MOTIF VATIONS

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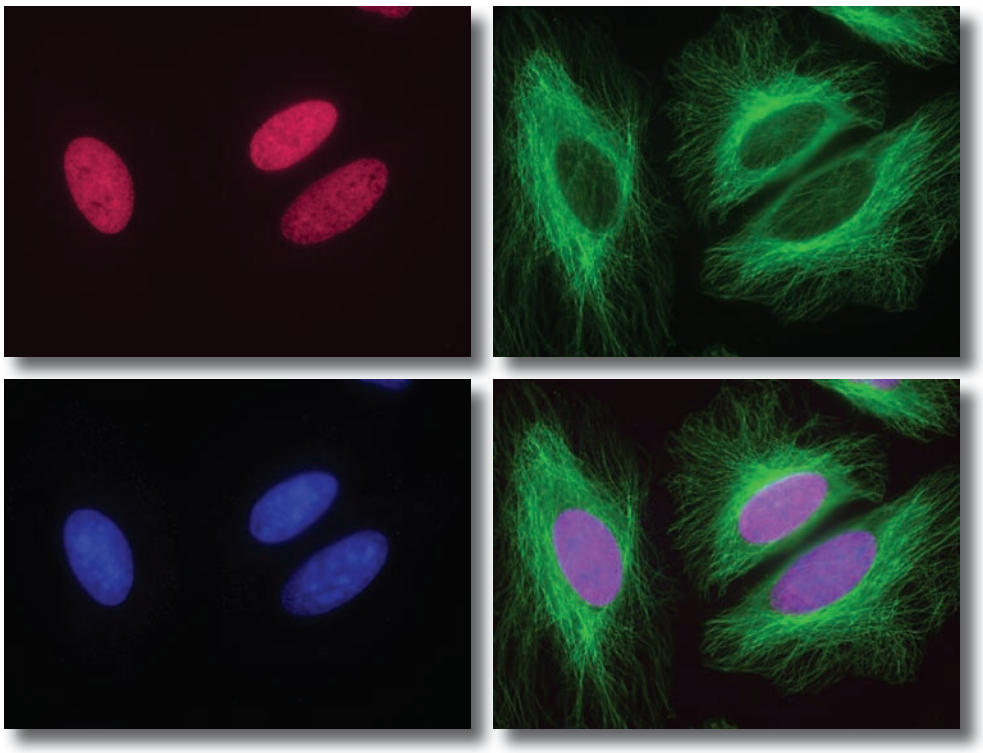
ACTIVE MOTIF®

Tools to Analyze Nuclear Function

High-quality Antibodies and Reagents for Epigenetics, Chromatin Biology & Cell Signaling



IN THIS ISSUE

Retroviruses, Chromatin Structure and Transcriptional Regulation

Retroviral DNA is integrated in its host genome, which allows the virus to escape from the host's immune system. Therefore, chromatin structure plays an important role in the transcriptional regulation of retroviral genes.

During retroviral infection of a eukaryotic cell, the integration of viral DNA into the cellular genome is an important step for the viral cycle. This allows the provirus to survive in the host cell and, with limited viral transcription, escape detection by the host immune system. Once integrated, the provirus is organized into chromatin, along with all cellular genes, and is transcribed by the host RNA polymerase II (RNA pol II). Transcription from eukaryotic promoters, including retroviral promoters, is regulated by different cellular mechanisms. Recruitment of transcription factors, chromatin structure, histone modifications and DNA methylation play important roles in this process.

Chromatin, the material into which genomic DNA is packaged in eukaryotes, is a very dynamic structure. The smallest subunit of chromatin is the nucleosome, 147 base pairs of DNA wrapped around an octamer of core histone proteins. Chromatin is subject to a variety of chemical modifications, including the post-translational modification of the histones and the methylation of cytosines in the DNA. Reported histone modifications include acetylation, methylation, phosphorylation, ubiquitylation, glycosylation, ADP-ribosylation, carbonylation and SUMOylation. Many modifications can and do influence others, and many are positively or negatively correlated with specific transcriptional states and the specific organization of repressive or open chromatin. Some modifications serve as signals for the binding of specific proteins, referred to collectively as the "histone code".

After integration of the viral DNA into the host genome, the provirus can be transcriptionally active or inactive (latent). For example, cells latently infected by Human Immunodeficiency Virus type 1 (HIV-1) serve as "reservoirs" of virus and are a permanent source of virus reactivation. Latency is a viral strategy to avoid the host immune response, allowing survival of the virus. The persistence of these latently HIV-infected cellular reservoirs, despite prolonged treatment with highly effective retroviral therapy, represents the major obstacle to virus eradication. Bovine Leukemia Virus (BLV) and Human T-Lymphotropic Virus type 1 (HTLV-1) infections are characterized by viral latency in the large majority of infected cells and by the absence of virus circulating in the blood. These features are thought to be due to the repression of viral gene expression *in vivo*.[1, 2]

In this review, we will focus on the role of some chromatin modifications in the initiation and maintenance of viral latency within the host cells.

DNA methylation

Inhibition of gene expression and a repressive chromatin state are often associated with DNA methylation, an epigenetic modification of DNA that occurs on cytosines within CpG dinucleotides. Addition of the methyl group to cytosines is carried out by the DNA methyltransferase enzymes (DNMT). There are at least two general mechanisms by which DNA methylation inhibits gene expression: first, modification of cytosine bases can inhibit the association of some DNA-binding factors with their cognate DNA recognition sequences;[3] and second, proteins that recognize methyl-CpGs can elicit the repressive potential of methylated DNA (reviewed in 4). The second mechanism is predominant and is generally associated with histone deacetylation, rendering the conformation of chromatin inaccessible to the transcriptional machinery. Additionally, there is a significant body of evidence linking DNA methylation with histone methylation, indicating that one might reinforce the other to cooperatively silence gene expression.[37-39]

An inverse correlation between gene expression and CpG methylation in the 5´LTR of retroviral genomes has been demonstrated in HTLV-1, in Moloney Murine Leukemia Virus (Mo-MuLV) and in Rous Sarcoma virus.

