Chromatin Immunoprecipitation (ChIP)
Histone Purification, DNA Methylation
Histone Antibodies, Histone ELISAs
Methylated Histone Proteins

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Histone Acetylation and Genome Function: *Transcription and Beyond*

Jim Bone, Ph.D.

The role of histone acetylation and its involvement in the regulation of transcription has long been a topic of research in cell and molecular biology labs. Recent studies have revealed the role of histone acetylation in other important processes regulating the structure and function of chromatin, and hence, the eukaryotic genome.

The process of organizing the millions (billions in the case of humans) of base pairs of genetic material in the eukaryotic nucleus has profound effects on DNA-dependent events, such as transcription, recombination, replication and repair. DNA is organized by its incorporation into chromatin, the basic subunit of chromatin being the nucleosome. A nucleosome is composed of 147 base pairs of DNA coiled around an octamer of histone proteins, two molecules each of histone H2A, H2B, H3 and H4. Histone H1 associates with chromatin outside the core octamer unit and regulates higher order chromatin structure. Chromatin and chromosomes undergo dramatic and dynamic changes in organization in response to a myriad of cellular signals. Chromosomes condense and relax during the cell division process. Damaged DNA adopts a unique structure that facilitates repair. Critical to cell function, most of the genome must remain in a transcriptionally silent state, save for specific combinations of genes, which vary significantly between cell types. These processes must be tightly regulated to maintain the integrity of the genome and the proper function of the cell.

Multiple mechanisms exist within the nucleus that allow the function and organization of the genome to be dynamically regulated, responding rapidly to different signals or inputs. Most notably, though, a large body of evidence dating back forty years and more has accumulated to indicate that post-translational modification of histones is crucial to all genome-based activity (see Kouzarides, 2007). The recruitment of transcriptional regulators and chromosomal proteins brings along enzymes that modify (by either addition or removal) specific functional groups on histones, and these dynamic addition and subtraction events have profound effects on the structure and function of chromatin.

Histone Acetylation and Transcription

The most widely studied histone modification is acetylation. Histones are covalently modified at the epsilon-amino group of conserved lysines by a class of enzymes called histone acetyltransferases (HATs). HATs come in two flavors, cytoplasmic and nuclear. The cytoplasmic HATs (*e.g.* Hat1) acetylate histones prior to nuclear localization and chromatin assembly, whereas the nuclear HATs acetylate histones in a manner associated with transcription and other DNA-dependent processes.

Some of the earliest observations made by Allfrey and colleagues linked histone acetylation to transcription (Allfrey *et al.*, 1964). A large amount of descriptive and correlational evidence accumulated in the thirty years after this initial work, until the identification of the first nuclear histone acetyltransferase, Tetrahymena p55, provided the first mechanistic link between histone acetylation and transcriptional activation (Brownell *et al.*, 1996). The yeast homologue of p55, Gcn5, had previously been identified as an important transcriptional co-activator, a protein recruited to gene promoters prior to transcription and necessary for activation. Further solidifying the connection between histone acetylation and transcription, it was determined that the HAT activity of Gcn5 is required for its ability to activate transcription (Candau *et al.*, 1997; Kuo *et al.*, 1998).

Tetrahymena p55 provided the first mechanistic link between histone acetylation and transcriptional activation.

Biochemical analysis of a variety of proteins and protein complexes involved in transcriptional activation led to the identification of a large number of histone acetyltransferases, strengthening the hypothesis that histone acetylation is tightly connected to (and likely part and parcel of) transcriptional activation (reviewed in Roth and Denu, 2001). Additional support for this hypothesis came from the opposite side of the histone acetylation coin, histone deacetylation. Contemporaneous with the identification of Gcn5 as the first HAT, the mammalian homologue of a known transcriptional repressor from yeast, Rpd3, was identified as the first enzyme that removes epsilon-acetylation from histones, a

histone deacetylase, or HDAC (Taunton *et al.*, 1996). Running parallel to the advances made with HATs, a wide variety of HDACs and HDAC-containing protein complexes were identified and linked to the repression of transcription (reviewed Yang and Seto, 2003).

Transcriptional Elongation

While the connection between histone acetylation and transcriptional activation has been known for some time, it has only recently been established that histone acetylation is involved in the process of transcriptional elongation. Whereas promoters of active genes are highly acetylated, the coding regions of genes need to be maintained in a hypoacetylated state to prevent the aberrant initiation of transcription. A histone methyltransferase (Set2) that travels with the elongating RNA polymerase marks the coding sequence with a unique modification, histone H3 methylated at lysine 36. A histone deacetylase complex (Rpd3S) is recruited and specifically deacetylates coding region chromatin through the recognition of lysine 36 by the chromodomain protein, Eaf3, a subunit of Rpd3S (Carrozza *et al.*, 2005; reviewed in Lee and Shilatifard, 2007).

Lys 16: An Acetylation Site with a Unique Molecular Identity

With regard to transcriptional activation, the acetylation of specific lysines appears to be less important than the overall acetylation level of histone proteins on the whole. There appears to be a functional redundancy between lysine residues, with no single lysine being more or less critical than the next. Indeed, HAT enzymes exhibit broad substrate specificity, although are apparently limited by which histone they are capable of acetylating. The exception to this rule is lysine 16 of histone H4, the acetylation and deacetylation of which have specific roles in important transcriptional processes (reviewed in Shia *et al.*, 2006). In yeast, acetylation of histone H3 at lysine 16 is required to maintain the boundaries between euchromatin (open and transcriptionally competent regions of chromatin) and heterochromatin (closed and transcriptionally inert). In Drosophila, lysine 16 acetylation is involved in the process of dosage compensation, the coordinate up-regulation of genes on the single male X chromosome that equalizes gene expression with that of genes on the two X chromosomes in females. In humans, the loss of lysine 16 acetylation appears to be a hallmark of specific types of cancer. The common thread between these findings may be found in the results of a recent study employing a unique biochemical method to study histone acetylation. Using a peptide-ligation method to synthesize recombinant chromatin acetylated homogeneously, it was determined that acetylation of lysine 16 inhibits the formation of higher-order chromatin. This result suggests that the unique role played by lysine 16 is to serve as a central regulator of chromatin structure (Shogren-Knaak *et al.*, 2006).

Genome Integrity, DNA Replication & DNA Damage Repair

There is a recent and growing body of evidence linking histone acetylation to DNA replication and repair. One of the first studies suggesting a link between acetylation and DNA repair came from a genetic study of histone H4 acetylation in yeast. A yeast strain containing mutations in the four amino-terminal lysines of histone H4 has severely compromised genome integrity, leading to continual activation of the DNA damage repair checkpoint (Megee *et al.*, 1995). A more recent study showed that this same yeast strain exhibits a dramatically reduced ability to repair double-strand breaks. It was determined that the histone acetyltransferase complex NuA4 was recruited to the site of double-strand breaks. Efficient repair of DNA damage was shown to require the catalytic subunit of the NuA4, Esa1, a HAT enzyme that acetylates histone H4. Thus, not only are proper levels of histone H4 acetylation required for maintaining genome integrity, it appears that acetylation of histone H4 participates directly in one or more steps required to repair broken chromatin (Bird *et al.*, 2002).

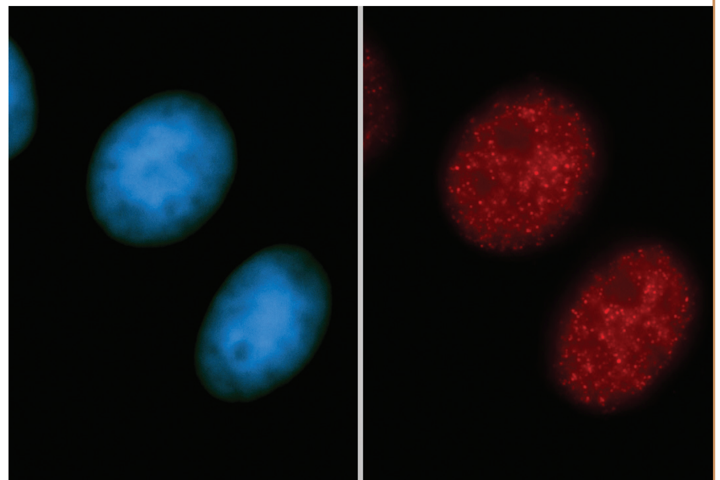

FIGURE 1:
Immunofluorescence of HeLa cells to visualize histone H3 acetylated at Lys 27. HeLa cells were grown, fixed and stained using the MAX Stain™ Universal Immunofluorescence System (Catalog No. 15250). The primary antibody used was the Histone H3 acetyl Lys27, symmetric pAb (Catalog No. 39133) at a dilution of 1:1,000.

The involvement of histone acetylation in DNA damage repair extends to mammals as well. The mammalian Esa1 homologue, TIP60, is involved with many aspects of the DNA damage repair pathway (reviewed in Squatrito *et*

al., 2006). In addition to a direct role in the repair of DNA damage similar to that of Esa1 (Murr *et al.*, 2006), TIP60 is also involved in the activation of the DNA damage repair pathway through the acetylation and activation of the ATM kinase (Sun *et al.*, 2005). TIP60 also acetylates the DNA damage-specific histone variant, H2AX, in response to DNA damage. Acetylation of H2AX leads to its subsequent ubiquitylation and remodeling of chromatin near the break, facilitating DNA repair (Ikura *et al.*, 2007).

The fascinating connection between histone acetylation and DNA damage repair has been made even more tantalizing by the identification of the importance of a single acetylation site in the process. This same single acetylation event is also required for DNA replication and chromatin assembly, which are associated with replication-coupled DNA repair. Li and colleagues found that acetylation of lysine 56 in yeast plays a specific role in chromatin assembly, dependant upon chromatin assembly factor-1 (CAF-1), a histone chaperone complex (Li *et al.*, 2008). It was determined that acetylation of lysine 56 increases the affinity of CAF-1 for histone H3, thus improving the efficiency of chromatin assembly. In a related study, it was found that acetylation of lysine 56 (and downstream chromatin assembly) is linked to proper cell cycle re-entry subsequent to double-strand DNA break repair (Chen *et al.*, 2008). The histone chaperone Asf1 (acting at a point before CAF-1) stimulates the acetylation of histone H3 at lysine 56 prior to its transfer to the nucleus and incorporation into chromatin. Lysine 56 acetylation is required for the timely deactivation of the DNA damage checkpoint after DNA repair is complete, allowing cells to re-enter the cell cycle. This suggests that the presence of H3 acetylated at lysine 56 is a hallmark of the properly re-assembled chromatin at the site of a newly repaired double-strand break, allowing the inactivation of the DNA damage checkpoint. However, in separate studies it was determined that unregulated lysine 56 acetylation is deleterious to cells and leads to spontaneous DNA damage (Celic *et al.*, 2008; Haldar and Kamakaka, 2008). It is possible that this is also the case in mammals as well as in yeast, as hyperacetylation of histones leads to the activation of the ATM DNA damage checkpoint protein (Bakkenist and Kastan, 2003). In addition to the specific role of acetylation at lysine 56 of histone H3 to facilitate chromatin assembly and serve as a marker for newly repaired chromatin, global acetylation levels must be kept at an intermediate, highly regulated level in order to maintain proper genome structure.

Involved as it is in the mechanism of DNA replication and chromatin assembly, there is evidence that histone acetylation also plays a role in the timing of DNA replication and replication origin activity. In general, increases in histone acetylation of chromatin surrounding an origin of replication tend to cause the origin to initiate replication earlier, compared to when the origin is within hypoacetylated chromatin (reviewed in Weinreich *et al.*, 2004). Origins located near or within heterochromatin replicate late, but this timing can be accelerated by artificially increasing the acetylation state of histones near the origin. As origin firing occurs throughout S-phase, rather than clustered at one time point, it is likely that individual origin firing is influenced by the local chromatin environment surrounding the origin (Vogelauer *et al.*, 2002).

