ACTIVE MOTIF®

MOTIFvations

The Newsletter of Active Motif October 2012 Volume 13 Number 2

Ribbon diagram of the nucleosome core particle structure (H2A.Z nucleosome, pdb entry 1F66) viewed down the superhelical axis (left) and rotated 90° (right). Original figure prepared by Dr. Karolin Luger, Department of Biochemistry and Molecular Biology, Colorado State Univ.

IN THIS ISSUE

Antibodies, Kits and Services for Epigenetic Research

NEW: ChIP-IT® High Sensitivity Kit for Difficult-to-ChIP Transcription Factors

As the field of epigenetics expands into the research areas of transcriptional regulation and stem cells, it has become more important to identify methods to obtain accurate and reliable chromatin immunoprecipitation (ChIP) results for difficult-to-ChIP transcription factors and limited cell numbers. Active Motif's new ChIP-IT® High Sensitivity Kit is designed to meet this need by enabling you to enrich for high-quality ChIP DNA even when working with low abundance transcription factors, low-affinity antibodies or limited starting material. Not only is the ChIP-IT High Sensitivity Kit capable of enriching DNA from as little as 1,000 cells, but the kit has been successfully ChIP-Seq validated with hundreds of samples and target antibodies.

Why another ChIP Kit?

Active Motif has decided to expand its ChIP-IT product offering to meet the needs of those researchers studying low abundance transcription factors, low affinity antibodies or using limited sample material. The ChIP-IT High Sensitivity Kit is specifically designed to give higher signal and reduced background, ensuring that low abundance binding events are detectable in downstream applications such as ChIP-Seq, ChIP-chip and qPCR.

ChIP-IT High Sensitivity uses specially formulated buffers for chromatin preparation from cultured cells or fresh or frozen tissue. Low background protein G agarose beads and an antibody blocker are used to minimize any non-specific binding during the immunoprecipitation reaction, enabling a higher degree of sensitivity than other commercially available ChIP Kits (Figure 1). Filtration columns provide a fast, easy and consistent solution for wash steps; DNA purification reagents are included to complete the workflow. Recommendations are included for the use of the high-quality ChIP DNA in qPCR and ChIP-Seq.

Works with limited sample material

Due to the high sensitivity of this ChIP kit, immunoprecipitation reactions can be performed from as little as 1,000 cells for highly abundant target proteins, such as histones, and as little as 50,000 cells for low abundance proteins (Figure 2).

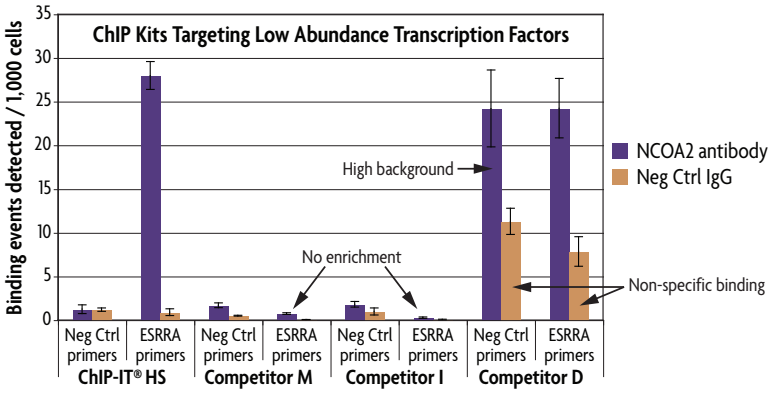

Figure 1: The ChIP-IT High Sensitivity Kit detects low abundance target proteins better than the competition. Chromatin was prepared according to the recommendations for each manufacturer's ChIP kit from 1.5 x 10^6 MCF-7 cells per assay. ChIP was performed with the optimal amount of chromatin suggested in each manufacturer's protocol using an antibody for the low abundance nuclear co-activator 2 (NCOA2) protein and a negative control IgG. Following enrichment, qPCR was performed using the ChIP-IT qPCR Analysis Kit (Catalog No. 53029, see page 3) to normalize the data for chromatin amounts and ChIP volume, as this enables a direct comparison of the competitor kit results. While NCOA2 is considered a difficult antibody for ChIP, NCOA2 binding was detected at the estrogen-related receptor alpha (ESRRA) promoter when ChIP was performed using ChIP-IT High Sensitivity; the enrichment was approximately 20-fold higher than the negative control primer set and the IgG. When using competitor kits, NCOA2 was either not enriched at all (Competitors M & I), or there was non-specific binding by the IgG and high background for the negative control primers (Competitor D). Data represents triplicate qPCR values and is expressed as binding events detected per 1,000 cells.

The ChIP-IT High Sensitivity advantage

- **Sensitivity** – optimized to obtain high signals and low background for the detection of low abundance proteins or when using antibodies with low binding affinities
- **Low Cell ChIP** – enrichment from as little as 1,000 cells per immunoprecipitation reaction
- **Ease of Use** – filtration columns make the wash steps quick and easy
- **Consistency** – extensive testing with multiple sample types and antibodies has validated the kit's performance in ChIP-Seq and qPCR

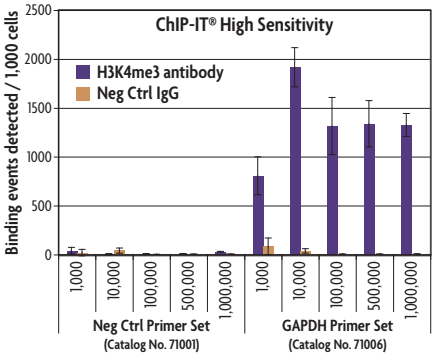

Figure 2: ChIP-IT High Sensitivity IP range of detection. MCF-7 chromatin was prepared according the ChIP-IT High Sensitivity manual using between 100,000 and 4 million cells. ChIP was performed using the number of cells indicated with Histone H3K4me3 antibody and IgG. Specific enrichment is detected in as few as 1,000 cells.