Methylation of retroviral promoter and enhancer sequences located in the 5´LTR (Long Terminal Repeat) is a mechanism of epigenetic silencing of provirus transcription, which allows the virus to become latent and avoid detection by the host immune system.[5-7] An inverse correlation between gene expression and CpG methylation in the 5´LTR of retroviral genomes has indeed been indeed demonstrated in HTLV-1,[7-9] in Moloney Murine Leukemia Virus (Mo-MuLV)[6, 10] and in Rous Sarcoma virus.[11] The role of HIV-1 promoter methylation in viral latency is quite controversial, however. CpG methylation within the HIV-1 promoter inhibits transcription of *in vitro*-methylated plasmids transfected

into cells,[12-14] and it was suggested as a mechanism to maintain HIV-1 latency in some infected cell lines.[15, 16] In contrast to these studies, Pion *et al.* showed using bisulfite sequencing, that transcriptional repression of HIV-1 is not associated with methylation of the 5´LTR.[17]

Histone acetylation

One factor influencing the modulation of chromatin structure is the reversible acetylation of conserved lysine residues on histone proteins. The acetylation reaction results in the transfer of the acetyl group from acetyl-coA to the ε-amino group of the lysine residue, neutralizing its positive charge. Steady-state levels of histone acetylation result from a balance between the activity of two families of antagonistic enzymes: histone deacetylases (HDACs) and histone acetyltransferases (HATs), which respectively remove or add acetyl groups to histones.

Histone acetylation is an important mechanism implicated in the regulation of retroviral promoters. Several studies have reported the regulation of BLV and HTLV-1 transcription by histone acetylation. Many groups have observed the strong effect of HDAC inhibitors on the BLV gene expression *in vitro* and *in vivo*.[18-21] They have also shown a role for the HATs CBP and p300 in the transcriptional activation of BLV and HTLV-1.[22-25] Recruitment of HDAC1, HDAC2 or HDAC3 to the HTLV-1 promoter has been observed and linked to repression of the viral promoter.[26, 27] Other retroviruses including HIV-1 (review in 28), the Rous Sarcoma virus[29] or MoMuLV[30, 31] are also regulated by histone acetylation. For example, it has been demonstrated by chromatin mapping experiments that a nucleosome positioned immediately downstream of the transcription start site is remodeled upon the activation of the HIV-1 promoter in response to HDAC inhibitors.[32] Lorincz *et al.* have demonstrated an inverse correlation between acetylation of histone H3 and proviral methylation density and transcriptional repression of MoMuLV.[30]

Histone Methylation

The methylation of histones can occur on arginine (R) and lysine (K) residues. Methylation at many residues of all the core histones has been observed (reviewed in 33). Histone lysine methyltransferases (HMTs) contain a catalytic SET domain, utilizing S-adenosyl-L-methionine (SAM) as a cofactor. Some sites of histone methylation are associated with transcriptional activation (*e.g.* H3 K4), whereas others (*e.g.* H3 K9, H3 K27) are associated with transcriptional repression and heterochromatin formation. The arginine methyltransferases are responsible for the transfer of methyl groups from SAM to the guanidinium group of arginine. There are several enzymes which methylate histones at arginine residues, PRMT1 and CARM1/PRMT4 being the most widely studied of these. Generally, methylation of histones on arginine residues is associated with transcriptional activation of genes.

Histone acetylation is also an important mechanism in regulation of retroviral promoters.

A few things are known about histone methylation within the BLV promoter. Merimi *et al.* demonstrated a change in lysine methylation leading to BLV activation after a treatment of the infected cells with a combination of trichostatin A (an HDAC inhibitor) and 5-azacytidine (a DNA methylation inhibitor).[34] An increase of H3 K4 methylation and a decrease of H3 K9 methylation accompanied a decrease of HDAC1 and mSin3 recruitment after treatment of the cells.[34] In the case of HTLV-1, two histone methyltransferases seem to be involved in the transcriptional regulation of the viral promoter.[35, 36] ChIP assays demonstrated the recruitment of CARM1 and Suv39H1 *in vivo* at the promoter region, together with the viral transcriptional activator protein, Tax. This recruitment is associated with strong H3 methylation on different arginine residues and with acetylation of H3 K9 in cells containing a single active integrated copy.

All these studies show the importance of epigenetic modifications in the transcriptional regulation of retroviruses. It is certain that similar future research will aid in treatment of viral diseases.

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NEW: HeLa Acid Extracts for Epigenetic Studies

Active Motif's commitment to providing researchers with quality products to complement their chromatin and epigenetic studies continues with the introduction of HeLa acid extracts. Active Motif is now offering HeLa acid extracts that are either untreated, or treated with chemicals known to affect epigenetic events. These acid extracts also serve as controls for many of the histone modification antibodies that Active Motif sells. To learn more about either the acid extracts, or associated histone modification antibodies, please visit www.activemotif.com.

Product	Format	Catalog No.
HeLa acid extract	100 µg	36200
HeLa acid extract (Paclitaxel treated)	100 µg	36201
HeLa acid extract (Sodium butyrate treated)	100 µg	36202
HeLa acid extract (Etoposide treated)	100 µg	36203
HeLa acid extract (Anacardic acid treated)	100 µg	36204

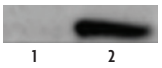


Figure 1: Western blot of HeLa acid extracts. 10 µg of HeLa acid extract that was either untreated (lane 1) or treated with 1 µg/ml paclitaxel for 20 hours (lane 2) was used in a Western blot with Histone H3 phospho Ser28 pAb (Cat. No. 39149). Paclitaxel is a mitotic inhibitor that arrests cells as they enter mitosis. Histone H3 serine 28 serves as a mitotic marker as it is highly phosphorylated during mitosis.