Future Directions

This review has discussed many of the important roles of histone acetylation with regard to regulating genome function, however many more questions have yet to be answered. With regard to etiology, what is the relationship between acetylation and transcriptional activation? Are there other acetylation sites with unique molecular identities, such as H3 lysine 56 and H4 lysine 16? And importantly, what are the molecular mechanisms by which acetylation mediates its diverse functions? Acetylation is the best studied of the histone modifications, but there is much more remaining to be learned.

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Antibodies to Study Chromatin and the Biology of the Nucleus

high-quality antibodies to histones, histone modifications and chromatin-modifying proteins

At Active Motif, we are committed to providing researchers with the highest quality antibodies for studying chromatin and the biology of the nucleus. We manufacture our to histone and histone modification antibodies in-house, so we control antibody quality and performance. We also offer a wide range of antibodies against transcription factors, chromatin modifiers and cell-cycle regulators.

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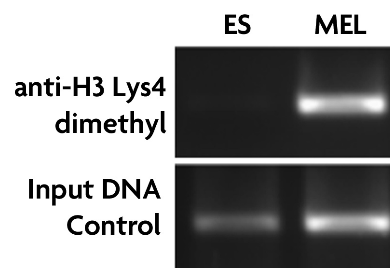


FIGURE 1:
Chromatin immunoprecipitation.
ChIP was performed with Histone H3 dimethyl Lys4 pAb (Catalog No. 39141) using chromatin prepared from mouse embryonic stem cells (left lane) or mouse erythroleukemia cells (right lane). PCR primers specific for the β-globin gene were used to amplify a 210 base pair region of the promoter following DNA isolation.

Antibodies to Chromatin Modifiers

There are many important non-histone chromatin proteins, from histone modifying enzymes like acetyltransferases, deacetylases and methyltransferases to chromatin binding proteins like HP1, which reads the "histone code". Because these proteins are important regulators of chromatin structure and function, Active Motif offers high-quality antibodies to these targets so you can study their roles.

DESCRIPTION	APPLICATIONS	CAT. NO.
HDAC11 Rabbit pAb	WB	39208
HMG-2 Rabbit pAb	WB	39029
HP1 alpha Rabbit pAb **NEW**	ChIP, IP, WB	39295
JARID1C Rabbit pAb **NEW**	WB	39229
JMJD2D Rabbit pAb **NEW**	WB	39247
JMJD2F Rabbit pAb **NEW**	WB	39257
L3MBTL1 Rabbit pAb	ChIP, IP, WB	39182
LSD1 Rabbit pAb	IP, WB	39186
MBD1 Mouse mAb	WB	39215
MBD3 Mouse mAb	WB	39216
MBD4 Rabbit pAb	WB	39217
MeCP2 Rabbit pAb	WB	39188
MeCP3 Rabbit pAb	WB	39218
MEP50 Rabbit pAb	WB	39190
Mi-2 beta Rabbit pAb **NEW**	ChIP, IF, IP, WB	39289
MRG15 Rabbit pAb **NEW**	WB	39361
MTA2 Rabbit pAb **NEW**	WB	39359
Nucleolin Mouse mAb (Clone 3G4B20)	IF, WB	39541
SIRT1 Mouse mAb (Clone 2G1/F7) **NEW**	IF, IP, WB	39353
SNF2h Mouse mAb	ChIP, IF, IP, WB	39543
Suz12 Rabbit pAb **NEW**	WB	39357
TRF2 Goat pAb	ChIP, IP, WB	39223

APPLICATIONS KEY: **ChIP** = Chromatin Immunoprecipitation; **IF** = Immunofluorescence; **IP** = Immunoprecipitation; **WB** = Western Blot

Histones and Histone Modification Antibodies

Histone post-translational modifications are important regulators of genome function. Active Motif is working to develop a panel of antibodies for all widely studied and biologically relevant modifications sites. All of our antibodies are rigorously screened for specificity and tested to verify which applications they work well in, such as ChIP, Western blotting and immunofluorescence.

ChIP-validated Antibodies

Chromatin immunoprecipitation is an extremely challenging technique, and only antibodies of the highest quality will do. For an antibody to work in chromatin IP, it needs to be of high titer, and be very specific, with no detection of non-target proteins. The most important characteristic is that it also has to recognize the target protein in its native context.

DESCRIPTION	APPLICATIONS	CAT. NO.
Histone H2A Rabbit pAb	WB	39209
Histone H2A Rabbit pAb (yeast)	ChIP, WB	39235
Histone H2A, acidic patch Rabbit pAb	WB	39111
Histone H2A acetyl Lys5 Rabbit pAb	WB	39107
Histone H2A acetyl Lys9 Rabbit pAb	WB	39109
Histone H2A phospho Ser129 Rabbit pAb (yeast) **NEW**	ChIP, IF, IP, WB	39271
Histone H2A/H4 phospho Ser1 Rabbit pAb	WB	39115
Histone H2AX phospho Ser139 Rabbit pAb	IF, WB	39117
Histone H2A.Z Rabbit pAb	WB	39113
Histone H2B Rabbit pAb	WB	39125
Histone H2B Rabbit pAb	WB	39210
Histone H2B Rabbit pAb (yeast)	ChIP, WB	39237
Histone H2B acetyl Lys5 Rabbit pAb	WB	39123
Histone H2B acetyl Lys16 Rabbit pAb	WB	39121
Histone H2B acetyl Lys120 Rabbit pAb	WB	39119
Histone H3 Rabbit pAb	WB	39088
Histone H3, C-terminal Rabbit pAb	ChIP, WB	39163
Histone H3 acetyl Rabbit pAb	WB	39139
Histone H3 phospho Thr3 Rabbit pAb	WB	39153
Histone H3 monomethyl Lys4 Rabbit pAb **NEW**	WB	39297
Histone H3 dimethyl Lys4 Rabbit pAb	ChIP, WB	39141
Histone H3 trimethyl Lys4 Rabbit pAb	ChIP, WB	39159
Histone H3 acetyl Lys9 Rabbit pAb	WB	39137
Histone H3 pan-methyl Lys9 Rabbit pAb **NEW**	IF, WB	39241
Histone H3 monomethyl Lys9 Rabbit pAb **NEW**	IF, WB	39249
Histone H3 dimethyl Lys9 Mouse mAb (Clone 6F12-H4)	ChIP, IF, IP, WB	39285
Histone H3 dimethyl Lys9 Rabbit pAb	ChIP, IF, WB	39239
Histone H3 trimethyl Lys9 Rabbit pAb	WB	39161
Histone H3 phospho Ser10 Rabbit pAb **NEW**	IF, WB	39253
Histone H3 phospho Ser10,28 Rabbit pAb	WB	39147
Histone H3 phospho Thr11 Rabbit pAb	WB	39151
Histone H3 acetyl Lys14 Rabbit pAb	WB	39127
Histone H3 dimethyl Lys14 Rabbit pAb **NEW**	WB	39349

DESCRIPTION	APPLICATIONS	CAT. NO.
Histone H3 acetyl Lys18 Rabbit pAb	IF, WB	39129
Histone H3 acetyl Lys23 Rabbit pAb	IF, WB	39131
Histone H3 acetyl Lys27 Rabbit pAb	ChIP, WB	39135
Histone H3 acetyl Lys27 Rabbit pAb	ChIP, IF, WB	39133
Histone H3 dimethyl Lys27 Rabbit pAb **NEW**	IF, WB	39245
Histone H3 trimethyl Lys27 Mouse mAb	IF, WB	39536
Histone H3 trimethyl Lys27 Mouse mAb	ChIP, WB	39535
Histone H3 trimethyl Lys27 Rabbit pAb	IF, WB	39155
Histone H3 trimethyl Lys27 Rabbit pAb	WB	39156
Histone H3 phospho Ser28 Mouse pAb	WB	39211
Histone H3 phospho Ser28 Rabbit pAb	WB	39149
Histone H3 phospho Ser28 Rat mAb (Clone HTA28)	IF, WB	39098
Histone H3 dimethyl Lys36 Rabbit pAb **NEW**	IF, WB	39255
Histone H3 acetyl Lys56 Rabbit pAb **NEW**	WB	39281
Histone H3 monomethyl Lys56 Rabbit pAb **NEW**	WB	39273
Histone H3 dimethyl Lys56 Rabbit pAb **NEW**	WB	39277
Histone H3 monomethyl Lys79 Rabbit pAb	WB	39145
Histone H3 monomethyl Lys79 Rabbit pAb **NEW**	WB	39367
Histone H3 dimethyl Lys79 Rabbit pAb	WB	39143
Histone H4 Rabbit pAb	WB	39212
Histone H4 pan-acetyl Rabbit pAb **NEW**	IF, WB	39243
Histone H4 tetra-acetyl Rabbit pAb	IF, WB	39177
Histone H4 tetra-acetyl Rabbit pAb	ChIP, WB	39179
Histone H4 dimethyl Arg3, symmetric Rabbit pAb **NEW**	IF, WB	39275
Histone H4 acetyl Lys5 Rabbit pAb	ChIP, IF, WB	39169
Histone H4 acetyl Lys8 Rabbit pAb	WB	39171
Histone H4 acetyl Lys12 Rabbit pAb	ChIP, WB	39165
Histone H4 acetyl Lys16 Rabbit pAb	ChIP, WB	39167
Histone H4 monomethyl Lys20 Rabbit pAb	ChIP, IF, WB	39175
Histone H4 dimethyl Lys20 Rabbit pAb	IF, WB	39173
Histone H4 dimethyl Lys20 Mouse mAb	WB	39539
Histone H4 trimethyl Lys20 Rabbit pAb	ChIP, IF, WB	39180

APPLICATIONS KEY: **ChIP** = Chromatin Immunoprecipitation; **IF** = Immunofluorescence; **IP** = Immunoprecipitation; **WB** = Western Blot

ChIP-IT™ Express

improved kits greatly reduce background

Active Motif has improved its ChIP-IT™ Express Kits by greatly reducing the background, which improves your results and enables you to use less starting material than ever before. In addition, the provided magnetic beads have made it possible to streamline the protocol so you can get results in half the normal time with much less sample manipulation. Also, ChIP-IT Express makes it easy to perform ChIP on many samples simultaneously.

ChIP-IT Express advantages

- No more need for pre-clearing, blocking or DNA purification steps
- Reduced background
- High throughput compatible
- Dramatically reduced hands-on time

The most efficient ChIP enrichment kit

ChIP is an enrichment technique, not a purification method. Thus, the less efficient your enrichment, the higher the sample background and the more material you will need to obtain an interpretable result. Conventional ChIP requires at least 2 million cells as starting material, which can be problematic with some cell lines. At the least, growing this many cells is labor intensive. Active Motif's improved ChIP-IT Express Kits, however, have been optimized to provide superior target gene enrichment, resulting in unmatched sensitivity. Using ChIP-IT Express, it is routine to perform ChIP on material from as few as 750,000 cells and the kits have even been shown to work with as few as 12,500 cells (Figure 1).

