MOTIF VATIONS

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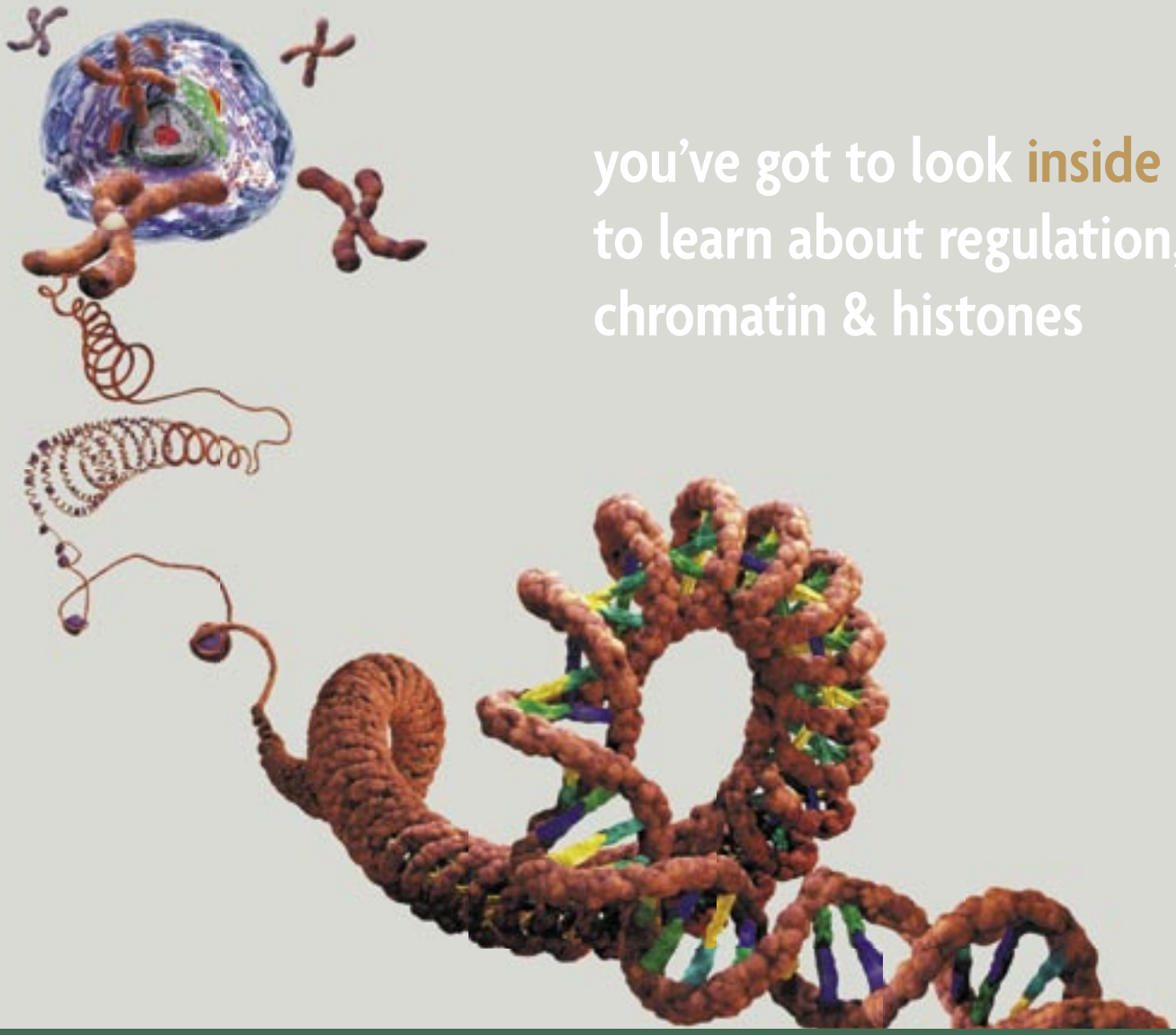
ACTIVE MOTIF®

Tools to Analyze Nuclear Function



you've got to look inside to learn about regulation, chromatin & histones

IN THIS ISSUE

NEW: DNA Damage Assay – an Easy Method to Assay for Apoptosis

Active Motif's DNA Damage Assay is a simple and fast 2-color fluorescent assay of H2AX phosphorylation, which occurs following double-stranded breaks in DNA. This makes it a sensitive and accurate assay of apoptosis.