Histones Containing Site- and Degree-specific Methylated Lysine Residues

Histones are subject to a variety of modifications including acetylation, phosphorylation, methylation, ubiquitination and ADP ribosylation. The type and number of modifications determine chromatin structure and the extent of gene activation or silencing. Generally, methylation on histones H3K4, H3K36 and H3K79 is associated with transcriptional activation while methylation on H3K9, H3K27 and H4K20 is associated with transcriptional repression. To better investigate how methylation patterns impact regulatory processes, assays will require histones with specific methylation states, such as the site- and degree-specific methylated lysine histones offered by Active Motif.

Figure 1: Ordered spacing of nucleosomes after enzymatic digestion of assembled chromatin. Recombinant Histone H3 dimethyl Lys9 (4.5 µg) was used to generate chromatin *in vitro* using the Chromatin Assembly Kit (Cat. No. 53500, see page 8). One µg of assembled chromatin was digested for 2 minutes (Lane 2) and 4 minutes (Lane 3). Lane 1 is 100 bp marker.

How is the methylation state made?

Active Motif now offers recombinant histones with site- and degree- specific lysine methylation. Recombinant methylated histones are created using a patented approach involving a chemical alkylation reaction that introduces an analog of methyl lysine. This specific treatment allows the site and degree of methylation to be precisely controlled. All recombinant histone modifications are confirmed by ESI-TOF mass spectrometry and dot blot or immunoblot. Because the methylation state closely mimics natural methylation, these recombinant proteins are perfect for studying the effects of specific lysine methylations on nucleosome remodeling, the binding of associated chromatin proteins, or for use as building blocks for *in vitro* chromatin assays (Figure 1).[1]

1 Lu, X. *et al.* (2008) *Nat. Str. & Mol. Biol.* doi:10.1038/nsmb.1489

Product	Format
Recombinant Histone H3 (C110A) or Histone H4	50 µg
Recombinant Histone H3 mono-, di-, or tri-methyl Lys4	50 µg
Recombinant Histone H3 mono-, di-, or tri-methyl Lys9	50 µg
Recombinant Histone H3 mono-, di-, or tri-methyl Lys27	50 µg
Recombinant Histone H3 mono-, di-, or tri-methyl Lys79	50 µg
Recombinant Histone H4 mono-, di-, or tri-methyl Lys20	50 µg

For additional details and ordering information, visit www.activemotif.com/recomhistones.

Analyzing DNA Methylation Aids the Study of Development and Disease

DNA methylation is an epigenetic event that affects cell function by altering gene expression. DNA methylation is involved in embryonic development and cell cycle regulation, while aberrant methylation is prevalent in many human cancers. During methylation, methyl groups are transferred from the cofactor, S-adenosyl-L-methionine, to the fifth-carbon of cytosine in a CpG dinucleotide. This reaction is catalyzed by one of the DNA methyltransferase enzymes. Due to the importance of methylation in development and disease, analyzing DNA methylation is necessary for understanding gene expression. Active Motif offers two techniques to aid in DNA methylation analysis.

Bisulfite Conversion for Accurate Analysis of Methylated DNA

What is bisulfite conversion?

One method to analyze DNA methylation is through bisulfite conversion. Bisulfite modification involves the conversion of unmethylated cytosines into uracils, while methylated cytosines remain unchanged. The DNA is then amplified by PCR and analyzed by sequencing or restriction digest. A comparison of the sequences of converted and untreated DNA will reveal the methylation profile of the sample.

The MethylDetector™ advantage

Active Motif's MethylDetector™ Bisulfite Kit simplifies analysis of DNA methylation by providing optimized reagents for performing DNA conversion, timesaving DNA purification columns and positive control PCR primers that are specific for bisulfite-converted DNA. This allows you to confirm the success of the conversion procedure before spending extra time and money on sequencing, or other analysis methods.

For complete product details, please visit www.activemotif.com.

Product	Format	Catalog No.
MethylDetector™	50 rxns	55001

Isolation of CpG-Methylated DNA Using Recombinant Methyl-binding Protein

Why isolate methylated CpG islands?

A second method for the analysis of DNA methylation is the isolation of CpG-methylated DNA. CpG islands are small regions of the DNA in which the CpG dinucleotide frequency is higher than would normally be expected. While CpG islands are only found in approximately 1% of the genome, more than 60% of human promoters contain CpG islands. Studying CpG island methylation is of considerable importance because CpG islands are normally not methylated. If a CpG island within a promoter becomes methylated, the gene associated with the promoter is permanently silenced, and this silencing can be transmitted through mitosis. This means that CpG island methylation is an epigenetic means of inheritance.

How does MethylCollector™ work?

Active Motif's MethylCollector™ Kit utilizes the recombinant Methyl-binding protein (MBD2b) to capture CpG-methylated DNA from enzymatically digested or sonicated cell or tissue samples. The kits' optimized buffers remove DNA fragments that have little or no methylation, eluting purified, methylated DNA that is ready for PCR analysis of the locus of interest (Figure 1). Positive control DNA and PCR primers are also included to help ensure success.

Learn more at www.activemotif.com.

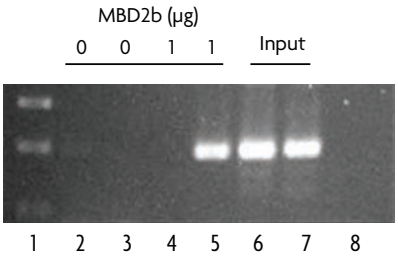


Figure 1: Isolation of Fully Methylated Jurkat DNA. MethylCollector was used to isolate methylated DNA from 100 ng genomic DNA (lanes 2 & 4) and Fully Methylated Jurkat DNA (lanes 3 & 5), with (lanes 4 & 5) or without (lanes 2 & 3) inclusion of 1 µg of the kit's His-tagged MBD2b protein. These samples and input genomic DNA (lane 6) and input Fully Methylated Jurkat DNA (lane 7) were then PCR amplified with the BRCA1 primers. A positive result is observed only with Fully Methylated Jurkat DNA captured by the kit's His-tagged MBD2b (lane 5). Lane 8 is a water-only PCR control.