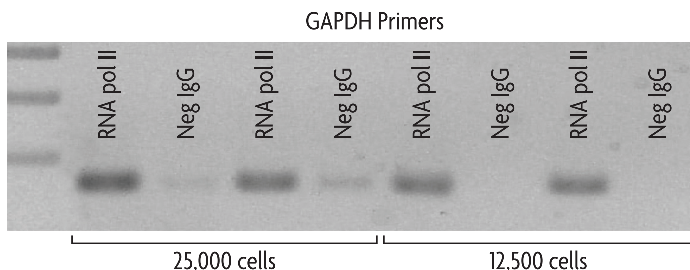


FIGURE 1:
ChIP-IT Express works with 12,500 cells.
ChIP-IT Express was performed in duplicate on decreasing amounts of sonicated HeLa cell chromatin. Two µg of RNA pol II and Neg IgG antibody was used for IP. GAPDH PCR primers were used to analyze the immunoprecipitated DNA. Using the improved ChIP-IT Express reagents and protocol, positive ChIP data was obtained from as few as 12,500 cells.

The magnetic bead advantage

The ChIP-IT Express magnetic beads have less background than standard agarose beads, so pre-clearing and blocking steps are no longer necessary. The magnetic pull-down occurs in just seconds, and re-formulated buffers allow steps to be combined and DNA purification to be eliminated. ChIP-IT Express is available in both sonication and enzymatic shearing formats.

Positive controls ensure success

Because interpreting ChIP data can be difficult, Active Motif has developed a complete set of controls to help you understand your results and troubleshoot your assays. To provide you with controls that are appropriate for your research, we offer our human, mouse and rat ChIP-IT Control Kits separately from ChIP-IT Express Kits. These provide positive and negative control antibodies and species-specific primers, PCR buffer and a convenient 10X DNA loading dye so your PCR reactions are gel-ready. All reagents are quality control tested and validated to ensure your ChIP assay is working properly. In addition, we also offer convenient Ready-to-ChIP Chromatin from a number of ENCODE cell lines, so you can be certain that the only variable in validating a new antibody for ChIP is the antibody itself.

Try the best ChIP kit today

For additional information on the new and improved ChIP-IT Express Kits, visit our website at **www.activemotif.com/chipit**.

Product	Format	Cat. No.
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
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™ Express HT

process up to 96 ChIP reactions at a time

Active Motif now makes it easy for you to perform your ChIP experiments on a high-throughput scale with the introduction of ChIP-IT™ Express HT. Based on the innovative ChIP-IT Express Kit, it uses the time-saving magnetic bead method for faster and cleaner chromatin IP experiments, enabling you to process up to 96 ChIP reactions at once.

ChIP-IT Express HT advantages

- Process up to 96 ChIP reactions
- Faster plate-based protocol
- Fewer cells required per ChIP
- Compatible with ChIP-chip and ChIP-seq methodologies

High-throughput ChIP

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. 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.

Improved ChIP

Chromatin Immunoprecipitation (ChIP) is an important technique, enabling the identification of the *in vivo* sites of chromatin binding proteins, or localization of histone modifications to specific regions of the genome. Recent improvements in ChIP protocols and reagents have made ChIP experiments faster and easier to perform, as well as more successful. In addition, the lower background enabled by improved ChIP techniques has made it possible to perform ChIP with fewer cells, and to perform more ChIP reactions at one time. Active Motif has been at the forefront of these advances with the introduction of magnetic beads for ChIP in its ChIP-IT Express Kit. The ChIP-IT Express HT Kit is the next stage in the development of kits for chromatin IP.

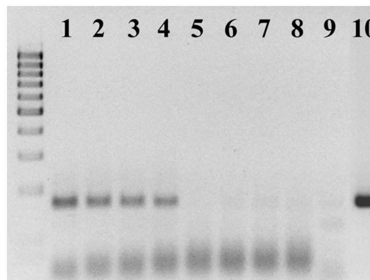


FIGURE 1:
Chromatin IP performed on HeLa chromatin with ChIP-IT Express HT. PCR carried out using primers specific for the GAPDH gene. Lanes 1-4: ChIP using 2 µg RNA Pol II antibody. Lanes 5-8: ChIP using normal mouse IgG as a negative control. Lane 9: no DNA control. Lane 10: input DNA control.

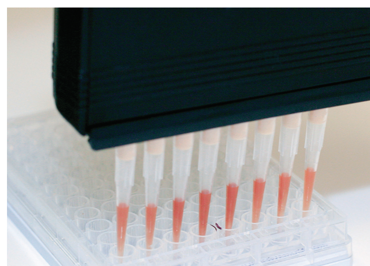


FIGURE 2:
True high-throughput ChIP with ChIP-IT Express HT.
With the efficient plate-based protocol of ChIP-IT Express HT, you can process up to 96 ChIP reactions at a time.

FREE 96-well magnetic sorter!

Order ChIP-IT Express HT and receive a free 96-well magnetic sorter. Available while supplies last.

For additional information on the new ChIP-IT Express HT Kit, go to: www.activemotif.com/htchip.

Product	Format	Cat. No.
ChIP-IT™ Express HT	96 rxns	53018

Re-ChIP-IT™

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

Performing sequential chromatin IP (also called Re-ChIP) was technically challenging and difficult, until now. Active Motif's 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.

Extend the utility of ChIP

When performing chromatin immunoprecipitation (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. Re-ChIP (aka Sequential ChIP, Chromatin Re-IP and ChIP Re-ChIP) is a relatively new technique that enables sequential chromatin immunoprecipitations to be performed using two different antibodies, enabling you to you assay for the simultaneous presence of two proteins or distinct histone modifications at the same genomic region of interest.

Active Motif's new Re-ChIP-IT Kit makes it simple for you to perform these types of experiments. All buffers for chromatin IP are included, making it easy to get started. And, the detailed protocol ensures you're successful the first and every time. Plus, Active Motif offers a variety of chromatin IP control kits to help you validate the results of your Re-ChIP experiments.

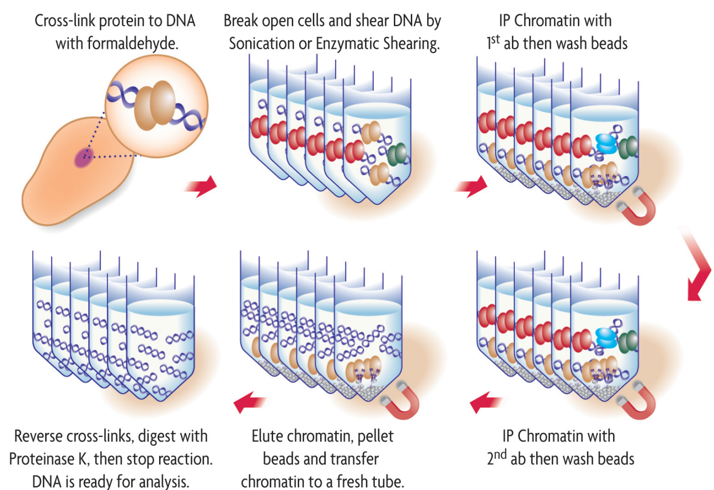


FIGURE 2:
Schematic representation of the Re-ChIP-IT procedure.

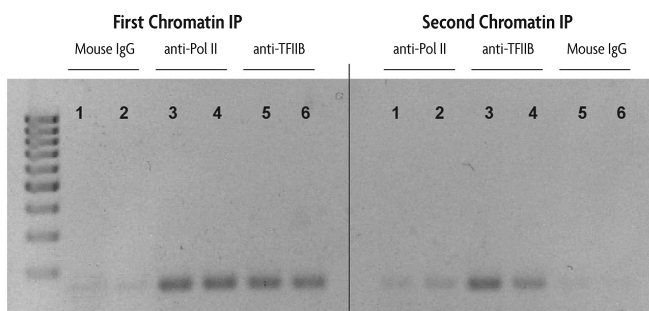


FIGURE 1:
Sequential chromatin immunoprecipitation using Re-ChIP-IT.
The lane numbers are the same in each panel to indicate that the DNA is from the same chromatin sample. The left panel shows the results of PCR performed on an aliquot of DNA removed from the experiment after the first ChIP step; the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

Sequential chromatin IP made easy

Re-ChIP-IT uses magnetic beads that have less background than standard agarose beads, so pre-clearing and blocking steps are not needed. Magnetic pull-down occurs in just seconds, and the method's low background has eliminated the need for DNA purification. And, Re-ChIP-IT can be used with chromatin prepared using our sonication or enzymatic shearing kits.

Get started with Re-ChIP

For additional information on the new Re-ChIP-IT Kit go to **www.activemotif.com/rechip**.

Product	Format	Cat. No.
Re-ChIP-IT™	25 rxns	53016

ChIP Accessory Kits and Reagents

maximize your chromatin IP experiments

ChIP & IP enhancer for mouse antibodies

Low antibody binding affinity can make getting good ChIP & IP results difficult. Because mouse IgM and IgG_1 antibodies do not have strong binding affinity for protein G, ChIP and regular immunoprecipitation (IP) can be challenging when using protein G-conjugated beads. The Bridging Antibody for Mouse IgG facilitates improved binding of mouse antibodies, especially IgG_1 and IgM isotypes, to protein G to generate more robust binding and better results in ChIP and standard IP experiments (Figure 1).

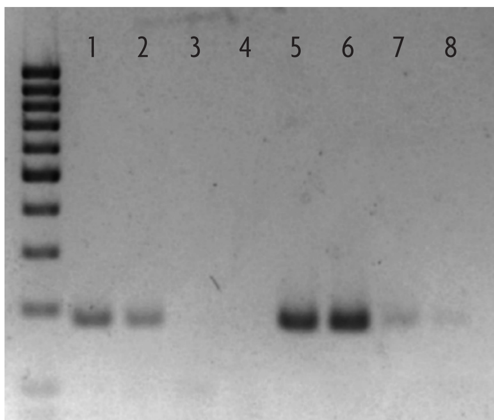


FIGURE 1:
Improvement in chromatin IP using an anti-mouse bridging antibody.
ChIP was performed using chromatin from U-937 cells induced with TNF-α. PCR was performed with primers corresponding to the human IL-8 promoter.
Lanes 1-4: beads not pre-incubated with bridging antibody.
Lanes 5-8: beads pre-incubated with 5 µg bridging antibody.
Lanes 1, 2 & 5, 6: ChIP performed using anti-p65 mouse mAb, 2 µg per IP.
Lanes 3, 4 & 7, 8: ChIP performed using negative control mouse IgG.

Product	Format	Cat. No.
Bridging Antibody for Mouse IgG	500 µg	53017

Beads, controls and shearing kits

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

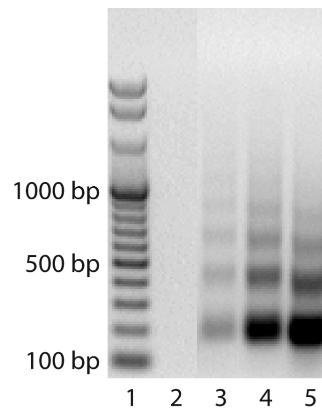


FIGURE 2:
Analysis of DNA sheared using the Enzymatic Shearing Kit.
HeLa cells were fixed for 10 minutes with 1% formaldehyde and then chromatin was prepared using the Enzymatic Shearing Kit protocol. Chromatin was sheared with the Enzymatic Shearing Cocktail for 5, 10 & 15 minutes and the reaction was stopped.

Lane 1: 100 to 1000 bp ladder.
Lane 2: Unsheared HeLa DNA.
Lane 3: HeLa DNA treated for 5 minutes.
Lane 4: HeLa DNA treated for 10 minutes (optimized).
Lane 5: HeLa DNA treated for 15 minutes.

Ready-to-ChIP Chromatin

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

Product	Format	Cat. 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

ACTIVE MOTIF®

Histone H3 phospho Ser28 & mono-, di- and tri-methyl Lys4 ELISA Kits

screen extracts for histone modifications

Active Motif has applied our histone-modification antibody expertise to identify optimal antibody pairs for the detection of specific histone modifications in a sandwich ELISA format. Now it is easy to quickly screen extracts for changes in histone H3 phosphorylation and methylation levels.