When cells are exposed to ionizing radiation or certain treatment compounds, double-stranded DNA breaks are created that rapidly result in the phosphorylation of the histone variant H2AX at serine 139. Because 0.03% of chromatin becomes phosphorylated at the serine 139 residue of H2AX per every double-stranded DNA break, phospho-H2AX is a sensitive marker for DNA damage, making it a useful marker for apoptosis[1]. By assaying for H2AX phosphorylation, Active Motif's new DNA Damage Assay makes screening compounds for their ability to induce DNA damage or apoptosis sensitive, accurate and very simple!

How does the assay work?

The DNA Damage Assay is a cell-based assay conducted in 96-well plates. Each kit includes all the reagents necessary for two 96-well plates. Cells are grown and treated in the plate, then fixed and incubated with the phospho-Histone H2AX (Ser 139) apoptosis marker antibody. Next, a short incubation with Chromeo™ 488 secondary antibody is performed for detection. The cells are then washed and stained with propidium iodide and read on a fluorescent plate reader.

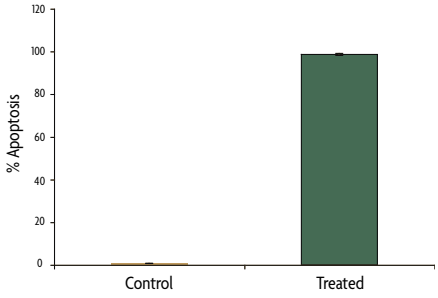


Figure 1: Percent induction of apoptosis in HeLa cells. Levels of phospho-H2AX measured in untreated (control) and cells treated with etoposide. Averages of quadruplicates are shown.

What's in the kit?

The DNA Damage Assay includes the polyclonal phospho-Histone H2AX (Ser 139) antibody, Chromeo 488 Goat anti-Rabbit secondary and etoposide, which is a control compound to induce >98% DNA damage (Figure 1). Propidium iodide is included to provide a total cell count. This simple detection method utilizing the superior Chromeo 488 fluorescence and propidium iodide stain makes the kit ideal for both high-throughput and high-content screening in all cells (Figure 2).

Specificity for low background

The assay relies on a high-quality rabbit polyclonal phospho-Histone H2AX (Ser 139) antibody which is subsequently detected with the Chromeo 488 Goat anti-Rabbit secondary. Our Chromeo fluorescent secondaries are highly specific with extremely low background, so your results are always accurate (Figure 3).

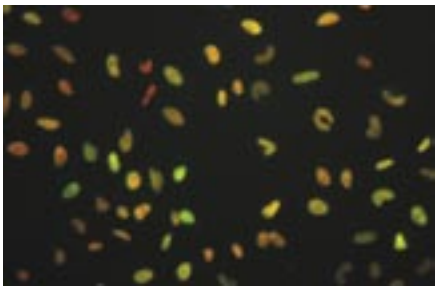


Figure 3: Merged image of etoposide-treated HeLa cells. HeLa cells stained only with propidium iodide (PI) appear red, while those stained with both PI and phospho-H2AX detected with Chromeo 488 appear more yellow.

Start screening compounds today!

Screening treatments for the induction of DNA damage and apoptosis has never been easier with our DNA Damage Assay. For more information about this assay and our other DNA Damage and Repair assays, please give us a call.

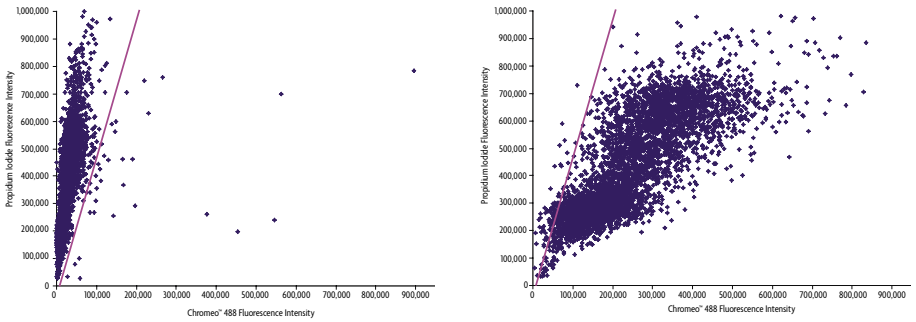


Figure 2: IsoCyte™ single cell analysis of phospho-H2AX detected with Chromeo 488 secondary in HeLa cells. Untreated HeLa cells (left panel) are detected mostly in the propidium iodide channel while etoposide-treated cells are seen in the green (488 nm) channel indicating a high degree of DNA damage.