Product	Format	Catalog No.
MethylCollector™	25 rxns	55002
Fully Methylated Jurkat DNA	10 µg	55003

Upgrade your Experiments using Magnetic Bead-based ChIP-IT™ Express Kits

Active Motif's line of ChIP-IT™ Express Kits and reagents make chromatin IP faster and more consistent. Kits are available in either sonication or enzymatic shearing formats, with or without positive and negative controls. ChIP-IT Express Kits employ protein G-coated magnetic beads, making it possible to simplify the ChIP protocol, which reduces your time and effort (Figure 1). We have also extended our ChIP-IT Express product line to include two new kits: ChIP-IT™ Express HT, for high-throughput ChIP, and Re-ChIP-IT™, making it easy to perform sequential IP.

ChIP-IT magnetic bead advantages

- **Faster procedure** – magnetic sorting is much faster than centrifugation
- **No pre-blocking needed** – magnetic beads are inert and exhibit low binding of proteins
- **Less effort required** – Our kits are compatible with multi-channel pipettors to streamline wash steps
- **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

The Innovation Leader in ChIP products

Active Motif was the first company to introduce a ChIP kit employing magnetic beads. We have recently strengthened our position as the leader in developing innovative chromatin IP kits with the release of two new kits that will allow you to expand the utility of the ChIP technique: ChIP-IT Express HT, allowing the processing of up to 96 ChIP reactions simultaneously, and Re-ChIP-IT, making it easy for you to perform sequential chromatin IP.

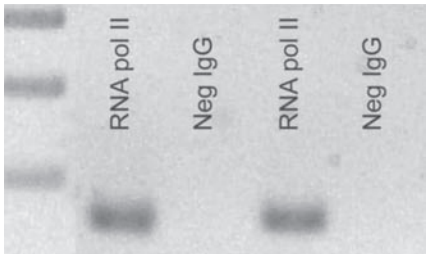


Figure 1: PCR of chromatin IP performed on 100,000 cells. Typically, ChIP requires 2 million cells per reaction. With ChIP-IT Express, it is possible to perform successful ChIP with 100,000 cells or less. In the gel above, ChIP was performed in duplicate on chromatin isolated from 100,000 HeLa cells using the Negative Control IgG, RNA pol II antibody and GAPDH PCR primers supplied in the ChIP-IT Control Kit – Human (Cat. No. 53010, see page 7).

High-throughput ChIP a reality

If you need to perform many ChIP experiments at once, the new ChIP-IT Express HT kit is your solution. It combines the time-saving, magnetic bead-based protocol of ChIP-IT Express with a high-throughput, 96-well microplate-based format (Figure 2). With ChIP-IT Express HT you can rapidly and efficiently process up to 96 ChIP reactions at a time. ChIP-IT Express HT is compatible with our enzymatic and sonication-based shearing kits for chromatin preparation, as well as with the ChIP-IT Control Kits.

Identify protein co-localization *in vivo* using sequential chromatin IP

When performing ChIP experiments, it is often useful to prove that two different proteins or histone modifications are present at the same site in the genome. Or, you may want to determine if a protein coincides with a specific histone modification at the same regulatory element. Sequential ChIP (also called Re-ChIP) is a relatively new technique in which sequential chromatin immunoprecipitation reactions are performed using two different antibodies, enabling you to assay for the simultaneous presence of two proteins or distinct histone modifications at the same genomic region of interest. Performing sequential chromatin IP was technically challenging and difficult, until now. Active Motif's new Re-ChIP-IT Kit makes it easy to perform sequential ChIP, so you can localize two different proteins or histone modifications to the same genomic locus.

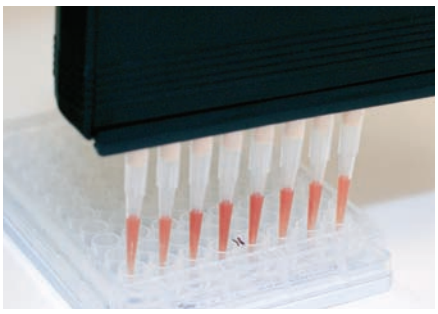


Figure 2: True high-throughput ChIP. With the efficient plate-based protocol of ChIP-IT Express HT, you can process up to 96 ChIP reactions at a time.

Are you ready to upgrade your ChIP experiments? Then please visit us at www.activemotif.com/chipitexpress to learn more about the ChIP-IT Express line of ChIP kits, reagents and ChIP-validated antibodies.

Product	Format	Catalog No.
ChIP-IT™ Express HT	96 rxns	53018
ChIP-IT™ Express	25 rxns	53008
Re-ChIP-IT™	25 rxns	53016
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014

Improve Your ChIP Experiments with ChIP Accessory Kits and Reagents

Active Motif offers a broad range of reagents and accessory kits to complement its ChIP-IT™ and ChIP-IT™ Express line of ChIP kits. These products will help you troubleshoot your ChIP experiments and make them more reproducible.

Superior ChIP-IT magnetic beads

When you use Active Motif's ChIP-IT Protein G Magnetic Beads, the protein G is directly conjugated to a low protein-binding magnetic bead, so pre-blocking of the beads is not required. ChIP-IT Protein G Magnetic Beads are validated for use in chromatin IP, and ready to use.

Simplified chromatin shearing

Employing sonication to shear chromatin for ChIP can be tedious and difficult to perform reproducibly. To eliminate the problems associated with sonication, Active Motif has developed a more robust and user-friendly method that uses our proprietary Enzymatic Shearing Cocktail to quickly shear chromatin. As enzymatic shearing is solely time and temperature dependent, inconsistencies associated with sonication are eliminated, helping improve your ChIP results.

ChIP-IT Control Kits

Chromatin IP is a complex technique, so it can be difficult to determine if the experiment worked. To make your ChIP experiments easier to troubleshoot, Active Motif sells ChIP-IT Control Kits for human, mouse and rat cells with positive and negative control antibodies and positive control PCR primers.