The Histone ELISA advantage

Screening extracts by standard immunoblotting methods is time consuming. Our Histone Modification ELISA kits enable you to efficiently screen up to 96 wells at once, with little hands-on time and colorimetric results in hours. Active Motif's high-quality histone antibodies guarantee specific results with extremely low background (Figures 1 & 2) For added convenience, Paclitaxel-treated HeLa acid extract is included in the Histone H3 phospho Ser28 ELISA Kit. Recombinant methylated histone H3 is included in the methylation-specific ELISA kits.

WHY USE HISTONE ELISA KITS?

- Sensitive – works with < 78 ng of acid extract
- Specific – C-terminal histone H3 capture
- Efficient – no time-consuming immunoblotting
- Convenient strip-well format – use only what you need

Histone H3 phospho Ser28

Cell division is a complex, tightly regulated process that is marked by mitosis. Two significant mitotic events include microtubule spindle formation and chromosome condensation. Histone H3 is phosphorylated on serine 28 during mitotic chromatin condensation before nuclear division occurs, which makes phosphorylation of histone H3 at Ser28 an important marker for cells undergoing mitosis. With this simple ELISA format, it is easy to assess levels of phosphorylated histone H3 Ser28 in cell extracts (Figure 1).

Histone H3 mono-, di- and tri-methyl Lys4

Methylation of Histone H3 at lysine 4 has been shown to indicate transcriptionally active chromatin. With the Histone H3 ELISA Kits for methylated Lys4, it is easy to screen for changes in the degree of methylation at lysine 4 in your acid extract samples (Figure 2). Histone H3 methylated Lys4 ELISA Kits are available for mono-, di- and tri-methylated lysine 4. Each kit includes a recombinant Histone H3 mono-, di- or tri-methylated lysine 4 (page 17) that can be used to quantitate the levels of specific methylation in your sample. The ELISA kits utilize the same histone H3 C-terminal antibody for capture, and each kit uses either the mono-, di- or tri-methyl Lys4 antibody for specific detection (Figure 3).

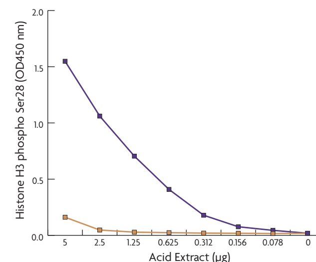


FIGURE 1:
Histone H3 phospho Ser28 levels in HeLa acid extracts.
78 ng to 5 µg untreated (copper line) and paclitaxel treated (purple line) HeLa acid extract assayed with the Histone H3 phospho Ser28 ELISA.

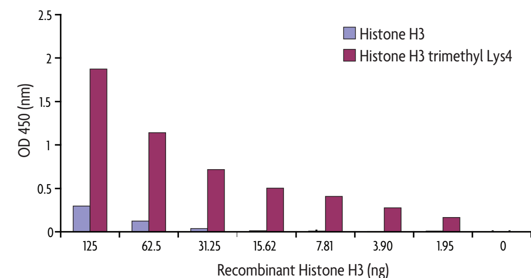


FIGURE 2:
Histone H3 trimethyl Lys4 detection.
Recombinant Histone H3 and Recombinant Histone H3 trimethyl Lys4 were assayed from 125 ng down to 1.95 ng using the Histone H3 trimethyl Lys4 ELISA.

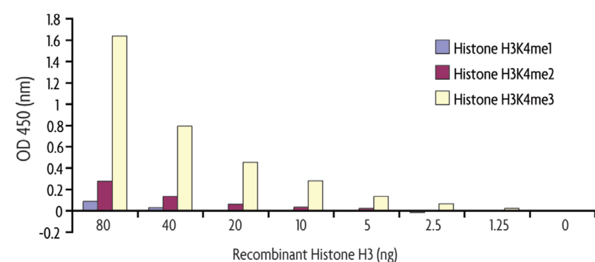


FIGURE 3:
Specific histone H3 trimethyl Lys4 detection.
Recombinant Histone H3 monomethyl Lys4 (H3K4me1), Recombinant Histone H3 dimethyl Lys4 (H3K4me2) and Recombinant Histone H3 trimethyl Lys4 (H3K4me3) were assayed from 80 ng to 1.25 ng with the Histone H3 trimethyl Lys4 ELISA. The assay is clearly very specific for the trimethyl Lys4 modification on Histone H3.

Product	Format	Cat. No.
Histone H3 phospho Ser28 ELISA	96 rxns	53100
Histone H3 monomethyl Lys4 ELISA	96 rxns	53101
Histone H3 dimethyl Lys4 ELISA	96 rxns	53102
Histone H3 trimethyl Lys4 ELISA	96 rxns	53103
HeLa acid extract	100 µg	36200
HeLa acid extract (Paclitaxel treated)	100 µg	36201

Chromatin Assembly Kit

generate high-quality chromatin for downstream success

Active Motif's Chromatin Assembly Kit enables the generation of chromatin *in vitro* from linear or supercoiled DNA. Based on an ATP-dependent method, the kit utilizes purified recombinant human chromatin assembly complex ACF and the histone chaperone NAP-1 (h-NAP-1) with purified HeLa core histones for *in vitro* assembly of extended, regularly ordered, periodic arrays of nucleosomes. The resulting chromatin closely resembles natural *in vivo* chromatin, enabling studies of histone modifications and associated proteins that are crucial to regulation of the target DNA sequence.

The assembly of genomic DNA and histones into chromatin is a fundamental process that affects a broad range of genome-dependent processes including DNA replication, DNA repair and gene expression. In general, there are ATP-dependent and ATP-independent methods for reconstituting or assembling chromatin *in vitro*. The ATP-independent method results in a random arrangement of histones on the DNA that does not accurately reflect the native core nucleosome. The resulting mononucleosomes are not regularly ordered, extended nucleosomal arrays and the DNA sequence used is usually no longer than 250 bp. To generate an extended array of ordered nucleosomes on a length of DNA greater than 250 bp, the chromatin must be assembled through the ATP-dependent process. The Chromatin Assembly Kit provides an easy and complete solution for ATP-dependent chromatin reconstitution and produces an excellent substrate for downstream assays.

The Chromatin Assembly Kit advantage

In the Chromatin Assembly method, recombinant h-NAP-1 and HeLa core histones are combined in a high-salt buffer, which is ideal for proper histone configuration. Recombinant ACF complex and sample DNA are then added with the complete ATP regeneration system to complete the Chromatin Assembly. After a four-hour incubation, the assembled chromatin is ready to use in downstream assays such as *in vitro* transcription assays, chromatin immunoprecipitation and histone acetyltransferase (HAT) assays. Chromatin assembly is verified by partial digestion with the provided Enzymatic Shearing Cocktail and analyzed by gel electrophoresis. High-quality chromatin should yield six or more distinct bands (Figure 1). For your convenience, supercoiled DNA is provided as a positive control.

Why use the Chromatin Assembly Kit?

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

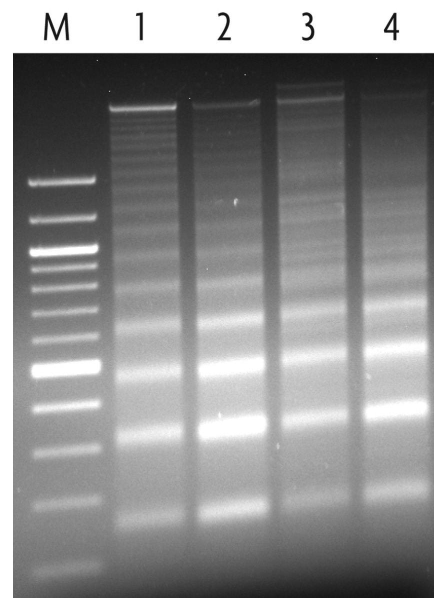


FIGURE 1:
Enzymatic digestion of assembled chromatin.
Chromatin assembled from 1 µg samples of circular DNA (Lanes 1 & 2) and linear DNA (Lanes 3 & 4) were digested for 2 and 4 minutes, respectively, deproteinated, phenol/chloroform extracted and run on an agarose gel. Each sample type processed with the Chromatin Assembly Kit resulted in regularly spaced nucleosomes.

Product	Format	Cat. No.
Chromatin Assembly Kit	10 rxns	53500

Histone Purification Kit

isolate pure fractions of core histone proteins while preserving post-translational modifications

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 states.

How does it work?

Active Motif's Histone Purification Kit enables you to isolate core histones from any cell culture or tissue sample (Figure 1). Unlike histone purification by acid precipitation, our method utilizes a unique purification resin and a series of proprietary elution buffers to isolate very pure histone fractions. The resin has a high binding capacity for histones, so core histones can be isolated from small cell culture samples on up to grams of tissue.

Sequential elution steps let you collect the core histones as one total population containing H2A, H2B, H3 and H4, or further separated into two populations: one enriched for H2A/H2B dimers and a second fraction containing > 90% pure H3/H4 tetramers.

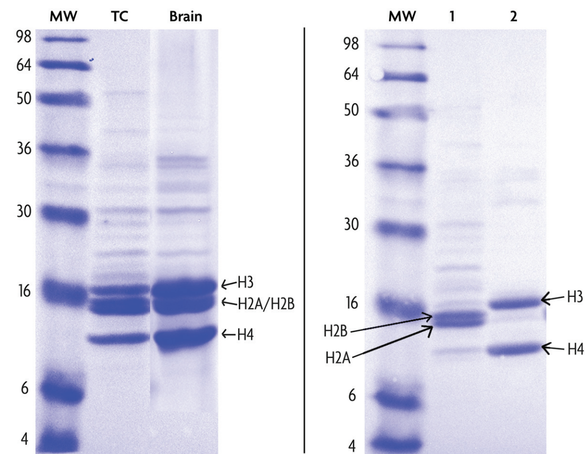


FIGURE 1:
SDS-PAGE of histone fractions purified using the Histone Purification Kit.
Ten µg of sample were loaded per lane and run on a 16% Tris-glycine gel. Left panel: core histones purified from logarithmically growing tissue culture cells (TC) and core histones isolated from rat brain tissue (Brain). Right panel: H2A/H2B (Lane 1) and H3/H4 (Lane 2) fractions purified from HeLa cells.

Preserve important modifications

Preserving post-translational modifications like acetylation, methylation and phosphorylation is critical when investigating the role of histones in transcription or chromatin biology. Our proprietary buffer system is optimized to maintain these modifications while separating the histones into pure fractions (Figure 2).

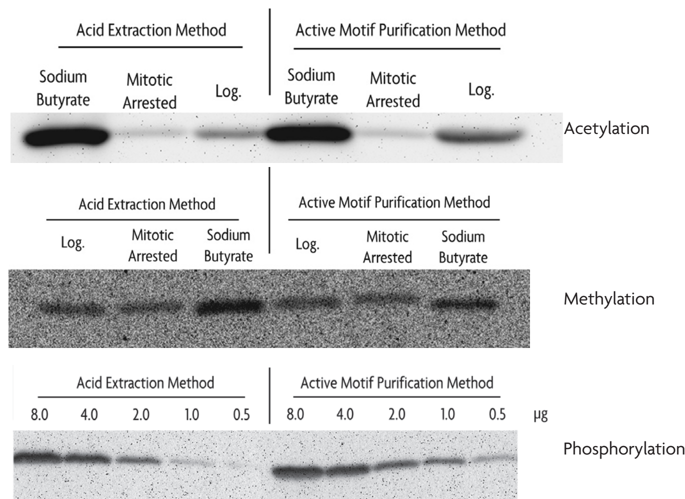


FIGURE 2:
Post-translational modifications preserved.
Acetylation, methylation and phosphorylation states are preserved as well or better with the Histone Purification Kit compared with standard acid precipitation method.