Product	Format	Catalog No.
DNA Damage Assay (Fluorescent)	2 x 96 rxns	18030

REFERENCES

1. Rogaku, E.P. *et al.* (1998) *J. Biol. Chem.* 273(10):5858-5868.

Simplified Study of SUMOylation Effects with SUMOlink™

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

The SUMOlink method

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

Everything you need to study SUMO

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

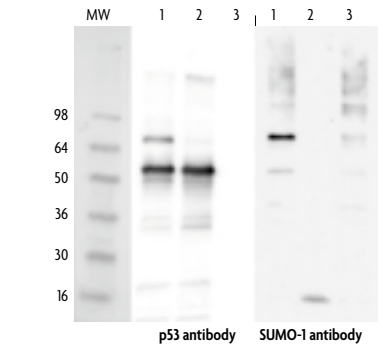


Figure 1: Specific SUMO-1 labeling of p53 by SUMOlink. Western blot analysis of *in vitro* SUMOylation of p53 protein by wild-type and mutated isoforms of SUMO-1: The two Western blots were incubated with p53 antibody (1:5000 dilution) and SUMO-1 antibody (1 :4000 dilution) Lane 1: Wild-type SUMO protein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

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

Easily Compare CpG Methylation of Your Samples

MethylCollector™ provides a fast, efficient protocol for isolating and comparing CpG-methylated DNA in cell or tissue samples. The kit uses a recombinant Methyl-binding protein (MBD2b) to capture DNA, rather than antibody-based immunoprecipitations, which improves assay sensitivity.

MethylCollector advantages

- Fast and easy protocol completed in less than 4 hours
- Flexible – enables detection from 5 ng to 1 µg of DNA
- Positive control DNA and PCR primers ensure success

The MethylCollector method

In MethylCollector, His-tagged recombinant MBD2b protein specifically binds to CpG-methylated DNA fragments prepared by enzymatic digestion or sonication. These protein-DNA complexes are captured with nickel-coated magnetic beads and washed with a stringent high-salt buffer to remove DNA fragments with little or no methylation. Ready-to-use methylated DNA is then eluted. 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 1).

MethylCollector applications

Specific isolation of methylated DNA by MethylCollector enables many powerful applications, including rapid screening of the methylation status of multiple loci in tumor tissue or cells and detecting changes in DNA methylation in other situations, such as normal cellular differentiation and aging. To find out more, please visit www.activemotif.com.

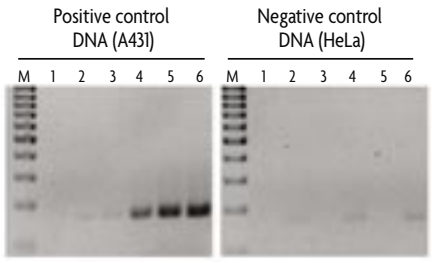


Figure 1: CpG-methylation in HeLa and A431 cells. Increasing amounts of genomic DNA from A431 (positive control DNA) and HeLa (negative control DNA) cells were assayed using MethylCollector and analyzed by PCR using the kit's control primers, which amplify a locus that is not methylated in HeLa but highly methylated in A431. MethylCollector-enriched A431 DNA generates robust signals proportional to the amount of starting material (left panel, lanes 4 to 6). No signal is observed in HeLa DNA (right panel, lanes 4 to 6) or in 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	Catalog No.
MethylCollector™	25 rxns	55002

NEW: Isolate Pure, Separate Fractions of Histone H2A/H2B and H3/H4 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.

Histone purification made easy

Active Motif's Histone Purification Kit enables you to isolate core histones from any cell culture or tissue sample (Figure 1). The core histones may be purified as one total population containing H2A, H2B, H3 and H4, or further purified into separate fractions of H2A/H2B and H3/H4 (Figure 2).