Product	Format	Catalog No.
ChIP-IT® High Sensitivity	16 rxns	53040

NEW: qPCR Analysis Strategy to Normalize ChIP Data for Easier Interpretation

Traditional methods to analyze ChIP qPCR results usually rely on fold enrichment of ChIP DNA over the enriched DNA from an IgG or non-specific antibody immunoprecipitation reaction. One disadvantage of this method is that the IgG reaction is performed separately, so any enrichment seen in the IgG reaction may not be directly relevant to what occurred in the ChIP reaction of interest. Another disadvantage is that this method also requires generating a standard curve for every primer set tested so as to determine the efficiency of each PCR primer pair. Finally, if different amounts of chromatin were used in the ChIP reactions, interpretation of results between samples or experiments is extremely difficult. To overcome these problems and simplify ChIP qPCR data analysis, Active Motif has developed the ChIP-IT® qPCR Analysis Kit and its associated Excel-based ChIP-IT® qPCR Analysis spreadsheet.

How does it work?

The ChIP-IT qPCR Analysis Kit contains known DNA standards to create a single standard curve per 96-well PCR plate; this curve is used to determine ChIP DNA quantity and to assess the efficiency of multiple PCR primer sets. ChIP DNA is tested using a gene-specific primer of interest and the provided negative control primer set. The negative control primer serves as a measure of background in the actual ChIP reaction of interest, and is an indicator of the quality of the ChIP DNA.

Following qPCR amplification, the values determined by the qPCR instrument are copied into the ChIP-IT qPCR Analysis spreadsheet. The spreadsheet performs calculations to normalize the data with respect to primer pair efficiency, the amount of chromatin in each reaction and the resuspension volume of the ChIP DNA, then generates a graph of the results. The spreadsheet template is designed to accommodate data from multiple 96-well PCR plates into a single analysis, making it easier to compare multiple sample types and experiments (see Figure 1 on page 2).

The ChIP-IT qPCR Analysis Kit includes DNA standards, a standard curve primer pair, as well as control qPCR primers for use with human and mouse samples. The kit contains sufficient reagents to perform ten qPCR standard curves.

Interpreting the ChIP DNA quality

Recommendations for data interpretation are provided to evaluate the success of the ChIP reactions and the quality of the ChIP DNA. Following these recommendations will provide confidence that the ChIP DNA is of a high quality before proceeding with expensive downstream applications such as ChIP-Seq.

The recommendations are based on the use of the ChIP-IT High Sensitivity Kit (Catalog No. 53040) to perform the chromatin immunoprecipitation reactions. If another method is used to perform ChIP that results in higher background levels and lower sensitivity, the recommended threshold levels may not apply.

Data from the ChIP-IT qPCR Analysis Kit is presented as binding events detected per 1,000 cells. To see published data using this qPCR normalization strategy, or to download the manual for an explanation of the calculations, please visit us at www.activemotif.com/qPCRanalysis.

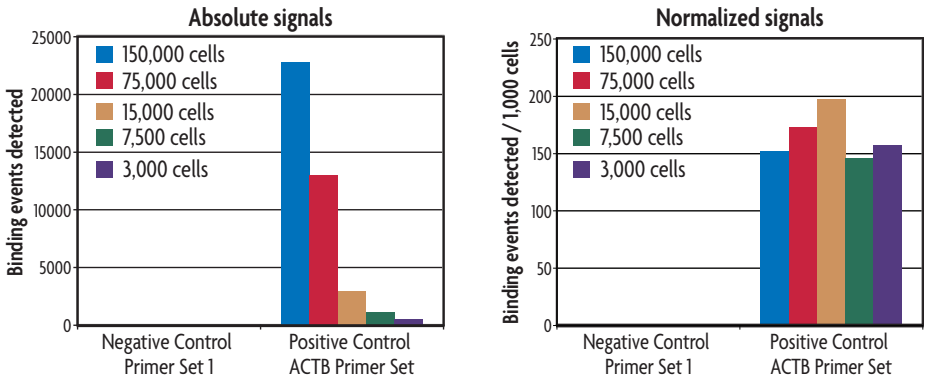

Figure 1: The ChIP-IT qPCR Analysis Kit enables direct comparison of ChIP efficiency.
ChIP was performed using an RNA pol II antibody with the ChIP-IT High Sensitivity Kit. Different amounts of chromatin were used in each ChIP reaction ranging from 3,000 cell equivalents to 150,000 cell equivalents. In the left graph, normalization was performed independent of the starting amount of chromatin, taking only the primer efficiency and resuspension volume into account. In the right graph, Active Motif's ChIP-IT qPCR Analysis Kit was used to also normalize for the starting amount of chromatin. This data demonstrates the dynamic range of the ChIP-IT High Sensitivity Kit and the accuracy of the normalization strategy in the ChIP-IT qPCR Analysis Kit.

Product	Format	Catalog No.
ChIP-IT® qPCR Analysis Kit	10 rxns	53029

NEW: Enhanced Gene Regulation Analysis with NOMe-Seq

Active Motif's NOMe-Seq assay is the first commercially available kit to simultaneously interrogate the relationship between nucleosome occupancy, transcription factor binding and DNA methylation within the same DNA molecule. It provides high-resolution mapping of nucleosome occupancy that enables even subtle changes in nucleosome position to be determined. By studying nucleosome occupancy in combination with DNA methylation at a specific gene locus, NOMe-Seq provides researchers with a tool to better understand the different states of chromatin and their effects on gene regulation. To learn more, visit us at www.activemotif.com/nome-seq.

What is NOMe-Seq?

NOMe-Seq stands for Nucleosome Occupancy and Methylome Sequencing. It is a technique that was developed by the Peter A. Jones lab at USC to simultaneously study nucleosome occupancy and CpG methylation on the same DNA strand. Traditional methods have only been able to look individually at DNA methylation (bisulfite sequencing) or at nucleosome positioning (MNase-seq or FAIRE-seq) without the ability to understand the relationship between the two.

In NOMe-Seq, fixed chromatin is treated with a GpC DNA methyltransferase enzyme to artificially methylate any GpC dinucleotides that are not protected by the binding of nucleosomes or proteins to the DNA. Because GpC dinucleotides are significantly more abundant within the genome than CpG dinucleotides, their use allows for better resolution of nucleosome positioning. Additionally, as GpCs are not endogenously methylated in mammalian genomes their artificial methylation does not interfere with the ability to establish an endogenous CpG methylation profile.

Following GpC methylation, the artificially methylated DNA is denatured and treated with sodium bisulfite. This converts unmethylated cytosines to uracil, while methylated cytosines remain unchanged. Gene-specific regions of interest are then PCR amplified, cloned and the DNA is sequenced (Figure 1).