Ready-to-ChIP Chromatin

For your convenience, Active Motif offers Ready-to-ChIP Chromatin from a variety of common human and mouse cell lines. Ready-to-ChIP Chromatin has been optimally sheared by sonication and validated in ChIP. As a result, you can more easily validate your own antibodies and primer sets.

Product	Format	Catalog No.
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014
ChIP-IT™ Control Kit – Human	5 rxns	53010
ChIP-IT™ Control Kit – Mouse	5 rxns	53011
ChIP-IT™ Control Kit – Rat	5 rxns	53012
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
Ready-to-ChIP Hep G2 Chromatin	10 rxns	53019
Ready-to-ChIP K-562 Chromatin	10 rxns	53020
Ready-to-ChIP NIH/3T3 Chromatin	10 rxns	53021
ChIP-IT™ Shearing Kit	10 rxns	53002
Enzymatic Shearing Kit	10 rxns	53005

Rigorous Testing Ensures Consistent Antibody Performance

Active Motif is committed to providing the highest quality antibodies for studying chromatin and the biology of the nucleus. We specialize in manufacturing antibodies against histones, histone modifications and chromatin proteins, many of which have been validated for use in ChIP and immunofluorescence. Each antibody we make is rigorously tested to ensure you won't waste your precious time and research dollars on antibodies that don't perform as specified.

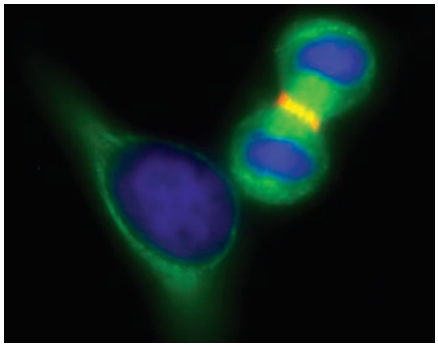
Our Antibody Commitment

- **Quality first** – we'd rather fail our project than sacrifice quality
- **Highly characterized** – all of our antibodies are stringently tested under multiple conditions
- **Controlled process** – we manufacture and test our own antibodies
- **Consistent** – we go to great lengths to minimize lot-to-lot variability

Expert manufacturers of:

- Histone and Histone Modification Antibodies
- Transcription Factor and Cell Signaling Antibodies
- Antibodies to Chromatin Modifying Proteins
- ChIP-validated Antibodies

To find the ones you need from the over 350 high-quality antibodies offered by Active Motif, use the convenient search tools found at www.activemotif.com/abs.



Immunofluorescence with Active Motif antibodies. HeLa cells stained with Aurora B pAb (Cat. No. 39261). Aurora B (stained in red, but appears yellow) is visualized in the spindle midzone of the mitotic cell on the right. Green: alpha Tubulin mAb (Cat. No. 39527). Blue: DAPI.

Reconstitute Chromatin Quickly and Easily with the Chromatin Assembly Kit

The Chromatin Assembly Kit enables you to generate chromatin *in vitro* from your linear or supercoiled DNA. It yields chromatin that closely mimics natural *in vivo* chromatin, so you can discover which histone modifications and associated proteins are crucial to the regulation of your target gene.

A simple way to generate chromatin

Now you can investigate regulation of your gene of interest in its native form by assembling it into chromatin using Active Motif's Chromatin Assembly Kit. The kit includes all the recombinant proteins, core histones, buffers and ATP-utilizing factors needed to generate chromatin *in vitro* from your DNA, and also to verify successful assembly. High-quality chromatin with regularly spaced nucleosomes is made by adding the supplied components to 1 µg of your linear or supercoiled DNA, then incubating for 4 hours. Micrococcal nuclease digestion of the resulting chromatin confirms the ordered spacing of nucleosomes (Figure 1).

Chromatin Assembly Kit advantages

- Generate chromatin from linear or supercoiled DNA
- ATP-dependent method results in an extended array of regularly spaced nucleosomes
- Easy protocol – simply incubate the kit components with your DNA
- Produces an excellent substrate for various gene regulation experiments

Try *in vivo* assembly of chromatin today

The Chromatin Assembly Kit makes it easy for you to assemble chromatin on your sequence of interest to investigate gene regulation in a context that closely resembles *in vivo* chromatin. For more information, please give us a call.

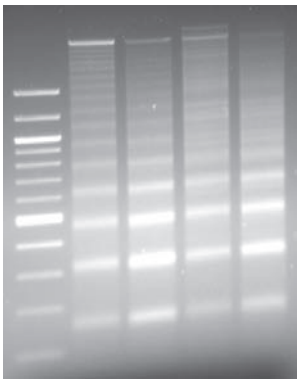


Figure 1: Enzymatic digestion of assembled chromatin. Limited micrococcal nuclease digestion was carried out on chromatin assembled using 1 µg samples of circular DNA (Lanes 1 & 2) or linear DNA (Lanes 3 & 4), resulting in regularly spaced nucleosomes.

Product	Format	Catalog No.
Chromatin Assembly Kit	10 rxns	53500

Easily Purify Core Histones from Any Cell Culture or Tissue Sample

Now you can easily purify histones and further separate the fractions of core histones from any cell culture or tissue sample while maintaining post-translational modifications like acetylation, methylation and phosphorylation. Active Motif has two versions of our Histone Purification Kit to meet your experimental needs.

How does it work?

Our unique kits utilize a patented method and proprietary binding columns and elution buffers to isolate very pure fractions of histones. Using the Histone Purification Mini Kit, core histones can be purified from as few as 8 x 10^5 cultured cells or tissue samples as a single population containing H2A, H2B, H3 and H4. With the original Histone Purification Kit, several milligrams of core histones can be purified as one total population or fractionated into separate pools of H2A/H2B dimers and H3/H4 tetramers (Figure 1). Both kits utilize a convenient spin-column format that greatly reduces the processing time required.

