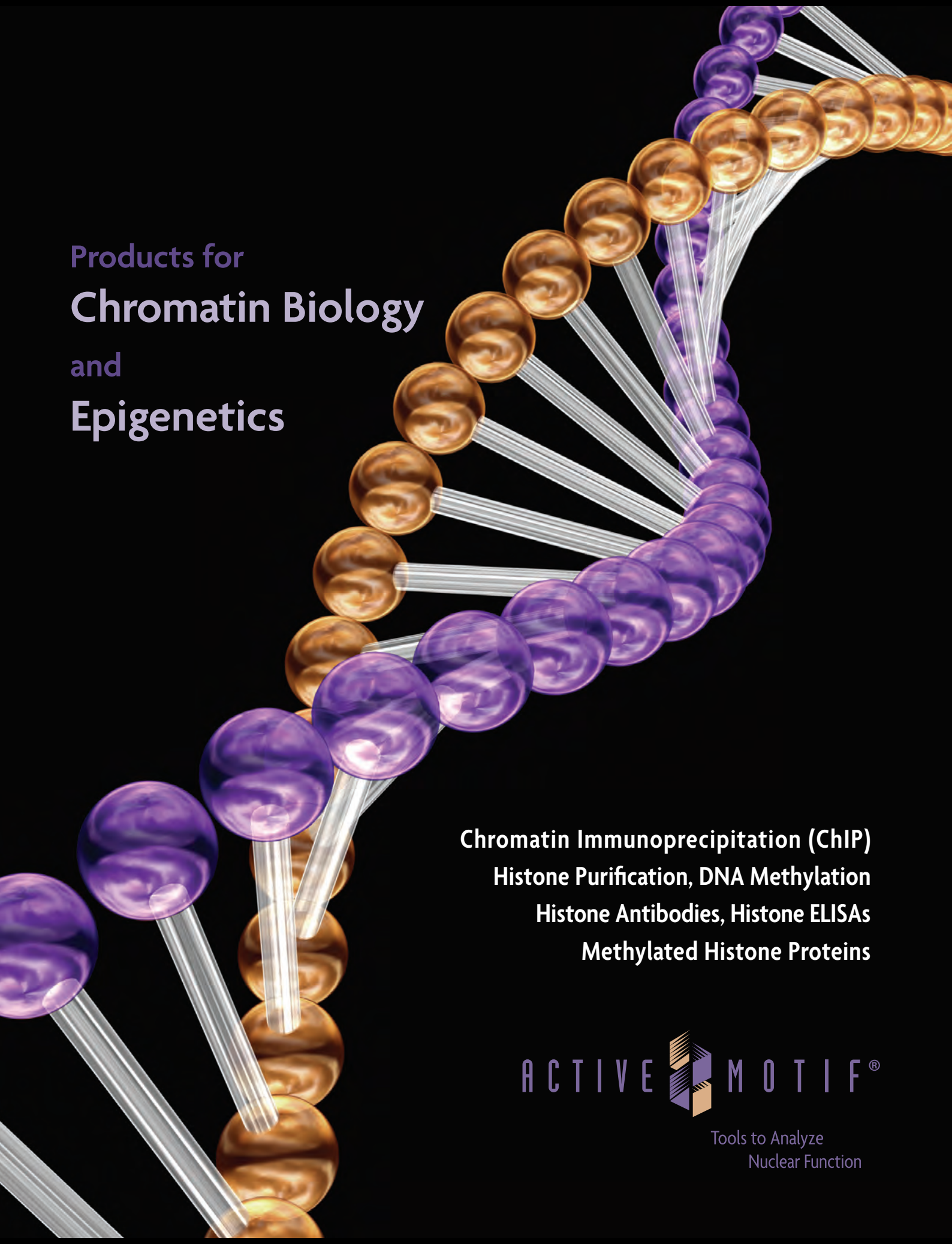

Products for Chromatin Biology and Epigenetics

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

ACTIVE MOTIF®

Tools to Analyze
Nuclear Function

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.

The Active Motif antibody difference

- Quality first – we'd rather fail our antibody project than sacrifice quality
- Highly characterized – all antibodies are tested stringently under multiple conditions
- Controlled process – we manufacture and test all our own antibodies
- Consistent – we go to great lengths to minimize lot-to-lot variability
- Convenient – most antibodies are available in two pack sizes

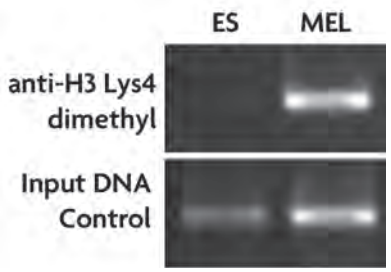

FIGURE 1:
Chromatin immunoprecipitation.
ChIP was performed with Histone H3 dimethyl Lys4 pAb (Catalog No. 39141) using chromatin prepared from mouse embryonic stem cells (left lane) or mouse erythroleukemia cells (right lane). PCR primers specific for the β-globin gene were used to amplify a 210 base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

DESCRIPTION	APPLICATIONS	CAT. NO.
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).