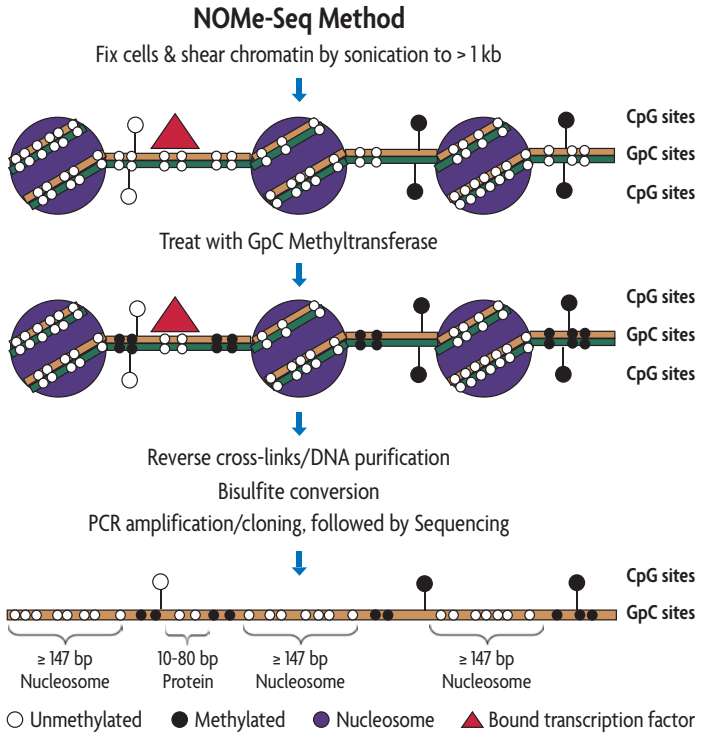

Figure 1: The NOMe-Seq method.
NOMe-Seq works by formaldehyde fixation of cells to preserve nucleosome position as well as protein/DNA binding interactions. Fragmented chromatin is enzymatically treated with a GpC methyltransferase to artificially methylate GpC residues not protected by bound nucleosomes or proteins. Following reversal of cross-links and DNA purification, bisulfite conversion is performed to identify the methylated cytosines on each DNA strand. For gene-specific analysis, the regions of interest are PCR amplified, cloned and sequenced. The GpC methylation profile will determine the nucleosome or protein binding site positions, while the CpG methylation reveals the endogenous methylation pattern of the gene of interest.

The sequence results are then mapped to compare the artificially methylated GpC and the endogenously methylated CpG residues. Regions of unmethylated GpC residues that are 147 bp or larger represent nucleosome occupancy, which prevented GpC methylation by the enzyme. Unmethylated regions of 10-80 bp indicate sites of transcription factor binding. By combining this with the sequence data of endogenous CpG methylation, one can correlate simultaneous nucleosome and transcription factor occupancy with DNA methylation all within the same DNA molecule. NOMe-Seq can also be used in whole-genome experiments, as demonstrated in a recent publication.

Product	Format	Catalog No.
NOMe-Seq	10 rxns	54000

NEW: MethylCollector™ Ultra-Seq is Now Available as a Service

Active Motif's popular MethylCollector Ultra Kit, which is used to specifically enrich for methylated DNA from fragmented genomic DNA, is now offered as an end-to-end, full service assay by Active Motif's Epigenetic Services. Customers can submit their cells or DNA and receive fully analyzed genome-wide methylation data in just weeks.

MethylCollector Ultra, based on the patented MIRA (Methylated CpG Island Recovery Assay) technology, offers the fastest, most efficient methylated DNA enrichment method on the market. Enrichment is achieved from as little as 1 µg of DNA using a purified methylated DNA binding complex of MBD2b and MBD3L1. This is followed by magnetic capture of double-stranded DNA that is compatible with Next-Gen Sequencing library preparation protocols.

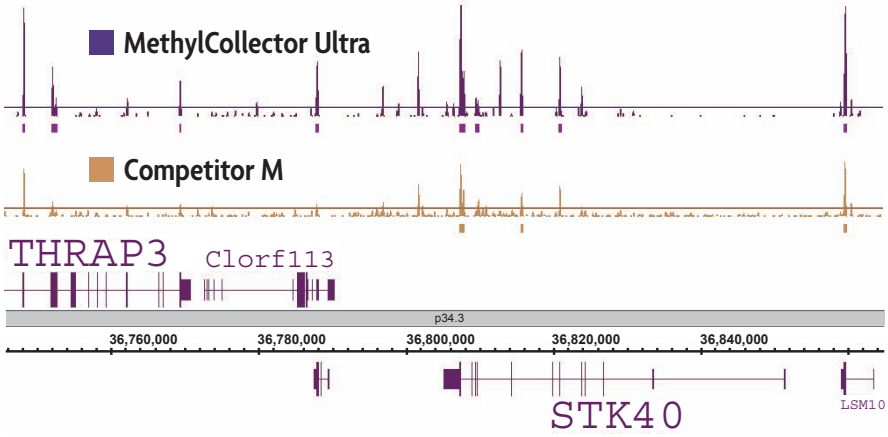

Figure 1: MethylCollector Ultra is more sensitive than other MBD-based enrichment methods.
To ensure that we provide scientists with the best possible results, the performance of MethylCollector Ultra was compared to that of an alternative MBD-Capture kit. Methylated DNA was isolated from 1 µg of human PBMC DNA using both enrichment kits, then the enriched DNA was sequenced using the Illumina HiSeq platform. Regions of enrichment from a 1.2 Mb region of the genome are shown above. The MethylCollector Ultra technology detected more methylated regions and the peaks were of greater intensity than those detected by the competitor's kit.

The New MIRA-Seq Service includes

- DNA isolation from cells or tissues
- Methylated DNA enrichment using MethylCollector Ultra
- qPCR analysis of positive and negative control sites
- Next-Gen Library generation
- Sequencing of at least 30 million tags using the Illumina HiSeq platform
- Analysis: mapping, peak calling, visualization files and Excel output

For more complete details on this and other services offered, please visit us at www.activemotif.com/services.