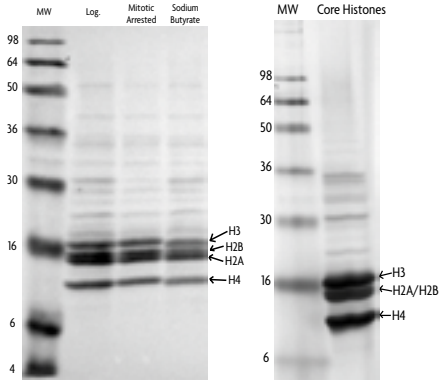


Figure 1: Core histones isolated from cells and tissue. Total core histones were isolated from HeLa cells (left gel) and rat brain tissue (right gel).

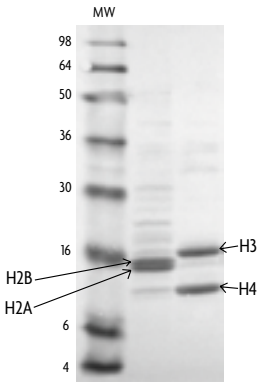


Figure 2: Separate H2A/H2B and H3/H4 fractions. H2A/H2B and H3/H4 fractions isolated from HeLa cells.

Preserve important modifications

Preserving post-translational modifications like acetylation, methylation and phosphorylation states is critical when investigating the transcriptional activity of a gene of interest. Our proprietary buffer system is optimized to maintain these modifications while separating the histones into pure fractions (Figure 3).

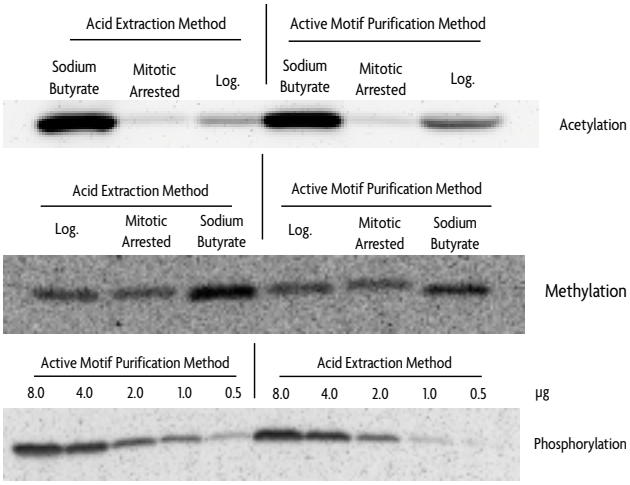


Figure 3: Post-translational modifications preserved. Acetylation, methylation and phosphorylation states are preserved as well or better with the Histone Purification Kit vs. a straight acid precipitation method.

How does it work?

Unlike histone purification by acid precipitation, our method utilizes 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 may be isolated from small cell culture samples up to grams of tissue. Sequential elution steps let you collect the core histones in either one step containing all core histones or into two separate populations: one enriched for H2A and H2B and a second fraction containing >90% pure H3 and H4.

What's in the kit?

Each kit contains all the necessary equilibration, neutralization and elution buffers plus a reusable purification column for 10 histone purifications.

Better substrate for downstream assays

Core histones isolated by the Histone Purification Kit method are highly pure and suitable for substrates for downstream assays. Purified histones can be used with the Chromatin Assembly Kit (page 5) to enable the generation of chromatin that very closely resembles native chromatin for functional assays (Figure 4).

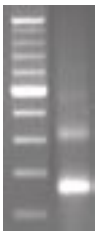


Figure 4: Chromatin assembled with purified histones. Histones were purified from HeLa cells and used in the Chromatin Assembly Kit. The ordered spacing of nucleosomes was confirmed and analyzed by agarose gel.

Try it today!

Call or visit us at www.activemotif.com to find out more about the Histone Purification Kit and related products that will benefit your research.

Product	Format	Catalog No.
Histone Purification Kit	10 rxns	40025

Create Chromatin *In Vitro* to Reveal Regulatory Mechanisms

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

A simple way to generate chromatin

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

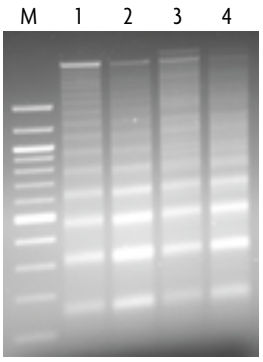


Figure 1: Enzymatic digestion of assembled chromatin. Chromatin assembled from 1 µg samples of circular DNA (Lanes 1 & 2) and linear DNA (Lanes 3 & 4) were digested for 2 and 4 minutes, respectively, deproteinated, phenol/chloroform extracted and run on an agarose gel. Each sample type resulted in regularly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

Why bother to make chromatin?