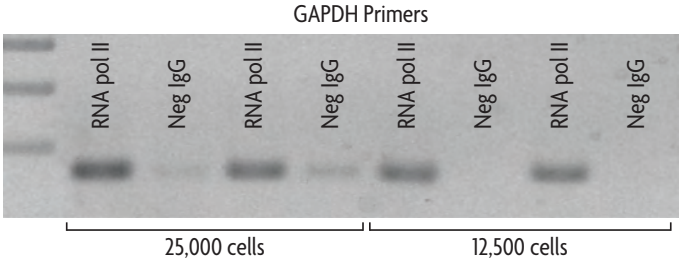

FIGURE 1:
ChIP-IT Express works with 12,500 cells.
ChIP-IT Express was performed in duplicate on decreasing amounts of sonicated HeLa cell chromatin. Two µg of RNA pol II and Neg IgG antibody was used for IP. GAPDH PCR primers were used to analyze the immunoprecipitated DNA. Using the improved ChIP-IT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

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.

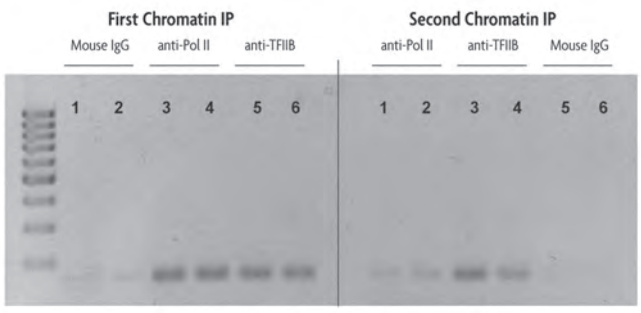

FIGURE 1:

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.

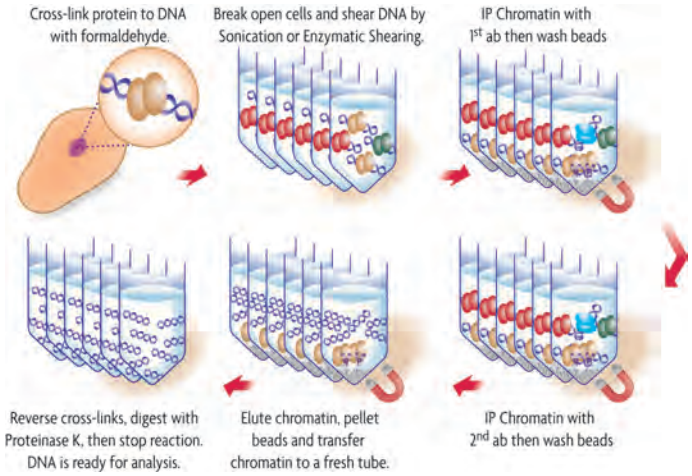

FIGURE 2:

Schematic representation of the Re-ChIP-IT procedure.

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

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.

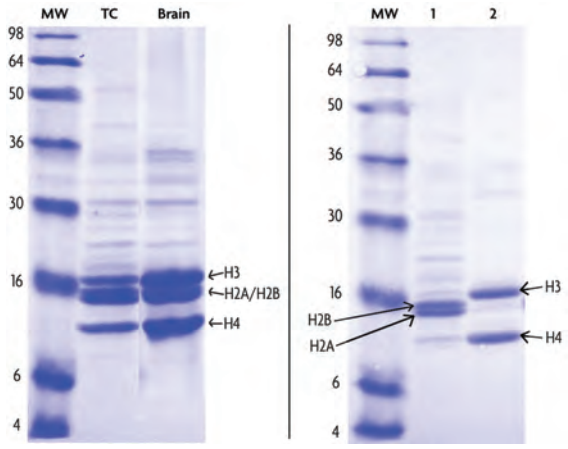

FIGURE 1:
SDS-PAGE of histone fractions purified using the Histone Purification Kit.
Ten µg of sample were loaded per lane and run on a 16% Tris-glycine gel. Left panel: core histones purified from logarithmically growing tissue culture cells (TC) and core histones isolated from rat brain tissue (Brain).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