Histone Purification Kit advantages

- Rapid and convenient protocol reduces effort
- Multiple kits to meet your histone purification needs
- Post-translational modifications remain intact
- Purified histones will improve your downstream assays

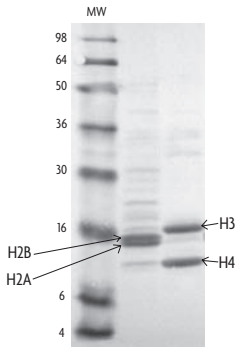


Figure 1: Separate H2A/H2B and H3/H4 fractions. H2A/H2B and H3/H4 fractions isolated from HeLa cells.

Product	Format	Catalog No.
Histone Purification Mini Kit	20 rxns	40026
Histone Purification Kit	10 rxns	40025

Assays to Measure HAT & HDAC Activity

Histone acetyltransferases (HATs) are enzymes that acetylate conserved lysine amino acids on histones by transferring an acetyl group from acetyl coenzyme A (acetyl-CoA). Generally, histone acetylation is associated with activation of gene expression, but it is also involved in DNA replication, histone deposition and DNA repair. Histone deacetylases (HDACs) remove the acetyl groups from histones creating hypoacetylated chromatin, which is transcriptionally silent. Because of the importance of histone acetylation in chromatin function, HATs and HDACs have major roles in the control of cell fate and compounds that activate or inhibit their activity are of great interest. Active Motif offers both HAT and HDAC Assay Kits that make it easy to accurately determine the activity of histone acetyltransferases and histone deacetylases in your sample, as well as the effect of inhibitor compounds.

How do the HDAC Assay Kits work?

The HDAC Assay Kits are available in both Fluorescent and Colorimetric formats. They utilize an acetylated lysine peptide substrate that can be deacetylated by Class I, II and IV HDAC enzymes. (Class III HDAC enzymes, or the Sirtuins, require the addition of the NAD+ cofactor into the assay). Once the substrate is deacetylated, the lysine reacts with the Developing Solution and releases either a chromophore or fluorophore. Trichostatin A is provided for use as an HDAC inhibitor (Figure 1).

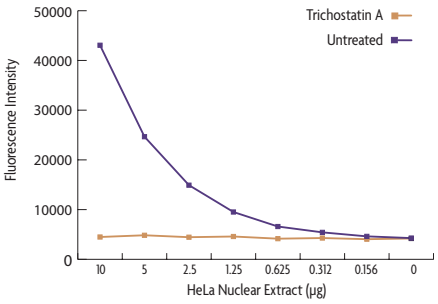


Figure 1: HDAC activity in HeLa cells.
HeLa nuclear extracts were assayed at 0 to 10 µg per well using the fluorescent version of the HDAC Assay Kit. Untreated extract results are shown with a purple line, while extracts inhibited by 1 mM Trichostatin A are shown with a copper line.

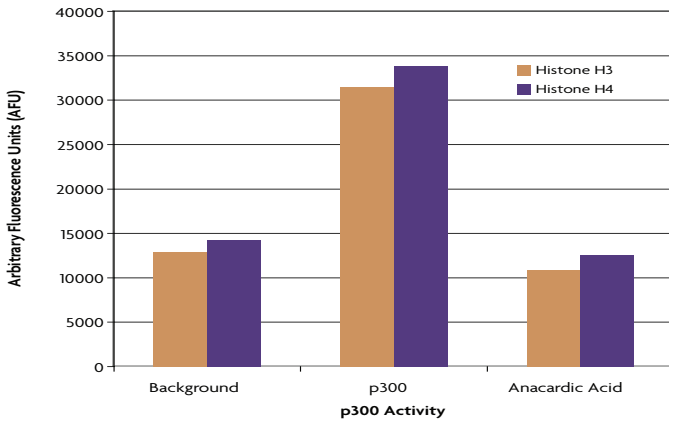


Figure 2: Effect of anacardic acid on p300 HAT activity.
50 ng of recombinant p300 protein was assayed with 50 µM acetyl-CoA and 50 µM Histone H3 or H4 peptide substrates in the absence or presence of 15 µM anacardic acid, a known HAT inhibitor.

How does the HAT Assay Kit work?

The HAT Assay Kit is a quick and simple assay that is performed in a 96-well plate. The kit includes Histone H3 and Histone H4 substrate peptides that are combined with your sample in the presence of acetyl-CoA. Activity is measured within 10-30 minutes by a fluorescent readout of the thiol-reactive dye with the Co-A-SH that is a by-product of the HAT reaction. Active recombinant p300 protein, which is capable of acetylating all four core histones, is included as a positive control. Anacardic acid, a potent HAT inhibitor, is also included for use as a negative control (Figure 2).

Order today!

Please give us a call or visit us at www.activemotif.com to get additional information on our HAT and HDAC Assays. Active recombinant p300 and GCN5 proteins are also available separately for use in other applications.

Product	Format	Catalog No.
HAT Assay Kit (Fluorescent)	1 x 96 rxns	56100
Recombinant p300 protein, catalytic domain	5 µg	31205
Recombinant GCN5 protein, active	5 µg	31204
HDAC Assay Kit (Fluorescent)	1 x 96 rxns	56200
HDAC Assay Kit (Colorimetric)	1 x 96 rxns	56210

In-cell ELISAs for More Quantitative Monitoring of Phosphorylation

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

FACE advantages

- **Cell-based** – no extraction, gels or blotting needed
- **Accurate** – fixation prevents additional protein modifications
- **Fast** – 5-hour protocol, with just 2 hours of hands-on time
- **Grow cells in 96-well plates** – save on reagents
- **Economical** – total and phospho-specific antibodies provided for 2 x 96 wells
- **Semi-quantitative results** – normalize to total protein and cell number

The "in-cell" method

Fast Activated Cell-based ELISAs (FACE™) are easy to use and require just 2 hours of hands-on time. Cells are grown in 96-well cell culture plates and treated to induce phosphorylation of the protein of interest. The cells are rapidly fixed with formaldehyde to preserve the phosphorylation state, and then each well is incubated with a primary antibody specific for either the total protein or its phosphorylated form. Subsequent incubation with a labeled secondary provides a colorimetric or chemiluminescent readout that is quantitative and reproducible (Figures 1 & 2). Data is 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 (Table 1). 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.