Better substrate for downstream assays

Core histones isolated by the Histone Purification Kit method are highly pure and suitable substrates for downstream assays. Purified histones can be used with the Chromatin Assembly Kit (page 13) to enable the generation of chromatin that very closely resembles native chromatin for functional assays (Figure 3).

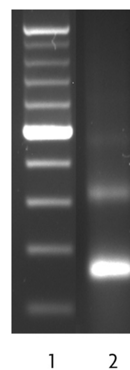


FIGURE 3:
Chromatin assembled with purified histones.
Histones were purified from HeLa cells and used in the Chromatin Assembly Kit. The ordered spacing of nucleosomes was confirmed and analyzed by agarose gel.

Product	Format	Cat. No.
Histone Purification Kit	10 rxns	40025

Histone Purification Mini Kit (Spin Column Format)

faster, easier histone purification while preserving post-translational modifications

Active Motif first brought you the ground breaking Histone Purification Kit, enabling the fast and easy isolation of core histones from any cell culture or tissue sample. Now, we are expanding upon that innovative product by introducing our new spin column-based Histone Purification Mini Kit, which is even faster, more sensitive and easier to use. Purification of histone from yeast cells is also possible with this new kit. And, like the original kit, post-translational modifications remain intact.

Histone Purification Mini Kit advantages

- Convenient spin column-based protocol
- Fast, efficient, requiring less hands on time
- Prepare histones from multiple samples simultaneously

How does it work?

Our unique method utilizes a purification column with a proprietary binding matrix (Figure 1) and a series of proprietary buffers to isolate very pure fractions of histones. Using the Histone Purification Mini Kit, core histones can be purified from cultured cells, yeast cells and tissue samples as a single population containing H2A, H2B, H3 and H4 (Figure 2). The column has a high affinity for histones, so histones can be purified from as little as $8x10^5$ cells. While you can not purify the same amount of histones as with the Histone Purification Kit, spin columns make it easier to isolate histones from multiple samples simultaneously.

Source	Yield
Adherent Cells	0.1 mg total core histones from 8 x 10^6 cells.
Suspension Cells	0.1 mg total core histones from 8 x 10^6 cells.
Yeast	0.5 mg total core histones from 8 x 10^7 cells.
Tissue	1 mg histone per gram of tissue*

TABLE 1:
Representative histone yields.
Yields are approximate. Results may vary according to cell or tissue type.

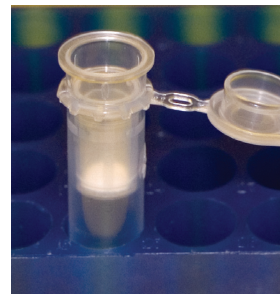


FIGURE 1:
Histone purification in a new spin column format.
Convenient spin column-based protocol enables faster purification of histones from multiple samples simultaneously.

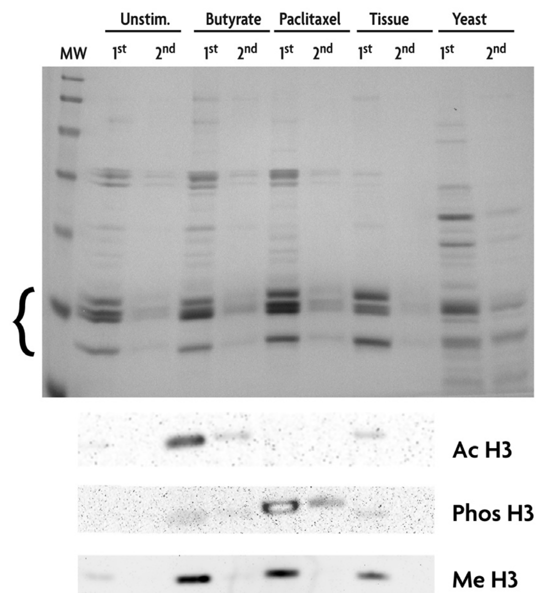


FIGURE 2:
Analysis of histone fractions by SDS-PAGE and Western blot.
Histones were purified from a variety of sources using the Histone Purification Mini Kit. (Unstim.: unstimulated HeLa cells, Butyrate: sodium butyrate-treated HeLa cells, Paclitaxel: paclitaxel-treated HeLa cells, Tissue: rat brain tissue, Yeast: budding yeast). The first and second elutions (labeled 1st and 2nd) from each sample were analyzed on a 16% Tris-glycine SDS-PAGE gel (top panel) and by Western blot (bottom panel). Antibodies recognizing acetyl-histone H3 (Ac-H3: Catalog No. 39139, 1:500 dilution), phospho-Ser28 histone H3 (Phos-H3: Catalog No. 39098, 1:1,000 dilution), and trimethyl-Lys4 histone H3 (Me-H3: Catalog No. 39159, 1:1,000 dilution). The migration of the histones in the SDS gel is indicated by the bracket to the left of the gel.

Post-translational modifications remain intact

Preserving post-translational modifications like acetylation, methylation and phosphorylation is critical when investigating the role of histones in transcription or chromatin biology. Our proprietary buffer system is optimized to maintain these modifications while separating the histones into pure fractions (Figure 2). Histones isolated with the Histone Purification Mini Kit are highly pure and are suitable as substrates in many downstream assays. Purified histones can be analyzed by Western blot or mass spectrometry, or assembled into chromatin that closely resembles native chromatin for functional assays using the Chromatin Assembly Kit (see page 13).

Product	Format	Cat. No.
Histone Purification Mini Kit	20 rxns	40026

HAT & HDAC Assay Kits

rapid, sensitive assays for HAT & HDAC activity and inhibitor compounds

Active Motif's HAT & HDAC Assay Kits are easy-to-use, sensitive assays that can be used to determine the activity of histone acetyltransferases and histone deacetylases in your cell and nuclear extracts, immunoprecipitates and purified enzymes, as well as to screen the effects of potential inhibitor compounds. The HAT Assay Kit uses a fluorescent readout, while HDAC Assay Kits are available in both fluorescent and colorimetric formats.

The HAT Family

Histone acetyltransferases (HAT) are enzymes that acetylate conserved lysine amino acids on histones. Generally, histone acetylation is associated with the activation of gene expression, as hyperacetylated chromatin is transcriptionally active. Histone deacetylases (HDAC) remove these acetyl groups from histones. Their action is opposite to that of histone acetyltransferases, as hypoacetylated chromatin is silent. Because HATs and HDACs are involved with other proteins in the regulation of gene expression, their activity is much studied, as are compounds that inhibit HAT and HDAC activity.

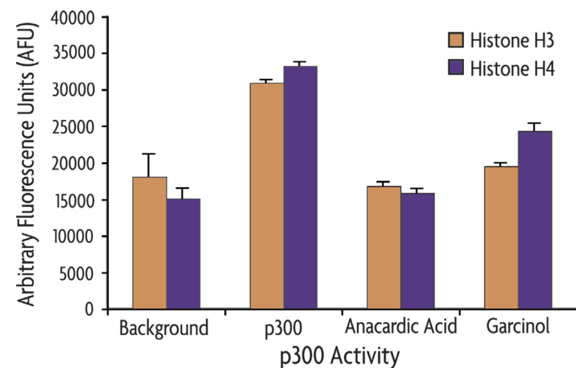


FIGURE 1:
HAT inhibitor effects on p300 activity.
50 ng p300 were assayed per well with 50 µM acetyl-CoA and 50 µM histone H3 or H4 peptide substrates in the absence or presence of 15 µM anacardic acid or 25 µM garcinol, known HAT inhibitor compounds.

How does the HAT Assay Kit work?

Assaying HAT activity is easy with this 96-well plate format. Simply incubate your HAT with your choice of the provided Histone H3 or Histone H4 substrate peptides and acetyl-CoA for 10-30 minutes, then develop. The HAT Assay Kit uses a thiol-reactive fluorescent dye that reacts with the Co-A-SH generated by the histone acetyltransferase activity to give a fluorescent readout. This makes it easy to generate standard curves with acetyl-CoA or β-mercaptoethanol, so you can relate the fluorescence of your HAT to pmol/min/µg specific activity.

Active recombinant p300 protein is provided as a control for use with your samples; enough is provided to use as a HAT to screen an entire 96-well plate of inhibitors. Anacardic acid is also provided for use as a control, as it is a potent HAT inhibitor (Figure 1).

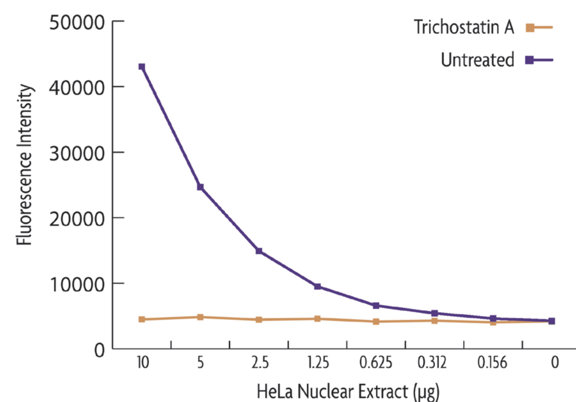


FIGURE 2:
HDAC activity in HeLa cells.
HeLa nuclear extracts were assayed at 0 to 10 µg per well using the fluorescent version of the HDAC Assay Kit. Untreated extract results are shown with a purple line, and extracts inhibited with 1 mM Trichostatin A are shown with a copper line.

How do the HDAC Assay Kits work?

The HDAC Assay Kits utilize a peptide substrate that contains an acetylated lysine residue 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 in the assay.) Once the substrate is deacetylated, the lysine reacts with the Developing Solution and releases either the chromophore or the fluorophore from the substrate, which produces either a colorimetric or fluorescent product. The colorimetric product absorbs maximally at 405 nm; the fluorescent product can be read with an excitation wavelength of 360 nm and emission wavelength of 460 nm (Figure 2).

Product	Format	Cat. 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

Recombinant Methylated Histones

new with site- & degree-specific methylated lysines

Active Motif is pleased to announce the first release in its new collection of Recombinant Histones, which focuses on Histone H3 proteins with site-specific mono-, di- and tri-methylated lysines. These proteins enable you to investigate how specific lysine methylations influence nucleosome remodeling and the binding of associated chromatin proteins.[1]

Histones & chromatin structure

Methyl-lysine residues in nucleosomal histones are thought to mediate interactions with the protein complexes involved in regulating transcription, replication and DNA repair. In order to investigate these complex functional questions, histones with specific methylation states are required to evaluate which methylation patterns are key to regulatory processes. Active Motif is pleased to be the first to offer methylated histone H3 proteins that can be used in nucleosome remodeling assays to investigate the implication of specific methylation on chromatin function.

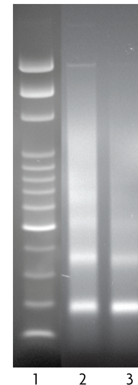


FIGURE 1:
Ordered spacing of nucleosomes after enzymatic digestion of assembled chromatin.
4.5 µg Recombinant Histone H3 dimethyl Lys9 was used to generate chromatin *in vitro* using the Chromatin Assembly Kit (see page 13). One µg of assembled chromatin was digested for 2 minutes (lane 2) and 4 minutes (lane 3). Lane 1 is 100 bp marker.

Methylated histone H3

Active Motif currently offers recombinant histone H3 proteins that are mono-, di- and tri-methylated at lysines 4, 9 & 27. Visit our website to see the latest releases of methylated histones.