When DNA sequences are assembled into chromatin by ATP-dependent factors, the resulting structure closely resembles the natural chromatin configuration. DNA that is in either a bare or unassembled state often cannot reveal the mechanism of transcriptional activation or repression with the associated factors and relevant histone modifications. However, properly assembled chromatin with regularly ordered nucleosomes is an excellent substrate for subsequent assays such as *in vitro* transcription assays, histone acetyltransferase (HAT) assays (Figure 2) and ChIP (Figure 3).

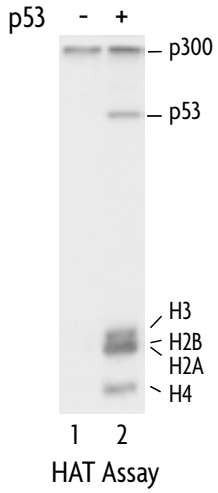


Figure 2: HAT assay using assembled chromatin. Chromatin was *in vitro* assembled with p300 and acetyl-CoA, in the presence (+) or absence (-) of p53 at 30°C for 30 minutes. The sample was then analyzed on an 18% SDS-PAGE gel and visualized following fluorography.

More natural for true results

Now you can use histones purified from actual samples with the Histone Purification Kit (page 4) to create chromatin that closely mimics the *in vivo* form, so you can confirm results and be certain about which factors are critically involved in regulation of your target sequence.

Chromatin Assembly Kit advantages

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

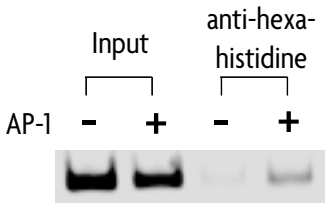


Figure 3: ChIP of *in vitro* assembled chromatin. *In vitro* ChIP was performed using *in vitro*-assembled chromatin containing an AP-1 binding site in the presence of p300 and acetyl-CoA, with or without hexa-histidine-tagged AP-1. The ChIP was performed with a hexahistidine antibody and PCR was conducted with primers flanking the AP-1 binding site.

Complete kit ensures your success

The kit includes recombinant h-NAP-1 chaperone protein, ACF assembly complex, HeLa core histones, and the buffers and enzymes necessary to generate assembled chromatin from your input DNA. Control Supercoiled DNA is also provided. To verify that your chromatin assembly reaction has been successful, reagents are provided to perform an easy partial enzymatic digestion to visualize the regular spacing of nucleosomes by agarose gel electrophoresis (Figure 1).

Start assembling chromatin now!

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

Product	Format	Catalog No.
Chromatin Assembly Kit	10 rxns	53500

Improved ChIP-IT™ Express Greatly Reduces 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. And, ChIP-IT Express makes it easy to perform ChIP on many samples at the same time.

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)!

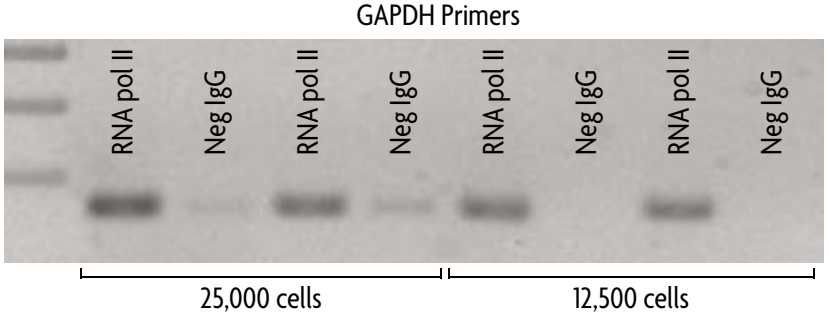


Figure 1: ChIP-IT Express works with 12.500 cells.
ChIP-IT Express was performed in duplicate on decreasing amounts of sonicated HeLa cell chromatin. Two µg of RNA pol II and Neg IgG antibody was used for IP. GAPDH PCR primers were used to analyze the immunoprecipitated DNA. Using the improved ChIP-IT Express reagents and protocol, positive ChIP data was obtained from as few as 12,500 cells.