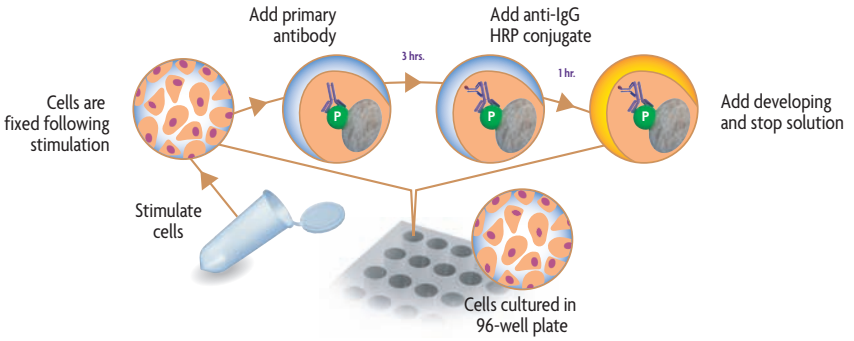
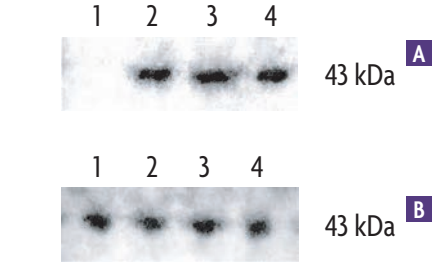


Figure 1: Flow chart of the FACE process.
Cells are grown, stimulated and fixed in the same 96-well plate. Addition of primary and secondary antibodies detects total protein as well as the phosphorylated form of the protein.



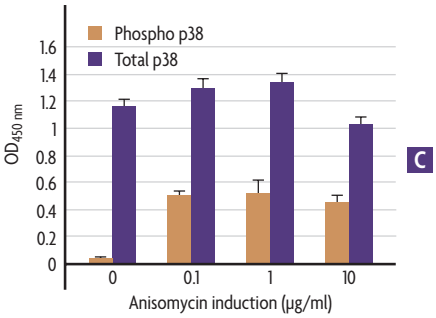


Figure 2: Phospho and total p38 MAPK assays.
Macrophage 4/4 cells were grown in 10 cm dishes to 80% confluency, serum-starved for 16 hours and stimulated with anisomycin for 15 minutes. Cell lysates were made and Western blots performed using phospho- (A) and total-p38 antibodies (B). For FACE, 4/4 cells 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.

Table 1: The FACE™ Product Line

FACE™ AKT (S473)	FACE™ ATF-2 (T71)	FACE™ Bad (S112)	FACE™ c-Jun (S63)	FACE™ c-Jun (S73)	FACE™ c-Src (Y418)
FACE™ EGFR (Y845)	FACE™ EGFR (Y992)	FACE™ EGFR (Y1173)	FACE™ ErbB-2 (Y877)	FACE™ ErbB-2 (Y1248)	FACE™ ERK1/2 (T202/Y204 & T185/Y187)
FACE™ FAK (Y397)	FACE™ FKHR (FOXO1) (T24)	FACE™ GSK3β (S9)	FACE™ HSP27 (S82)	FACE™ JAK1 (Y1022/Y1023)	FACE™ JNK (T183/Y185)
FACE™ MEK1/2 (S217/S221)	FACE™ NFκB Profiler (S468 & S536)	FACE™ p38 (T180/Y182)	FACE™ PI3 Kinase p85	FACE™ STAT2 (Y869)	FACE™ STAT4 (Y693)
FACE™ STAT6 (Y641)	FACE™ Maker	Suspension Cell FACE™			

Simplified Study of SUMOylation Effects with SUMOlink™

SUMOlink™ Kits are a simple, effective method for generating SUMOylated proteins *in vitro*. These fast, efficient and robust assays contain all necessary reagents for SUMOylation of target proteins, and include positive and negative controls that help to ensure your success.

The SUMOlink method

SUMOlink™ Kits enable you to easily perform and detect post-translational modifications by SUMO (small ubiquitin-like modifier). With SUMOlink, you simply add the assay components to a microcentrifuge tube with your protein of interest. After a 3-hour incubation, the reaction is stopped and results can be analyzed by Western blot (Figure 1). With the kit's p53 antibody and either SUMO-1 or SUMO-2/3 antibodies, you can easily see the extent to which your target protein has been SUMOylated.

Everything you need to study SUMO

The kits contain E1 activating and E2 conjugating enzymes along with wild-type and mutant SUMO-1 (SUMO-1 Kit) or SUMO-2 and -3 proteins (SUMO-2/3 Kit). Antibodies for SUMO-1 or SUMO-2/3 modifications, as well as control p53 protein and antibody, are included. For complete information, please give us a call or visit us at www.activemotif.com.

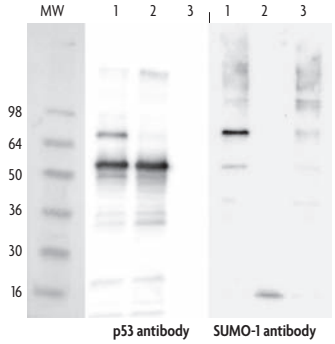


Figure 1: Enzymatic digestion of assembled chromatin. Western blot analysis of *in vitro* SUMOylation of p53 protein by wild-type and mutated isoforms of SUMO-1: The two Western blots were incubated with p53 antibody (1:5000 dilution) and SUMO-1 antibody (1 :4000 dilution). p53 is SUMOylated only by wild-type SUMO-1 protein. Lane 1: Wild-type SUMO protein conjugation reaction. Lane 2: Mutated SUMO protein conjugation reaction. Lane 3: No p53 control protein used in conjugation.