How is the methylation state made?

Recombinant methylated histones are created via a chemical alkylation reaction* that introduces an analog of methyl lysine. This specific chemical treatment enables the site and degree of methylation to be controlled precisely, so each methylation reaction is over 99% complete, as verified by high-resolution ESI-TOF mass spectrometry. All recombinant histones are also confirmed by dot blot or immunoblot (Figure 2). As the methylation state closely mimics natural methylation, these recombinant histones are perfect for any functional assay.

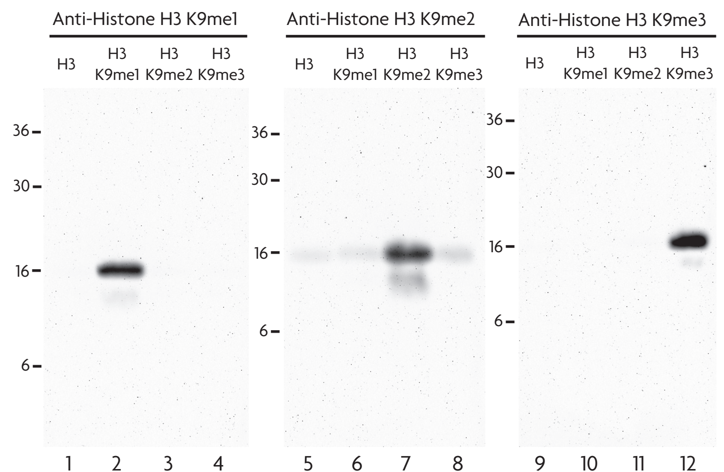


FIGURE 2:
Analysis of mono-, di- and tri-methylated Recombinant Histone H3.
Western blot analysis of 1 µg Recombinant Histone H3 (lanes 1, 5 & 9), 1 µg Recombinant Histone H3 monomethyl Lys 9 (lanes 2, 6 & 10), 1 µg Recombinant Histone H3 dimethyl Lys9 (lanes 3, 7 & 11) and 1 µg Recombinant Histone H3 trimethyl Lys9 (lanes 4, 8, and 12) probed with Histone H3 monomethyl Lys9 pAb (Cat. No. 39249) (lanes 1-4), Histone H3 dimethyl Lys9 pAb (Cat. No. 39239) (lanes 5-8) and Histone H3 trimethyl Lys9 pAb (Cat. No. 39161) (lanes 9-12).

Product	Format	Cat. No.
Recombinant Histone H3 (C110A)	50 µg	31207
Recombinant Histone H3 monomethyl Lys4	50 µg	31208
Recombinant Histone H3 dimethyl Lys4	50 µg	31209
Recombinant Histone H3 trimethyl Lys4	50 µg	31210
Recombinant Histone H3 monomethyl Lys9	50 µg	31211
Recombinant Histone H3 dimethyl Lys9	50 µg	31212
Recombinant Histone H3 trimethyl Lys9	50 µg	31213
Recombinant Histone H3 monomethyl Lys27	50 µg	31214
Recombinant Histone H3 dimethyl Lys27	50 µg	31215
Recombinant Histone H3 trimethyl Lys27	50 µg	31216

*Patent pending.

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

MethylDetector™

simplified bisulfite conversion of DNA with easily verified results

Active Motif's MethylDetector™ Bisulfite Modification Kit simplifies analysis of DNA methylation. It comes complete with optimized reagents for performing DNA conversion with bisulfite, plus time-saving DNA purification columns and positive control PCR primers to validate your results.

DNA methylation is a naturally occurring event that affects cell function by altering gene expression. A methyl group is added to the fifth-carbon of cytosine in a CpG dinucleotide by DNA methyltransferase. As aberrant methylation is prevalent in many human cancers, and because methylation is also involved in embryonic development and cell cycle regulation, much research depends on accurately quantifying DNA methylation. Many DNA methylation analysis methods begin by using bisulfite to convert unmethylated cytosines to uracils.[1,2] Unmethylated cytosine is changed to uracil, which base pairs with adenosine, not guanosine. During conversion, methylated cytosines remain unchanged. The DNA is then amplified by PCR and analyzed by sequencing or restriction digest. A methylation profile of the sample can then be created by comparing the sequence of the converted DNA to untreated DNA. However, bisulfite conversion can be technically challenging, and it is desirable to confirm that the process was successful before spending time and money on sample analysis. To help ensure your success, the MethylDetector Kit provides optimized conversion reagents, an easy-to-use protocol and positive control PCR primers that are specific for bisulfite-converted DNA. Because these primers produce a PCR product only if conversion has occurred, you can confirm the procedure worked before performing sequencing or other analysis methods.

The MethylDetector™ advantage

In the MethylDetector method, DNA of interest is rapidly heat denatured in a thermocycler in the presence of the bisulfite conversion reagent. The temperature is then lowered and the conversion reaction is performed. Unlike other methods, MethylDetector does not require a separate denaturation step as the conversion reagent includes a DNA denaturant, saving you time and effort. After DNA conversion, the sample is added to the included DNA purification columns, and a simple, on-column desulfonation reaction is performed. Ready-to-use DNA is then eluted from the columns. For your convenience, the included positive control PCR primers can be used to assess the success of the bisulfite conversion before you spend time and money on DNA sequencing. The included primers only anneal to converted human DNA (Figure 1).

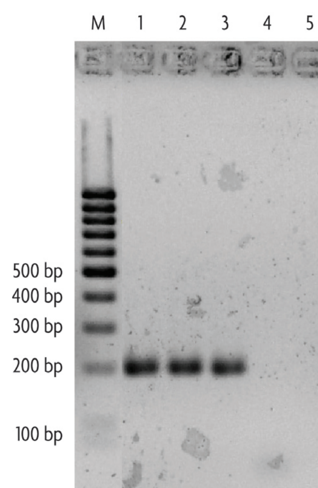


FIGURE 1:
Agarose gel analysis of PCR products generated with MethylDetector.
Three different DNA conversions were performed (Lanes 1-3) and compared to an unconverted DNA control (Lane 5) and to a no DNA control (Lane 4). The presence of PCR product in only the converted samples demonstrates the conversion efficiency and reproducibility of MethylDetector.

Why use MethylDetector™?

- Works efficiently with high G/C content sequences and uncut DNA
- Reproducible assay consistently provides 99% conversion efficiency of unmethylated cytosines
- Optimized reagents and protocol with proven human controls
- Combined thermal denaturation and conversion reaction eliminates NaOH-mediated denaturation and streamlines procedure
- DNA purification columns eliminate the need for precipitation and a separate desulfonation step
- High yield of converted DNA ideal for downstream analysis

Product	Format	Cat. No.
MethylDetector™	50 rxns	55001
Fully Methylated Jurkat DNA	10 µg	55003

REFERENCES
1. Frommer, M. *et al.* (1992) *PNAS* **89**: 1827.
2. Clark, S.J. *et al.* (1994) *Nuc. Acids Res.* **22**: 2990-2997.

MethylCollector™

rapid and efficient comparison of methylation in various samples

Active Motif's MethylCollector™ Kit provides users with a fast and efficient method* for isolating and comparing CpG-methylated DNA from various cell or tissue samples. MethylCollector uses a recombinant Methyl-binding protein (MBD2b) to capture DNA, rather than traditional antibody-based immunoprecipitations, improving sensitivity.

MethylCollector™ Advantages

- Fast and easy protocol completed in less than 4 hours
- Flexible – enables detection from 5 ng to 1 µg of DNA
- Suitable for use with DNA fragmented by sonication or enzymatic digestion
- Positive control DNA and PCR primers help ensure your success

The MethylCollector™ method

In the MethylCollector method, His-tagged recombinant MBD2b protein specifically binds to CpG-methylated DNA fragments that have been prepared by enzymatic digestion or sonication. These protein-DNA complexes are captured with nickel-coated magnetic beads and subsequent wash steps are performed with a stringent high-salt buffer to remove DNA fragments that have little or no methylation. Ready-to-use methylated DNA is then eluted from the beads (Figure 1). MethylCollector is highly efficient, enabling analysis of the methylation state of any specific locus on genomic DNA isolated from less than 800 cells (~5 ng DNA, Figure 2).

Applications of MethylCollector™

The highly specific isolation of methylated DNA by MethylCollector enables powerful applications, including rapid screening of the methylation status of multiple loci in tumor tissue or cells. It can also be used to detect changes in DNA methylation in other situations, *e.g.* cellular differentiation, aging and cancer.

*Technology covered under U.S. Patent No. 7,425,415.

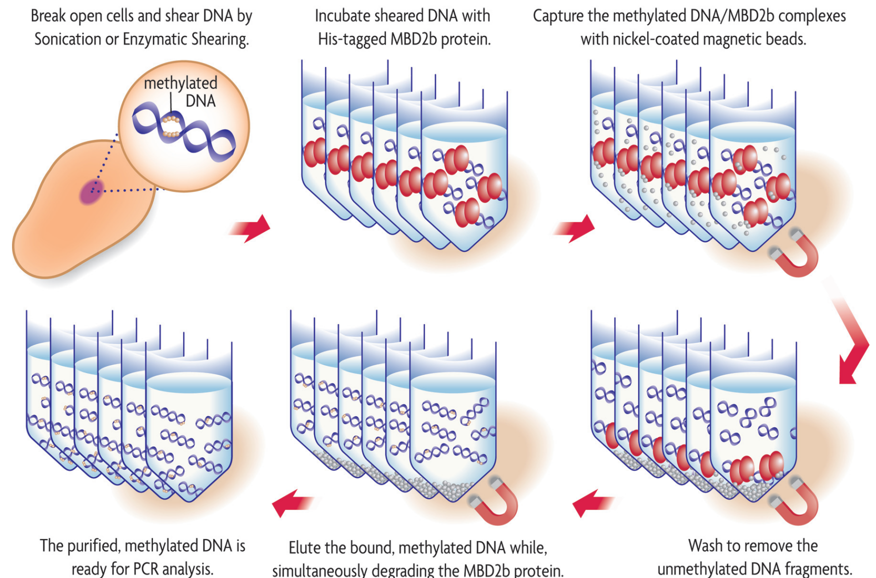


FIGURE 1:
Flow chart of the MethylCollector process.
In a MethylCollector assay, genomic DNA of interest is sheared by either enzymatic digestion or sonication. The sheared DNA is then incubated with His-tagged recombinant MBD2b protein, which has a strong affinity for CpG-methylated DNA. These protein-DNA complexes are captured with nickel-coated magnetic beads and stringent washes are performed to remove DNA fragments that have little or no methylation. The methylated DNA is then eluted from the beads and PCR is performed on the resulting supernatant, using primers that are specific to amplify the locus of interest.

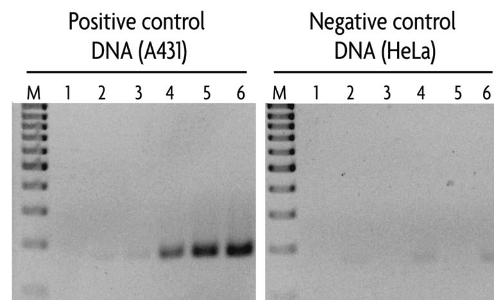


FIGURE 2:
Comparison of CpG-methylated DNA isolated from HeLa and A-431 cells using MethylCollector.
Genomic DNA from A-431 (positive control DNA) and HeLa (negative control DNA) cells were enzymatically digested by *Mse* I for 2 hours. Increasing amounts of fragmented DNA (from 5, 40 and 100 ng) were then incubated for 1 hour with 1 µg His-MBD2b recombinant protein in the presence of nickel-coated magnetic beads. After washing, CpG-methylated complexes were eluted. The isolated DNA was then analyzed by 36 cycles of PCR using the kit's control primers, which amplify a locus that is not methylated in HeLa but highly methylated in A-431. PCR on MethylCollector-enriched A-431 DNA generates robust signals that are proportionate to the amount of DNA starting material (left panel, lanes 4 to 6). No signal is observed with enriched HeLa DNA (right panel, lanes 4 to 6) or in those samples where His-MBD2b protein was omitted from the binding reaction (left and right panels, lanes 1 to 3). Taken together, these results indicate that MethylCollector specifically enriches for methylated DNA fragments, and that this enrichment is due to the presence of the kit's His-MBD2b protein.