Product	Format	Catalog 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

The magnetic bead advantage

The ChIP-IT Express magnetic beads have less background than standard agarose beads, and this means 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 removed the human-only controls from ChIP-IT Express Kits and now offer human, mouse and rat ChIP-IT Control Kits separately. 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 HeLa Chromatin, 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.

Simplified Co-Immunoprecipitation of Intact Nuclear Complexes

The Nuclear Complex Co-IP Kit simplifies co-immunoprecipitation of intact nuclear protein complexes because its reagents have been optimized for preparing nuclear extracts and immunoprecipitating DNA-bound proteins.

Co-Immunoprecipitation (Co-IP) is often used to study protein/protein interactions. In Co-IP, a first antibody is used to immunoprecipitate a target antigen, which also co-precipitates any bound, interacting proteins. These are then detected by Western blot using antibodies targeted against the interacting proteins. However, traditional Co-IP methods are not optimal for studying DNA-binding proteins because their complexes are very fragile, so are frequently disrupted during extraction. In addition, these complexes can be altered by the salt and detergent composition of the immunoprecipitation buffers, which can further complicate their analysis.

Perform Co-IP of DNA-bound proteins

To overcome these problems, the Nuclear Complex Co-IP Kit contains extraction reagents that were designed to help maintain nuclear protein complexes. The kit's extraction process provides a simple, effective method for isolating intact protein complexes contained in nuclear compartments of the cell, specifically those previously bound to DNA. After isolation of the intact complexes, the supplied Co-IP reagents were designed so that you can vary the stringency of the Co-IP buffers. This improves your results by making it easy for you to study any protein complex, whether its members are tightly or weakly bound.

"The Nuclear Complex Co-IP Kit improves co-immunoprecipitation of DNA-binding proteins by providing extraction and immunoprecipitation components that are optimized to maintain nuclear protein complexes."

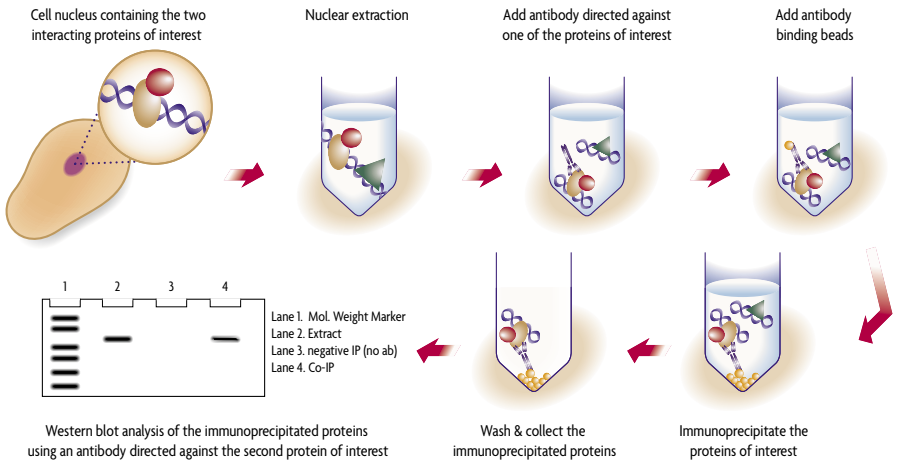


Figure 1: Flow chart of the Co-Immunoprecipitation process.

Product	Format	Catalog No.
Nuclear Complex Co-IP Kit	50 rxns	54001

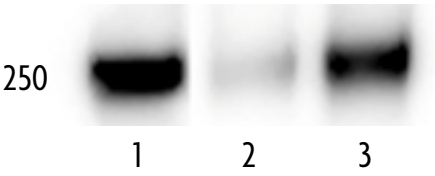


Figure 2: Analysis of p33 in the RNA pol II complex. HeLa cells were grown to confluence on 100 mm plates and nuclear extracts were prepared using the kit's extraction reagents. For IP experiments, the stringency of the IP High Buffer was increased by supplementing with NaCl and Detergent. 100 µg of nuclear extract was used per IP reaction and incubated with either 2 µg p33 antibody or no antibody. Following the IP, Western blot analysis was performed using RNA pol II mouse mAb at 0.1 µg/ml followed by anti-mouse HRP at 1:1000. Detection of the p33/RNA pol II complex by the RNA pol II antibody (lane 3) demonstrates that the Co-IP was successful in maintaining the protein complex. The input HeLa extract (lane 1) was run as a control for the Western blot using 0.1 µg/ml RNA pol II.