NEW: Targeted Next-Gen Bisulfite Sequencing Now Offered as a Service

Identify differentially methylated regions of DNA

The identification of differential methylation across multiple samples is an important strategy for determining the underlying mechanisms of development and disease. However, while a complete understanding of these mechanisms may require base pair resolution of DNA methylation, the current costs for whole genome bisulfite sequencing remain prohibitive. A great alternative to whole genome bisulfite sequencing is to use MethylCollector Ultra to identify regions of differential methylation followed by targeted bisulfite sequencing of such identified regions in a larger population of samples. Active Motif's Targeted Next-Gen Bisulfite Sequencing Service combines many bisulfite-converted amplicons from multiple samples into a single Next-Gen sequencing run. It is ideal for use as a follow-up assay to MethylCollector Ultra, providing single base pair resolution of DNA methylation across many cell populations.

Active Motif Epigenetic Services

- **Experience** – over 1500 genome-wide data sets generated
- **Quality** – QC steps ensure high quality whole-genome data
- **Support** – All services include bioinformatics analysis

For more complete details, please visit www.activemotif.com/services.

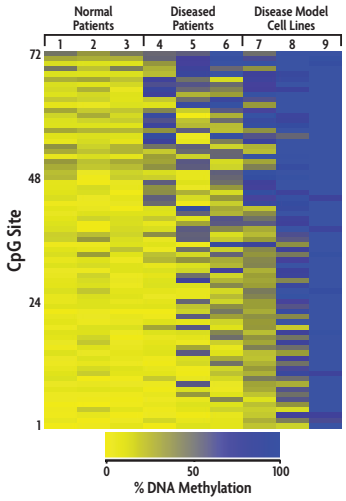

Figure 1: Targeted Next-Gen Bisulfite Sequencing.
MethylCollector Ultra-Seq was performed on 3 samples of interest to identify differentially methylated regions. One identified region was used for Targeted Next-Gen Bisulfite Sequencing on a broader population of 9 samples. This heat map shows the single base pair resolution of the 72 CpGs in this region across the 9 samples.

NEW: Multiplex Transcription Factor Assays for Use with Luminex® Instruments

Active Motif is pleased to announce the release of its new Transcription Factor Multiplex Assays to study NFκB and AP-1 transcription factor binding events using either the Luminex® 200™ or MAGPIX® instruments. The assays work as pull-down reactions of biotinylated DNA probe-transcription factor complexes with transcription factor-specific antibodies conjugated to fluorescently labeled magnetic beads. Because each antibody-conjugated bead contains a different fluorescent label, the assay can be multiplexed to study the entire transcription factor family within a single well of the assay. Alternatively, individual family members can be assayed on their own as a singleplex.

Bead-based transcription factor assays

The Transcription Factor Multiplex assays work as pull-down reactions where cell lysates are combined with a biotinylated double-stranded consensus binding sequence for the transcription factor family of interest. Fluorescently labeled magnetic beads, which have been conjugated to antibodies that target the individual transcription factor family members, are used to capture the transcription factor-bound DNA. Streptavidin-phycoerythrin is used to bind the biotinylated oligo and determine the magnitude of signal (Figure 1).

Because each antibody-conjugated bead contains a different fluorescent label, antibodies for multiple family members can be added to the same sample for multiplexing. The readout from the streptavidin-phycoerythrin is proportional to the amount of transcription factor binding. The data can be used to compare transcription factor binding activity among the various family members.

The Luminex® Principle

The Luminex Principle is based on the use of tiny, 6.4 micron superparamagnetic beads, each containing their own distinct dye ratio. Inside the Luminex 200™ instrument, the beads are individually passed through a small shaft where a light source excites the internal dye that is used to identify each bead. Based on the dye ratios, each bead will exhibit a unique fluorescence that can be identified with a unique bead number. A second wavelength is used to determine the magnitude of the streptavidin-phycoerythrin signal (Figure 2). A minimum of 100 beads are read for each well of the assay. The Luminex xPONENT software program will provide a real-time readout of signal as median fluorescent intensity (MFI) (Figure 3, page 7).

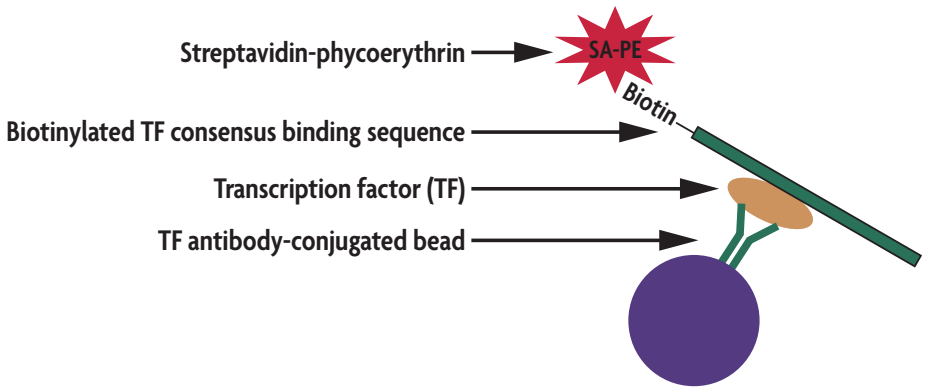

Figure 1: Transcription Factor Multiplex Assays are bead-based assays to measure TF-DNA binding activity.

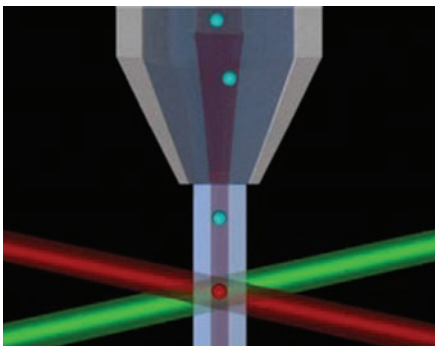

Figure 2: The Luminex 200™ instrument. The Luminex 200 instrument uses two distinct wavelengths to analyze individual beads as they pass through the machine for bead identity and phycoerythrin signal.

What's in the box?

The Transcription Factor Multiplex assays include a 96-well assay plate, a biotinylated oligonucleotide containing the consensus binding sequence for the transcription factor family of interest, and wild-type and mutant competitor oligos to confirm the specificity of the reactions. Streptavidin-phycoerythrin, assay buffers and a positive control cell lysate are also included.

The transcription factor antibody-conjugated beads are purchased separately. This allows the assay to be customized to fit the individual needs of each researcher. All antibody-conjugated beads have been confirmed to work when multiplexed without any negative effects on the assay results. Simply select the bead sets of interest and the Transcription Factor Multiplex Buffer Kit and you will have all the reagents needed to perform the assay.

