyes are also available as NHS-Esters, Carboxylic Acids, Azides and Alkynes for Click-Chemistry. Biotin and Streptavidin conjugates are also available. To see a complete list of available fluorescent dyes, please visit us at www.activemotif.com/dyes.

ACTIVE MOTIF®

Tools to Analyze Nuclear Function

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
ChIP kits and ChIP-validated antibodies

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, 5-hmC & 5-mC antibodies