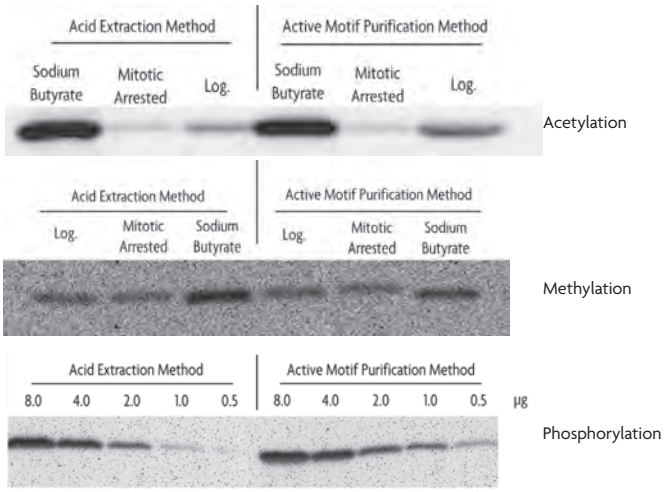

FIGURE 2:
Post-translational modifications preserved.
Acetylation, methylation and phosphorylation states are preserved as well or better with the Histone Purification Kit compared with standard acid precipitation method.

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).

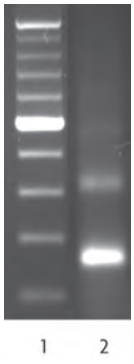

FIGURE 3:
Chromatin assembled with purified histones.
Histones were purified from HeLa cells and used in the Chromatin Assembly Kit. The ordered spacing of nucleosomes was confirmed and analyzed by agarose gel.

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.

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).

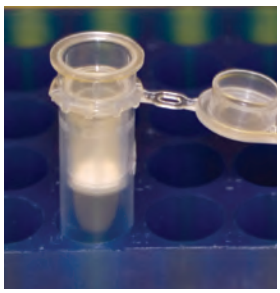

FIGURE 1:
Histone purification in a new spin column format.
Convenient spin column-based protocol enables faster purification of histones from multiple samples simultaneously.

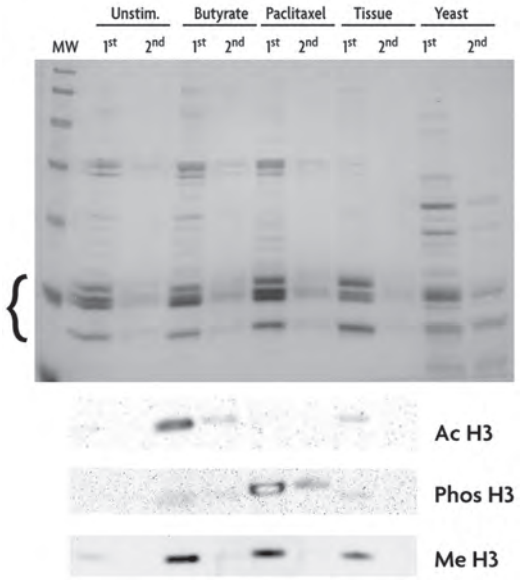

FIGURE 2:
Analysis of histone fractions by SDS-PAGE and Western blot.
Histones were purified from a variety of sources using the Histone Purification Mini Kit. (Unstim.: unstimulated HeLa cells, Butyrate: sodium butyrate-treated HeLa cells, Paclitaxel: paclitaxel-treated HeLa cells, Tissue: rat brain 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.

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.

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).

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).

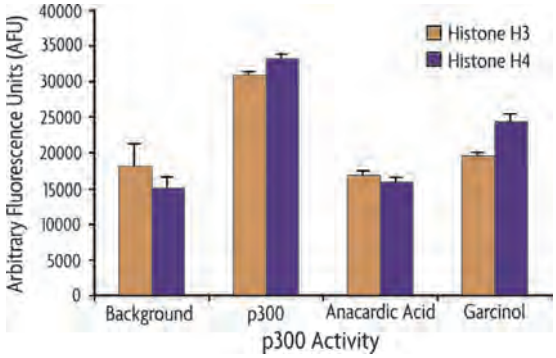

FIGURE 1:
HAT inhibitor effects on p300 activity.
50 ng p300 were assayed per well with 50 µM acetyl-CoA and 50 µM histone H3 or H4 peptide substrates in the absence or presence of 15 µM anacardic acid or 25 µM garcinol, known HAT inhibitor compounds.

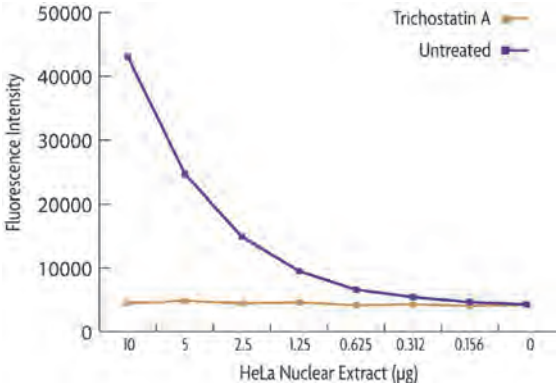

FIGURE 2:
HDAC activity in HeLa cells.
HeLa nuclear extracts were assayed at 0 to 10 µg per well using the fluorescent version of the HDAC Assay Kit. Untreated extract results are shown with a purple line, and extracts inhibited with 1 mM Trichostatin A are shown with a copper line.