Transcription Factor AP-1

AP-1 is a transcription factor family consisting of a mixture of homodimeric and heterodimeric protein complexes derived from the Fos and Jun families. The AP-1 proteins play a role in the expression of genes involved in regulation of differentiation, proliferation and apoptosis. AP-1 expression is induced by multiple stimuli, including the presence of serum, growth factors, phorbol esters and oncogenes. Phosphorylation of AP-1 family members by kinases is required for transactivation activity. Once the AP-1 family members have translocated into the nucleus they are able to bind DNA. Active Motif offers antibody-conjugated beads for each of the following AP-1 family members: c-Fos, FosB, Fra-1, JunB and JunD.

Transcription Factor NFκB

NFκB is a widely studied transcription factor family due to its implications in the regulation of genes that control inflammation, cell proliferation and cell survival. The NFκB family is comprised of a mixture of homodimeric and heterodimeric subunits that are members of the structurally related Rel family of transcription factors. NFκB p65 (RelA), RelB and c-Rel contain a transactivation domain that is required for translocation of active NFκB complexes into the nucleus where they are able to bind DNA. The p50 and p52 subunits do not contain the transactivation domain and must form heterodimers. Active Motif currently offers antibody-conjugated beads for NFκB RelB and NFκB p52 subunits, with other NFκB family members to become available soon.

TF Multiplex advantages

- Reduction in both costs & labor
- Simultaneously analyze different subunits of the same transcription factor family in the same sample
- Individual bead sets enable the assay to be customized

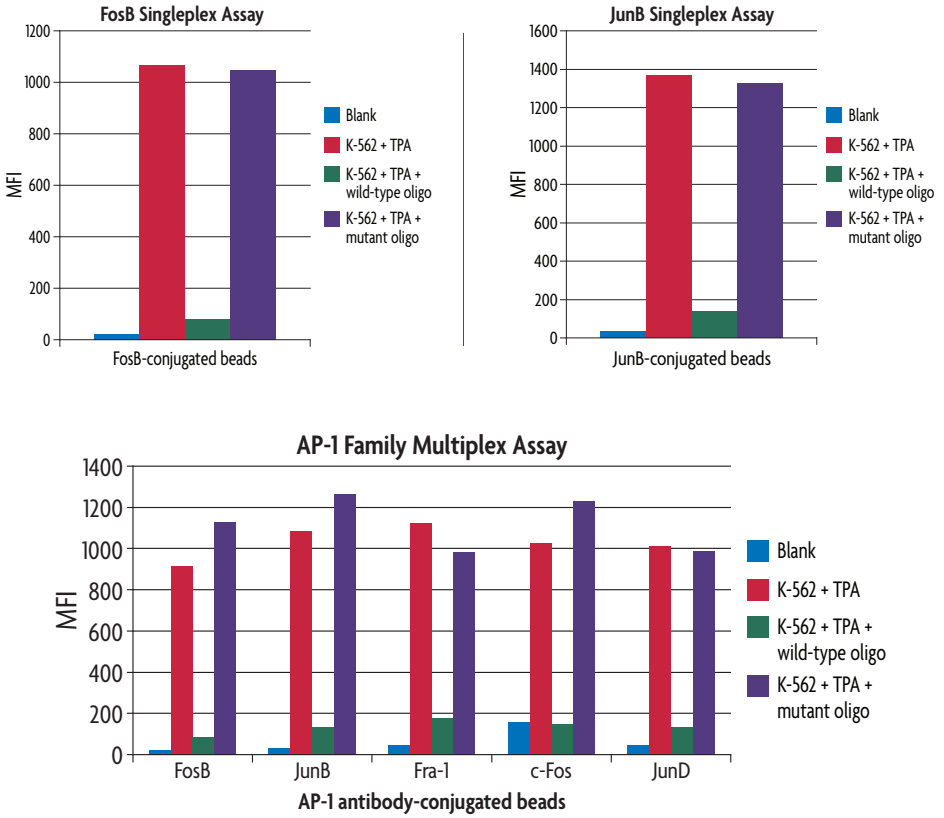

Figure 3: Transcription factor AP-1 studied as singleplex or multiplex assay.
The Transcription Factor Multiplex Kit – AP-1 was used to study the DNA binding activity of various members of the AP-1 transcription factor family. The top images show singleplex data for FosB and JunB assays run individually. The positive control lysate, K-562 nuclear extract treated with TPA, is tested in the absence or presence of the provided competitor oligos. The wild-type oligo shares the same consensus sequence as the biotinylated consensus oligonucleotide. Therefore, it competes for binding with the transcription factor, inhibiting the ability of the transcription factor to bind the biotinylated consensus oligonucleotide. The mutant oligonucleotide has a modified consensus sequence that is not recognized by the transcription factor and therefore does not compete for transcription factor binding. The use of the wild-type and mutant oligos within the assay confirm the specificity of the assay. The bottom image shows a multiplex assay in which multiple transcription factor antibody-conjugated beads were combined into a single well of the assay to study the transcription factor activity of the various AP-1 family members within the same sample. A comparison of the top and bottom images shows that multiplexing does not have any negative effects on the assay results.

To learn more about the new Transcription Factor Multiplex Assays for use with Luminex instruments, please call or visit us at www.activemotif.com/luminex.

Product	Format	Catalog No.
Transcription Factor Multiplex Kit – AP-1	100 rxns	33100
c-Fos-conjugated beads	48 rxns	33101
FosB-conjugated beads	48 rxns	33102
Fra-1-conjugated beads	48 rxns	33103
JunB-conjugated beads	48 rxns	33104
JunD-conjugated beads	48 rxns	33105
Transcription Factor Multiplex Kit – NFκB	100 rxns	33110
RelB-conjugated beads	48 rxns	33111
NFκB p52-conjugated beads	48 rxns	33112

Arrays, Antibodies and Kits for the Study of Histones and their Modifications

Histone modifications such as acetylation, phosphorylation and methylation at specific amino acid residues on the histone globular domain and the N-terminal tails have been shown to influence and regulate transcription, chromosome packaging and DNA damage repair. Due to the importance of histone modifications in regulating chromatin structure and disease, Active Motif has developed a variety of products to simplify histone analysis including MODified™ Histone Peptide Arrays and a large number of ELISAs, kits, antibodies and proteins (page 9).