Product	Format	Cat. No.
MethylCollector™	25 rxns	55002

Nuclear Extract Kit

high yields of specifically segregated extracts

The Nuclear Extract Kit is ideal for the preparation of nuclear, whole-cell and cytoplasmic extracts from mammalian cells and tissues. The resultant high-quality extracts may be used with Active Motif's TransAM™ Kits or in gelshift assays, Western blots, DNA footprinting or as a starting point for transcription factor purification.

The Nuclear Extract Kit eliminates the need to optimize reagents and ensures consistently high yields. The detailed protocol helps ensure that your extract is not contaminated with proteins from other cellular compartments (Figure 1).

The Nuclear Extract Kit advantage

In the Nuclear Extract Kit, cells are collected in ice-cold PBS in the presence of phosphatase inhibitors to limit further protein modifications. Next, the cells are resuspended in hypotonic buffer to swell the cell membrane. Addition of detergent causes leakage of the cytoplasmic proteins into the supernatant. After collection of the cytoplasmic fraction, the nuclei are lysed and the nuclear proteins are solubilized in lysis buffer in the presence of the protease inhibitors. Whole-cell extracts can also be prepared by collecting the cells in the PBS/phosphatase inhibitors solution and lysing in lysis buffer. Solubilized proteins are separated from the cell debris by centrifugation. The concentration of protein in the cell extract is then measured by Active Motif's ProStain Protein Quantification Kit (Cat. No. 15001) or with a Bradford-based assay.

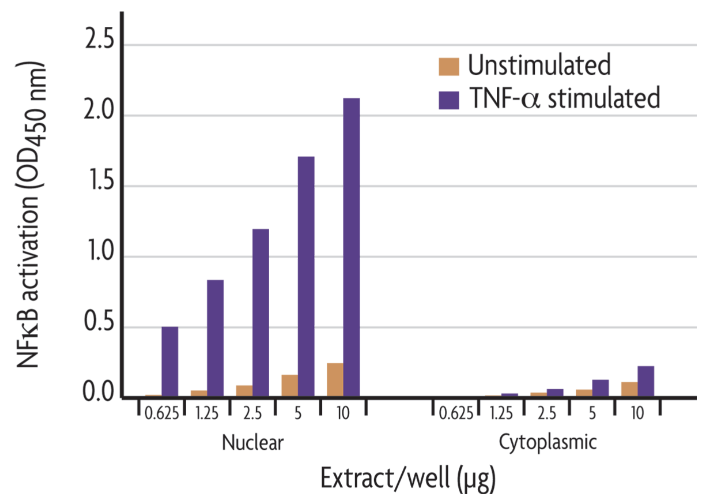


FIGURE 1:
Specific extraction of nuclear and cytoplasmic extracts.
Nuclear and cytoplasmic extracts were prepared using the Nuclear Extract Kit from HeLa samples unstimulated or stimulated with TNF-α for 30 minutes and assayed using the TransAM™ NFκB p50 Kit. Because activated NFκB translocates to the nucleus, only nuclear extract from stimulated cells should contain activated NFκB.

Why use the Nuclear Extract Kit?

- Quality-controlled reagents ensure reproducibility
- No need to optimize your own procedure
- Complete kit contains all required reagents
- Prepare nuclear, cytoplasmic or whole-cell extracts with one kit
- Ability to prepare extracts from both cultured cells and tissue samples

Product	Format	Cat. No.
Nuclear Extract Kit	100 rxns	40010
	400 rxns	40410

Universal Magnetic Co-IP Kit

lower background; suitable for both nuclear & whole-cell complexes

The Universal Magnetic Co-IP Kit improves co-immunoprecipitation (Co-IP) through the use of protein G-coated magnetic beads, which speed and simplify the IP and wash steps while greatly reducing background. The kit includes optimized reagents for making both nuclear & whole-cell extracts from cells or tissue, giving you the flexibility to Co-IP any protein complex, whether it was originally bound to DNA (Figure 1) or in the cytoplasm.

Co-IP cytoplasmic AND nuclear complexes

Co-IP is often used to study cytoplasmic protein complexes. But, traditional methods are not optimal for studying DNA-binding proteins because nuclear complexes are very fragile, causing them to be disrupted during extraction. For this reason, in addition to containing components for preparing whole-cell extracts, the Universal Magnetic Co-IP Kit provides nuclear extraction reagents that have been optimized to preserve nuclear protein complexes. The kit's Enzymatic Shearing Cocktail uses DNA digestion to gently release the nuclear protein complexes from the DNA, so they are intact and ready for Co-IP.

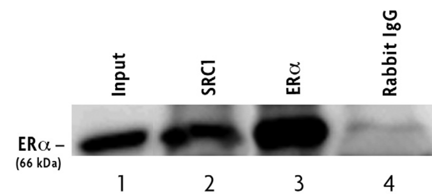


FIGURE 1:
Nuclear Co-IP of SRC-1 and ERα.
The Universal Magnetic Co-IP Kit was used to make nuclear extract from MCF-7 cells induced 1 hour with 10 nM Estradiol. IP was performed on 300 µg samples using 2 µg SRC-1 pAb, ERα pAb and rabbit IgG (as a negative control). Western blot was then performed using the ERα pAb on 10 µg Input Extract (Lane 1), SRC-1 IP (Lane 2), ERα IP (Lane 3) and the rabbit IgG IP (Lane 4).

Universal Magnetic Co-IP Kit advantages

- Magnetic beads simplify procedure and reduce background
- Optimized extraction method maintains nuclear protein complexes
- Preserve protein modifications

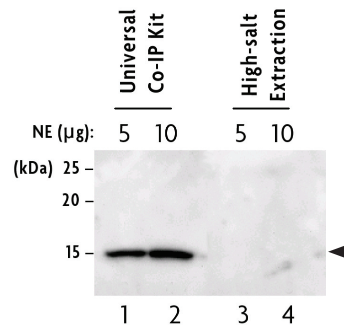


FIGURE 2:
Detection of acetylated Histone H3.
HeLa nuclear extracts were made using the Universal Magnetic Co-IP Kit and a traditional high-salt extraction protocol, each supplemented with 1 µM trichostatin A, a deacetylase inhibitor. Five and ten µg samples of each extract were used in Western blot with Histone H3 acetyl rabbit pAb (Cat. No. 39139). The pan acetyl-H3 (arrow) was detected only in samples made using the kit's gentle nuclear extraction procedure.

Simpler procedure, lower background

The Universal Magnetic Co-IP Kit utilizes protein G-coated magnetic beads, which simplify Co-IP by enabling the IP and wash steps to be performed in seconds, rather than having to use centrifugation. Because these beads have very low non-specific binding, background is reduced even while using the kit's low-salt Co-IP/Wash Buffer, which helps maintain weaker complexes.

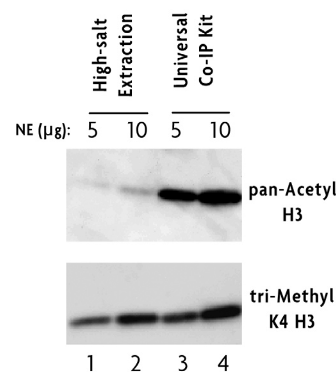


FIGURE 3:
Preserve acetylation and methylation.
Nuclear extracts were made from HeLa cells treated with 500 nM trichostatin A for 20 hours using either the Universal Magnetic Co-IP Kit (with its deacetylase inhibitor) or traditional high-salt extraction. Five and ten µg samples of these extracts were used in Western blot with Histone H3 acetyl pAb (Cat. No. 39139) and Histone H3 trimethyl Lys4 pAb (Cat. No. 39159). The acetylated protein was detected only in the sample made using the kit. Methylation was slightly better maintained in the sample made using the kit.

Complete kit for better results

The Universal Magnetic Co-IP Kit has both nuclear and whole-cell extraction reagents, so you can perform IP on all types of protein complexes. The kit also includes protein G-coated magnetic beads, a unique Co-IP/Wash Buffer as well as phosphatase, protease and deacetylase inhibitors that preserve the integrity of the proteins and protein modifications (Figures 2 & 3). Finally, the kit includes a strong bar magnet, so you can take advantage of the improved wash and IP steps enabled by the magnetic beads. This makes the Universal Magnetic Co-IP Kit a simple, flexible and complete solution for getting more from your Co-IP.

Product	Format	Cat. No.
Universal Magnetic Co-IP Kit	25 rxns	54002

ACTIVE MOTIF®

DNA Damage Assay

simple and fast 2-color fluorescent assay of H2AX phosphorylation

When cells are exposed to ionizing radiation, double-strand DNA breaks occur that stimulate the phosphorylation of the histone variant H2AX at serine 139 by the ATM kinase. As H2AX is present at approximately 10% of the level of histone H2A, detection of phosphorylated H2AX is an extremely sensitive marker to assess the effects and timing of DNA damage-inducing agents.

Active Motif's DNA Damage Assay makes screening compounds for their ability to induce DNA damage or apoptosis sensitive, accurate and reproducible. This assay can also be used to detect cells committed to undergo programmed cell death, as chromatin is cleaved by caspase-dependent nucleases, also leading to H2AX phosphorylation.

How does the assay work?

The DNA Damage Assay is a cell-based assay conducted in 96-well plates. Each kit includes all reagents necessary for two 96-well plates. Cells are grown and treated in the plate, fixed, then incubated with the phospho-histone H2AX (Ser 139) antibody. Next, a short incubation with Chromeo™ 488-conjugated secondary antibody is performed, followed by a readout on a standard or high-content fluorescence plate reader.

High specificity and low background

The DNA Damage Assay relies on a high-quality rabbit polyclonal phospho-histone H2AX (Ser 139) antibody that is subsequently detected with the Chromeo 488-conjugated Goat anti-Rabbit secondary. Our Chromeo fluorescent secondaries are highly specific with extremely low background, so your results are always accurate (Figure 1).

The assay includes a control compound, etoposide, which induces high levels of DNA damage and apoptosis (Figure 2). The phospho-histone H2AX (Ser 139) antibody is extremely specific, resulting in very low background in the assay. Propidium iodide is included to provide a marker for total cell count. This simple detection method utilizing Chromeo 488 fluorescence and propidium iodide stain makes the kit ideal for both high-throughput and high-content screening in all mammalian cell types (Figure 3).

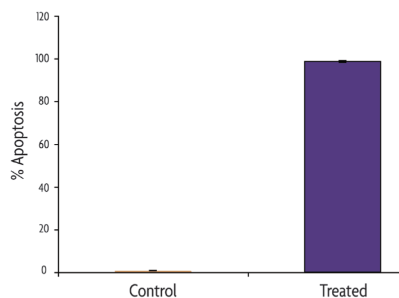
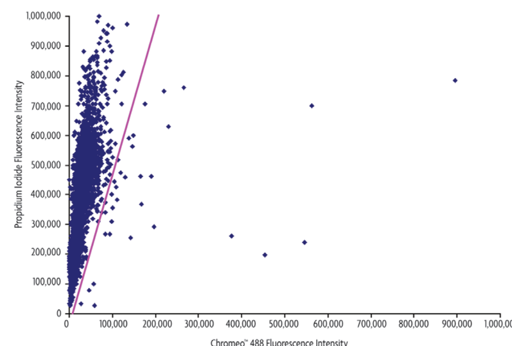


FIGURE 2:
Etoposide-induced DNA damage in HeLa cells.
Levels of phospho-H2AX measured in untreated (control) and cells treated with etoposide. Averages of quadruplicates are shown.