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

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).

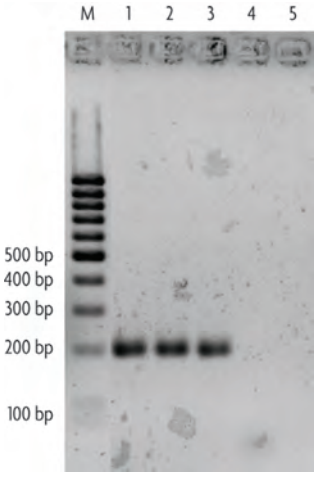

FIGURE 1:
Agarose gel analysis of PCR products generated with MethylDetector.
Three different DNA conversions were performed (Lanes 1-3) and compared to an unconverted DNA control (Lane 5) and to a no DNA control (Lane 4). The presence of PCR product in only the converted samples demonstrates the conversion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

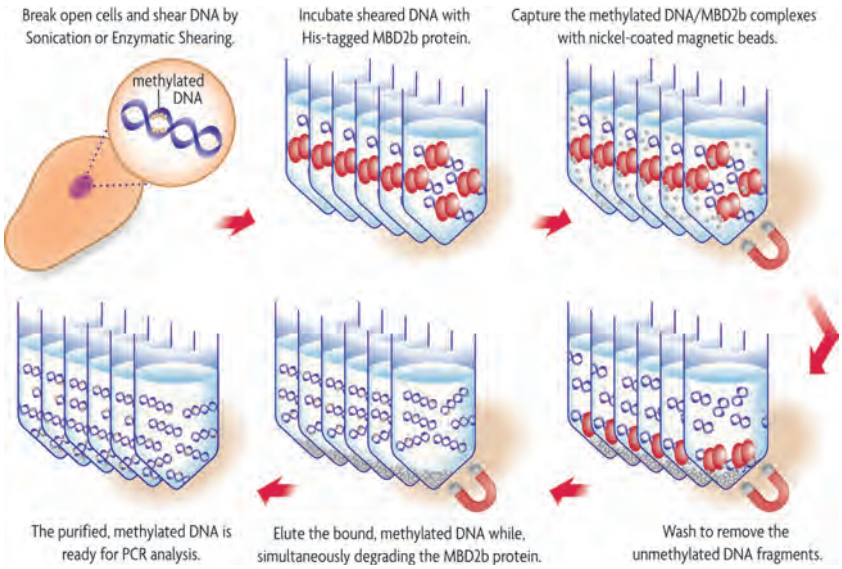

FIGURE 1:

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.

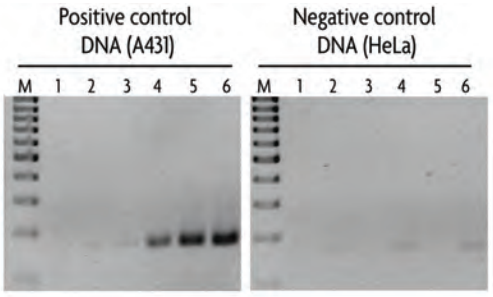

FIGURE 2:

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

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.

UNIVERSAL MAGNETIC CO-IP KIT ADVANTAGES

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

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.

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.

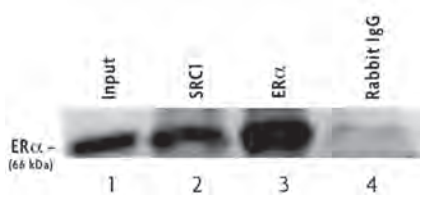

FIGURE 1:

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).

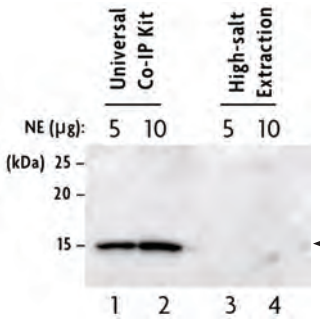

FIGURE 2:

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.

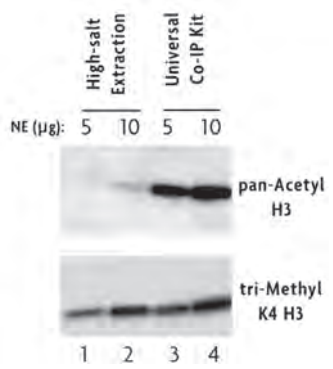

FIGURE 3:

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.

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

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