Unique array to screen interactions

The MODified™ Histone Peptide Array* can be used to screen antibodies, proteins and enzymes for interactions with histones and their post-translational modifications. Each array contains 384 different combinations of acetylation, phosphorylation, methylation and citrullination modifications on the N-terminal tails of histones H2A, H2B, H3 and H4. Because each peptide can contain up to 4 different modifications, the array can be used to study not only individual modifications, but also to determine if neighboring modifications alter site recognition and binding.

Verify antibody specificity first

MODified Arrays are ideal to screen histone modification antibodies for non-specific cross-reactivity with alternate modifications. Using a simple protocol that works like a Western blot, an image of the array is captured and the antibody binding is quantified by the provided software (Figure 1). This valuable data lets you know if your antibody is specific or not before investing your resources using it in downstream assays.

Study proteins and enzymes that interact with histones

In addition to screening antibody specificity, MODified Arrays can be used to study proteins and enzymes that interact with and/or modify histones as well as their inhibitors. The activity of methyltransferases, demethylases, acetyltransferases, *etc.* can be assayed at specific histone modifications simply by incubating the compound(s) of interest on the array followed by detection with a specific antibody (Figure 2).

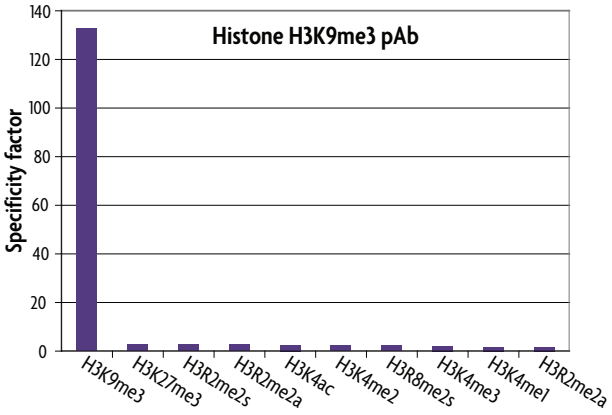

Figure 1: Peptide array analysis confirms the specificity of H3K9me3 pAb for its intended modification. Histone H3 trimethyl Lys9 antibody (Catalog No. 39161) was applied at a dilution of 1:2,000 to the MODified Histone Peptide Array and scanned with Array Analyse Software 15. The results plotted in the adjacent graph show specificity data for the ten most reactive peptides, demonstrating that this antibody is specific for H3K9me3, with little binding at other sites.

An entire collection of products for studying histone modifications

Active Motif also offers Histone Modification ELISAs for histone quantification, kits for purifying core histones, assembling chromatin *in vitro*, and for studying HATs, HDACs and demethylases. In addition, we offer over 160 highly specific histone modification antibodies that have been validated using the MODified Array and/or dot blots, as well as a large collection of recombinant histone proteins (page 9) and histone modifying enzymes. For complete details, please visit us at www.activemotif.com/histone.

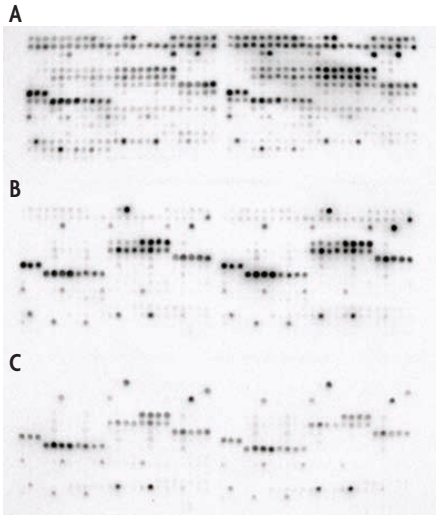

Figure 2: Detection of G9a histone methyltransferase activity using the MODified Histone Peptide Array. MODified Histone Peptide Arrays were treated with A) 25 µM G9a methyltransferase, B) 25 µM G9a mutant methyltransferase or C) a no enzyme control overnight in the presence of 1 mM AdoMet. The arrays 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.

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

*CelluSpots™ arrays are manufactured under license by INTAVIS Bioanalytical Instruments AG and sold through Active Motif as MODified™ Histone Peptide Arrays

Recombinant Proteins to Analyze Histone Post-translational Modifications

Understanding the implications of histone post-translational modifications as they relate to gene regulation and chromatin remodeling is critical to determining how these modifications affect or contribute to development and disease. Active Motif's expanded line of recombinant proteins for use in epigenetics research now includes biotinylated histones and recombinant bromodomain proteins to specifically meet the needs of researchers interested in the analysis of histone modifications. For complete details, visit us at www.activemotif.com/info to download our Histone Analysis Products brochure or request a copy of by mail.

Recombinant Histone Proteins to Analyze Site-specific Modifications

Recombinant histone proteins with site-specific modifications

To broaden your choice of histone analysis tools, Active Motif offers unmodified recombinant histones as well as recombinant histones that include site- and degree-specific methylation, acetylation, and phosphorylation modifications. Each recombinant protein is prepared using either our patented Expressed Protein Ligation (EPL) or Methylated Lysine Analog (MLA) technologies.

New biotinylated H3 proteins

To expand the versatility of our recombinant histones, Active Motif now offers biotinylated versions of unmodified Recombinant Histone H3 as well as Recombinant H3 with methyl modifications at Lys4 and Lys9. The biotinylated recombinant histones are engineered using the patented EPL technology that enables the incorporation of site-specific modifications to the histone tail while preserving its native structure. The modified tail is ligated to the histone globular domain using a method known as "natural chemical ligation" that preserves the native peptide bond. The recombinant histones are biotinylated at the N-terminus and, as a result of EPL synthesis, more closely mimic "natural" histones, making them ideally suited as controls for the analysis of histone modifications, as substrates for histone modification enzymes, and for use in *in vitro* chromatin assembly.

Product	Format	Catalog No.
Recombinant Histone H3 biotinylated	25 µg	31271
Recombinant Histone H3K4me1 biotinylated (EPL)	25 µg	31284
Recombinant Histone H3K4me2 biotinylated (EPL)	25 µg	31283
Recombinant Histone H3K4me3 biotinylated (EPL)	25 µg	31282
Recombinant Histone H3K9me1 biotinylated (EPL)	25 µg	31286
Recombinant Histone H3K9me3 biotinylated (EPL)	25 µg	31285

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

NEW: Recombinant Bromodomain Proteins to Study Bromodomain Function

What are bromodomains?