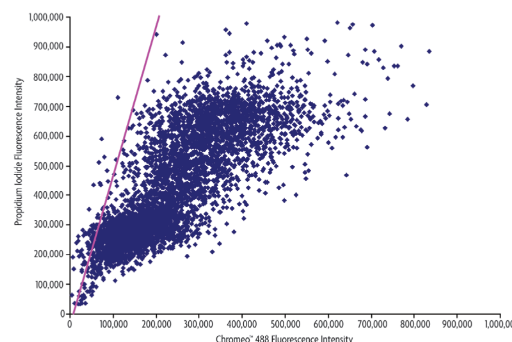


FIGURE 3:
Single cell analysis of phospho-H2AX in HeLa cells by the IsoCyte™ scanner.
Untreated HeLa cells (upper panel) are detected mostly in the propidium iodide channel while etoposide-treated cells are seen in the green (488 nm) channel, indicating a high degree of DNA damage.

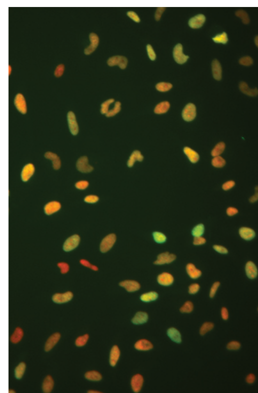


FIGURE 1:
Merged image of etoposide-treated HeLa cells.
HeLa cells stained only with propidium iodide (PI) appear red, while those stained with both PI and phospho-H2AX detected with Chromeo 488 appear more yellow.

REFERENCE

1. Rogaku, E. P. *et al.* (1992) *J. Biol. Chem.* **273(10)**: 5858-5868.

Product	Format	Cat. No.
DNA Damage Assay (Fluorescent)	2 x 96 rxns	18030
Histone H2AX phospho Ser 139 pAb	200 µl	39117

Mitotic Index Kits

quickly determine the proportion of cells in a population undergoing mitosis

Cell division is a complex and tightly regulated process, the final step being mitosis. The ability to detect cells undergoing mitosis is a simple way to characterize the effect of drug treatments on cell division. During the progression of interphase and G2 into M phase, chromosomes are condensed to allow nuclear division to continue. During this event, histones are subject to post-translational modifications in a specific and temporally regulated manner. Studies have shown that histone H3 is phosphorylated at serine 28 (and several other sites) during mitosis. Therefore, the phosphorylation of histone H3 at serine 28 is a useful marker for mitosis, and this specific phosphorylation event can be used to determine quickly and accurately the mitotic index of a population of cells.

The Mitotic Index Kit advantage

The Mitotic Index Assay Kit is a simple and accurate method to determine the percentage of cells undergoing mitosis within a population. The kit uses a monoclonal antibody (clone HTA28) that is specific for phosphorylation at serine 28 of histone H3 and an exceptionally bright fluorescent secondary antibody, Chromeo™ 488 Goat anti-Rat IgG, for detection. Propidium iodide is used to stain all cells, and the mitotic index is determined by calculating the percentage of cells undergoing mitosis within the total population (Figure 1). For added convenience, the microtubule poison paclitaxel is included in the kit to arrest cells in mitosis, providing a high mitotic-index reference population (Figure 2).

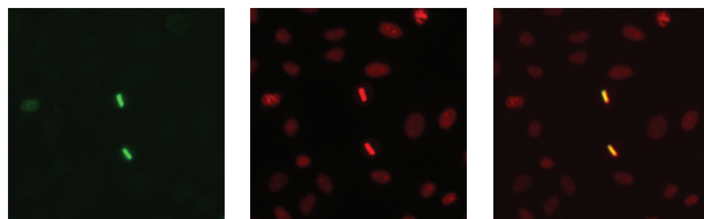


FIGURE 1:
HeLa cells fixed with methanol and assayed with the Mitotic Index Assay Kit. Phospho-histone H3 (Ser28) detected with Chromeo 488 Goat anti-Rat IgG is shown in the first image. Nuclear staining of all cells with propidium iodide is shown in the center image. The merged image is shown on the right; cells with both green Chromeo 488 signal and red propidium iodide signals appear yellow.

Why use the Mitotic Index Kit?

- Highly specific monoclonal phospho-Histone H3 (Ser28) clone HTA28
- Exceptionally bright Chromeo™ 488 detection
- High-throughput compatible method that is easily adaptable to any plate format
- Whole cell analysis with no cell lysis steps
- Available in chemiluminescent and colorimetric formats

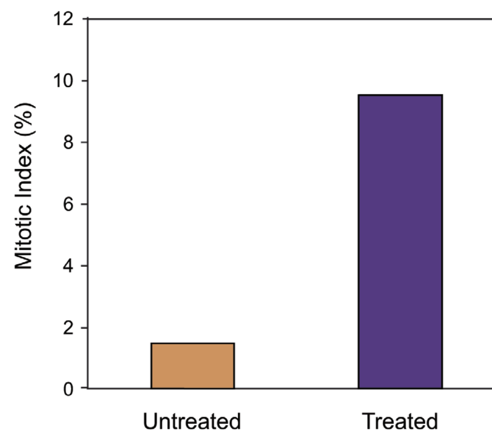


FIGURE 2:
Whole well analysis of paclitaxel-treated vs. untreated HeLa cells.
HeLa cells were treated with 1 µM paclitaxel diluted in complete medium for 6 hours prior to analysis using the Mitotic Index Assay Kit.

Please browse our complete line of Chromeo-labeled fluorescent antibodies at: www.activemotif.com/chromeo.

Product	Format	Cat. No.
Mitotic Index Assay Kit	5 x 96 rxns	18020
Mitotic Assay Kit (Color)	2 x 96 rxns	18021
Mitotic Assay Kit (Chemi)	2 x 96 rxns	18022

Distribution Network

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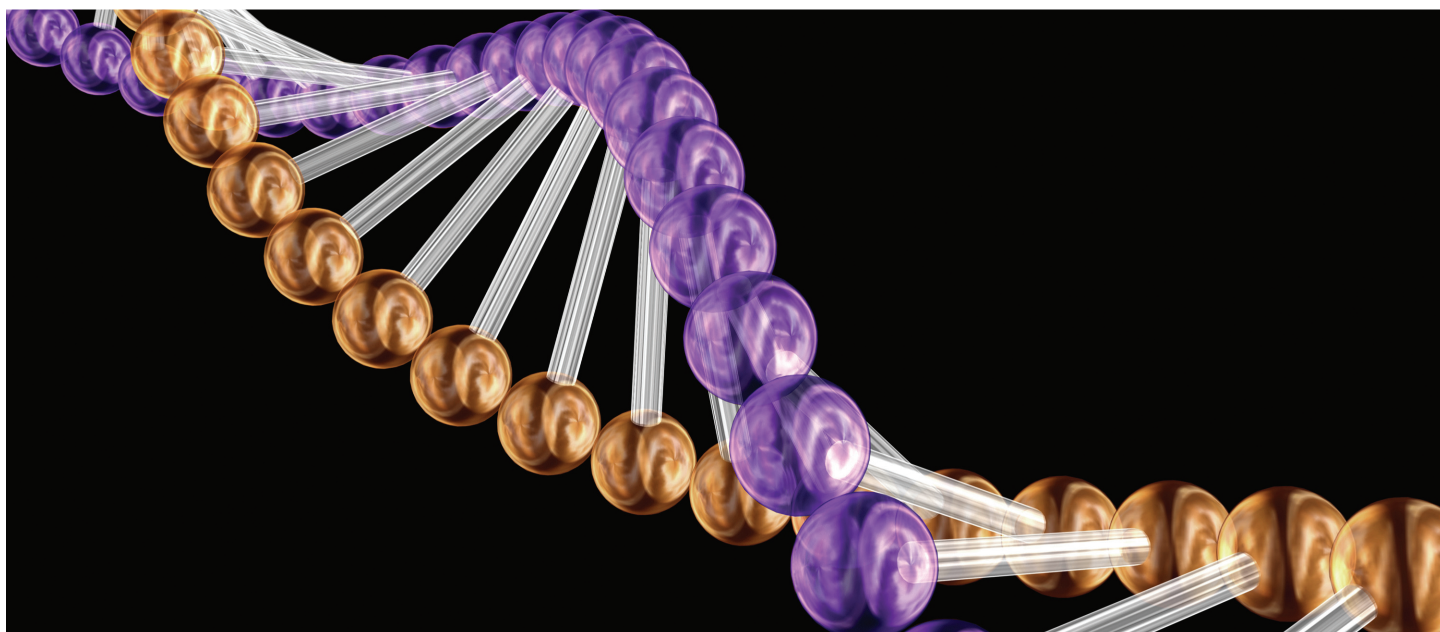

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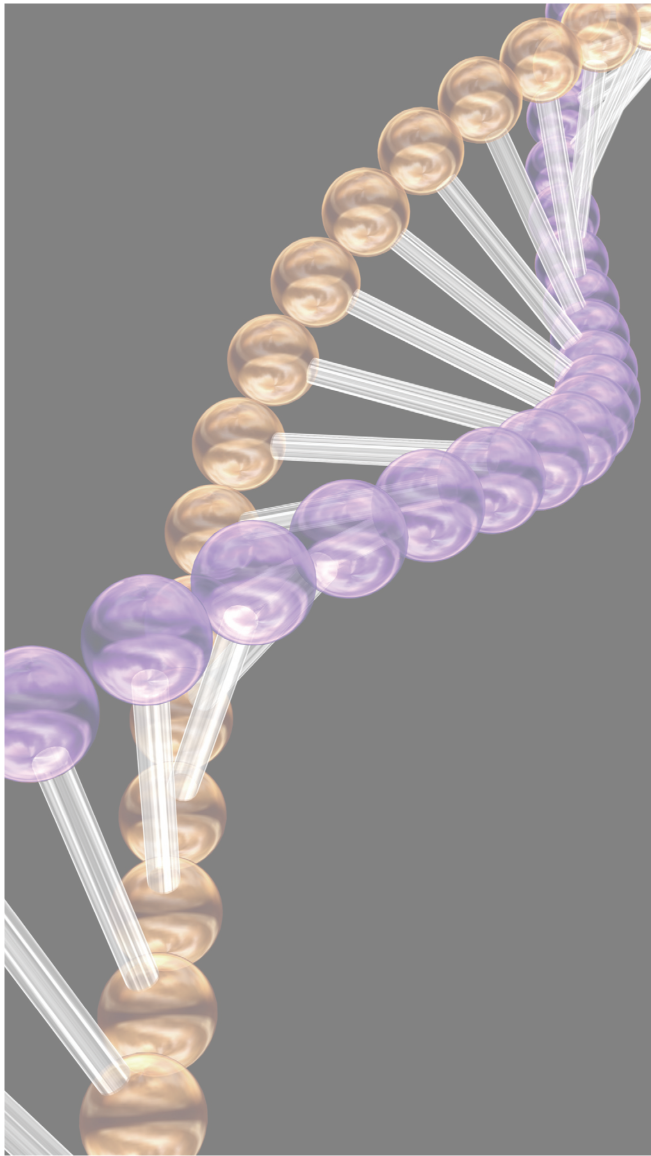

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