Product	Format	Catalog No.
SUMOlink™ SUMO-1 Kit	20 rxns	40120
SUMOlink™ SUMO-2/3 Kit	20 rxns	40220

Accurately Measure DNA Repair Protein Activity

Active Motif's DNA Repair Protein Kits are DNA-binding ELISAs that detect DNA repair protein activity in both mammalian tissue and cell culture extracts. They combine a fast, user-friendly format with a sensitive, specific assay that is 10-fold more sensitive than gelshift, without the need for gels or radioactivity. Quantitative results are achieved in less than 5 hours.

The DNA Repair Protein Kit method

DNA Repair Protein Kits provide a quick and easy method for sensitive DNA repair protein detection. Each kit contains a 96-well plate with multiple copies of an immobilized oligonucleotide containing a DNA lesion. Cell extract is added to each well and the repair protein of interest binds specifically to the oligonucleotide on the plate. Each well is then incubated with a primary antibody directed against the repair protein being studied. Addition of an HRP-conjugated secondary antibody and developing solution provides an easily quantified colorimetric readout (Figure 1).

DNA Repair Protein Kit advantages

- ELISA format eliminates gels, blotting and radioactivity
- From cell extract to completed assay in less than 5 hours
- Up to 10-fold greater sensitivity than gelshift assays
- Ability to assay both cultured cells and tissue samples

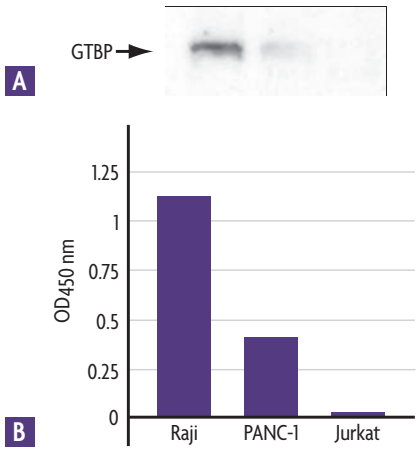


Figure 1: Measuring GTBP activity in several cell lines. Jurkat, PANC-1 and Raji nuclear extracts were assessed for GTBP activity using Western blot (A) and the GTBP DNA Repair Protein Kit (B). The DNA Repair Protein Kit is clearly more quantitative and sensitive.

Product	Format	Catalog No.
GTBP DNA Repair Kit	1 x 96 well-plate 5 x 96 well-plates	51096 51596
Ku70/86 DNA Repair Kit	1 x 96 well-plate 5 x 96 well-plates	51196 51696
RPA DNA Repair Kit	1 x 96 well-plate 5 x 96 well-plates	51296 51796

Epigenetics, Chromatin Biology & Cell Signaling

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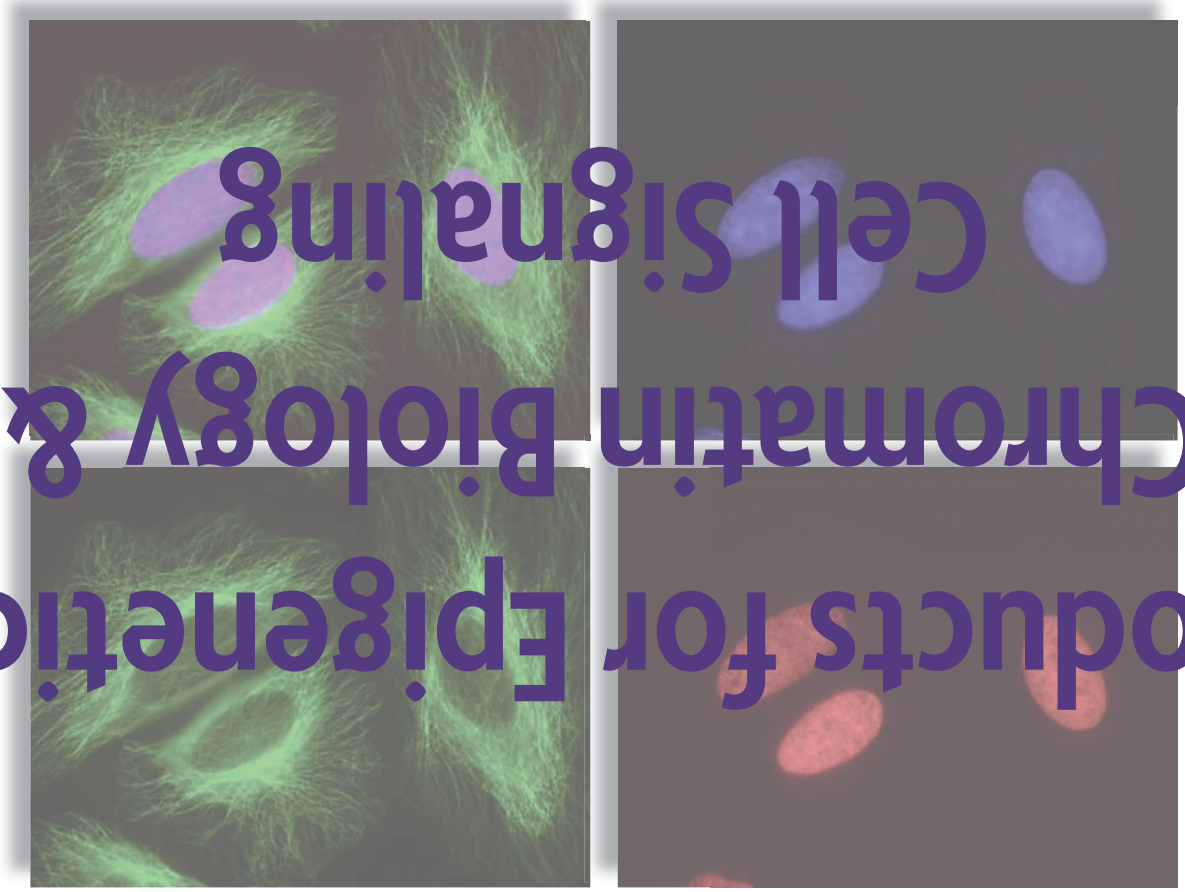

Products for Epigenetics, Chromatin Biology & cell signaling