Active Motif has expanded its line of recombinant proteins to include 15 new recombinant bromodomain (BRD) proteins. BRDs function as "readers" of the histone code. They are the only protein domains capable of specifically recognizing and binding to acetylated lysine residues on histone tails. BRDs regulate gene expression and chromatin remodeling events by recruiting associated protein complexes to acetylated chromatin.

Why use recombinant bromodomains?

Aberrant acetylation has been implicated as the cause of various diseases, including neurological disorders, cancer and inflammation. Therefore, BRD-containing proteins have emerged alongside HATs and HDACs as therapeutic targets for drug development and discovery. To aid in studies of BRD function, Active Motif now offers recombinant bromodomain(s) of several common BRD-containing protein families, including HATs, transcriptional coactivators and BET family proteins, to name a few. These highly purified recombinant bromodomains are suitable for use in various applications, including studies of enzyme kinetics, inhibitor screenings and selectivity profiling.

For an up-to-date list of our available recombinant bromodomains and complete details, please visit us at www.activemotif.com/brd.

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 demanding high-resolution fluorescence microscopy (e.g. **STED** and **GSDIM**). We use these reagents in our labs to help us develop our novel assays and to evaluate our antibodies in a consistent and reliable manner.

Fluorescent secondary antibodies

Active Motif's goat anti-mouse and goat anti-rabbit secondary antibodies 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 validated for use in a variety of 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.
To see a complete list of fluorescent secondary antibody conjugates, please visit www.activemotif.com/fluorabs.

Better slide prep for the best IF images

MAX Stain™ Immunofluorescence Tools will help you to produce high-quality IF images every time by providing you with a complete set of optimized reagents for slide preparation. 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. For details, visit us at www.activemotif.com/maxstain.

Advantages of MAX Stain reagents

- **MAXblock™ Blocking Medium** – effectively blocks non-specific antibody binding without reducing signal intensity or specificity
- **MAXbind™ Staining Medium** – increases the specificity of antibody binding as well as the fluorescent 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

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

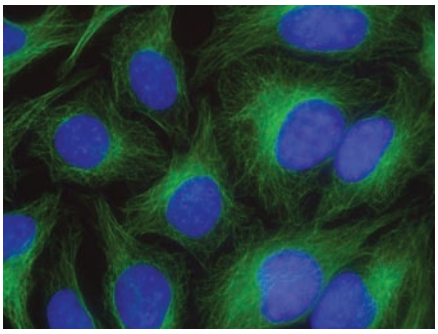

Figure 1: Chromeo fluorescent secondaries and MAX Stain Tools provide bright, high-quality images.
HeLa cells were stained with alpha Tubulin mouse mAb (Catalog No. 39527) and Chromeo 488 Goat anti-mouse IgG (Catalog No. 15031). The nuclei have been counter-stained with DAPI. In all steps of slide preparation,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

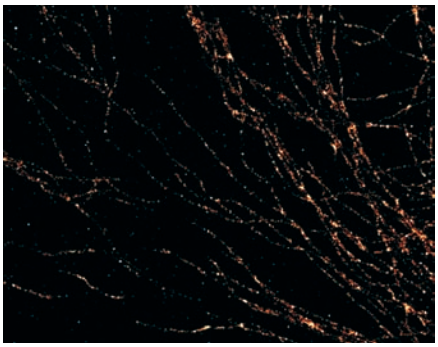

Figure 2: Chromeo fluorescent secondaries in super-resolution GSDIM microscopy.
Tubulin was stained with a primary monoclonal mouse antibody and with Chromeo 546 Goat anti-mouse IgG (Catalog No. 15033) secondary antibody. Image courtesy of Leica Microsystems, Germany.

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

NEW: Long-term Staining of Membranes in Live or Fixed Cells & Tissue

The Chromeo™ LT Live Cell Membrane Staining Kit* features a cell permeable fluorescent complex that selectively stains lipid structures like plasma and internal membranes with high specificity. Such fluorescent stains specifically intercalate into membranes so they can be used not only to label the different cellular compartments, but also as a versatile and powerful tool for long-term (LT) live cell imaging. The Chromeo LT Live Cell Membrane Staining Kit can be used to monitor cell number, cell proliferation and the effect on cell growth or death in response to treatment by drugs, activators, inhibitors or radiation. In addition, it can serve as a classical counter-stain in immunofluorescence experiments to visualize lipid structures in live or fixed cells as well as in FFPE tissue sections.

The Chromeo LT Live Cell Membrane Staining Kit utilizes a proprietary, water-soluble fluorescent complex that rapidly diffuses into live or fixed cells and tissue. The stain accumulates in lipid structures such as plasma and internal membranes, lysosomes or lipid bodies, allowing them to be specifically labeled and imaged with low background. Its affinity for and retention in lipid structures, and its stability towards photobleaching, make it possible to monitor cells over a period of several days.

Why use the Chromeo LT Live Cell Membrane Stain?

- Water-soluble fluorescent stain for live cells or Formalin-Fixed, Paraffin-Embedded (FFPE) cells and tissue
- Low toxicity
- Easy to use
- Excitable at 488 nm
- Use FITC filter sets
- Long-term labeling experiments

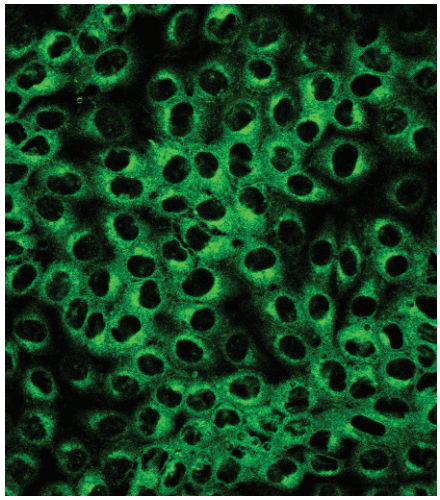

Figure 1: Staining of live NRK cells with the Chromeo LT Live Cell Membrane Stain.
NRK cells were plated in 3 cm cell culture dishes and washed once with pre-warmed PBS before adding 30 nM Chromeo LT Live Cell Membrane staining solution. After incubation for 10 minutes at 37°C, cells were washed once with pre-warmed PBS and imaged in PBS with a Nikon CLSM (60x objective).

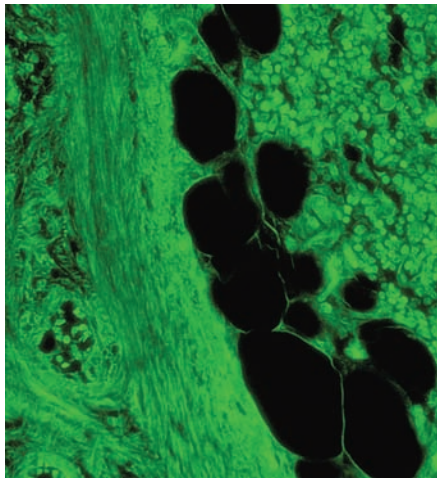

Figure 2: Staining of Formalin-Fixed Paraffin-Embedded (FFPE) epithelial carcinoma with the Chromeo LT Live Cell Membrane Stain.

In addition to the Chromeo LT Membrane Stain, Active Motif offers several other fluorescent cell and organelle stains (Table 1). For more complete information and a vast amount of application data, please visit www.activemotif.com/stains.

Cell and Organelle Stain	Live cells	Fixed cells	Long-term staining	Excitation	Emission
Chromeo™ LT Live Cell Membrane Staining Kit	√	√	√	420 - 490 nm	510 nm
Chromeo™ Live Cell Mitochondrial Stain	√	√	√	470 - 550 nm	610 nm
Chromeo™ Red Fluorescent Fixed Cell Staining Kit	–	√	–	520 - 550 nm	627 nm
LavaCell™ Live Cell Membrane Staining	√	√	√**	405, 488, 532 nm	610 nm

** While LavaCell is an excellent tool for long-term studies, the dye must be kept in the culture medium during the entire experiment.

Table 1: Fluorescent properties and performance overview of Active Motif's organelle-specific dyes.

Product	Format	Catalog No.
Chromeo™ LT Live Cell Membrane Staining Kit	1 kit	15007
Chromeo™ Live Cell Mitochondrial Stain	1 kit	15005
Chromeo™ Red Fluorescent Fixed Cell Staining Kit	1 kit	15006
LavaCell™ Live Cell Membrane Staining	200 µg	15004

* The Chromeo™ LT Live Cell Membrane Stain is manufactured under license by OnkoTec GmbH.

Prevent Wasted Time and Effort with our ChIP Antibody Validation Services

Finding an antibody that performs well in ChIP is one of the biggest challenges when initiating a ChIP project. Traditional ChIP antibody validation using qPCR can be akin to "finding a needle in a haystack". It requires substantial time searching the literature for good binding sites in an appropriate cell type and stimulation conditions, and can be inconclusive if the incorrect binding sites are interrogated. That is why, as part of our broader ChIP-Seq service, Active Motif offers ChIP antibody validation services by ChIP-Seq and ChIP-chip (Diagram 1).

Antibody validation by ChIP-Seq

ChIP-Seq antibody validation is a small scale, ChIP-Seq reaction.

- More robust and more sensitive than ChIP-chip
- Sequencing performed on an Illumina MiSeq
- 3-4 week turnaround
- Recommended when the antibody target is expected to be challenging

Antibody validation by ChIP-chip

ChIP-chip antibody validation is based on hybridization to an array.

- Less expensive than ChIP-Seq
- Hybridization to a single Affymetrix tiling array
- 2-3 week turnaround
- Recommended when antibody performance is expected to be robust

Active Motif Epigenetic Services has over 8 years of experience providing end-to-end ChIP services. We have processed over 10,000 ChIP samples, including over 4,000 ChIP-on-chip tiling arrays and over 1,000 ChIP-Seq reactions. To avoid wasting time and effort, start your next project off right by utilizing our expertise to ChIP validate your antibody. For details, please visit www.activemotif.com/services.

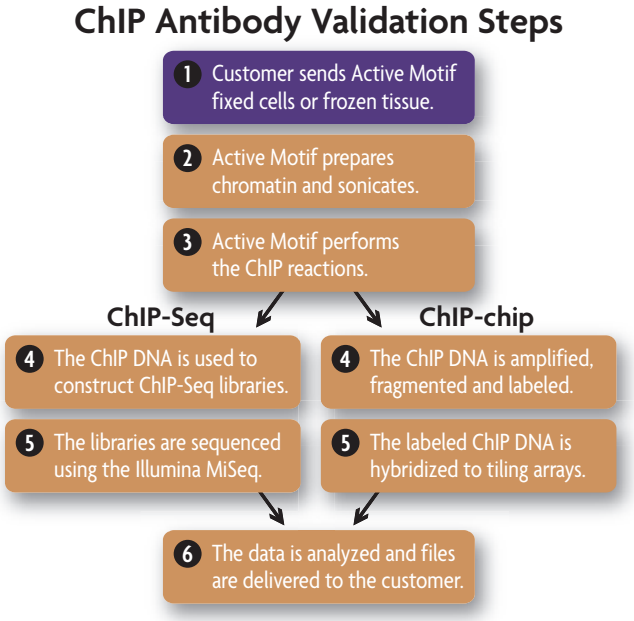

Diagram 1: Active Motif performs all steps of the ChIP experiment from chromatin preparation to data analysis, and provides an evaluation of the antibody's performance.

Monitor Binding of 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 binding. TransAM Family Kits enable you to measure the activity of entire families of transcription factors in a single experiment. TransAM is by far the most referenced transcription factor DNA-binding assay, with over 800 literature citations. In addition, Active Motif 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.

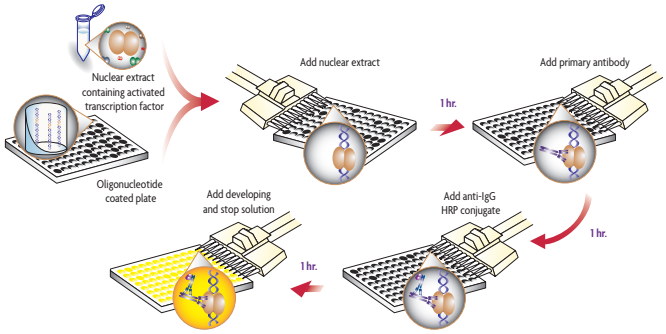

Figure 1: Flow chart of the TransAM process.
Activated transcription factor in the sample binds to the immobilized oligo, containing the transcription factor consensus-binding site, in each well. Incubation with the supplied primary and secondary antibodies and addition of developing solutions provides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