Lane 1 Western blot control
Lane 2 Negative Control (no antibody used in IP)
Lane 3 Co-IP: IP using p33/WB using RNA pol II

Easy to optimize for ideal stringency

With the Nuclear Complex Co-IP Kit, nuclear extracts are prepared using low-salt buffers and enzymatic shearing. The low-salt buffers keep the protein complexes intact, while digestion gently releases them from the DNA. Immunoprecipitation is then carried out to detect the bound proteins. The kit contains high- and low-stringency IP buffers, as well as salt and detergent. Addition of salt and detergent is ideal for robust protein/protein interactions because higher stringency reduces background. However, as unstable protein complexes may not withstand high stringencies, the kit makes it simple to optimize the stringency as required for each particular protein complex.

Find the complex members you missed

The Nuclear Complex Co-IP Kit offers you a simpler, more flexible alternative than traditional co-immunoprecipitation methods. To find out more about how it can help you, please give us a call or visit us on the web at www.activemotif.com.

Learn More Faster With AlmaKnowledgeServer 2

AKS[2] lets you dig deeper than other search tools. It utilizes advanced text mining to automatically examine the thousands of new abstracts added to PubMed every day. AKS applies rules-based analysis to determine which words are bioentities, such as genes, chemicals, drugs, diseases, *etc*. This content is added to the AKS database, which is searched to reveal relationships between bioentities and their relevance. A variety of graphical tools make it easy to quickly find the information that is important to you (Figures 1-4). AKS can even discover links that have not been published.

The new AKS v2.1 includes:

- Automatic abstract alerts via email
- PubChem compound structures
- Publication trend analyses
- Improved export tools and graphics

Why not give it a try? For a **free 7-day demo**, visit www.activemotif.com/aks.

Figure 1: Chemical structure and trend analyses.

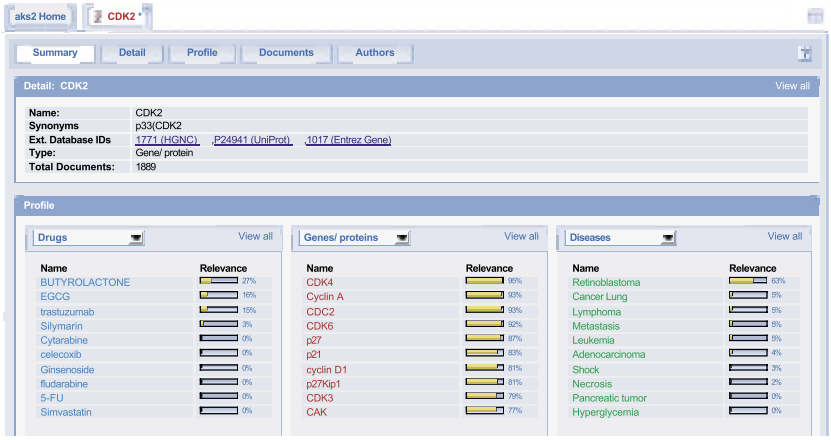


Figure 2: Summary results for the gene/protein CDK2, which shows the relevance of co-occurring bioentities.

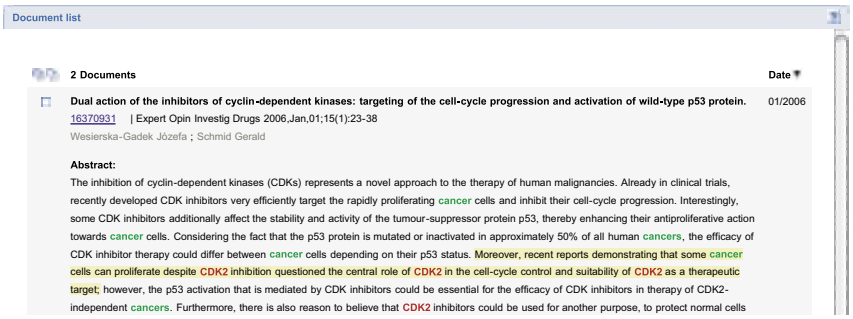


Figure 3: Abstracts are displayed with your choice of bioentity types highlighted, so you can quickly find relevant information.

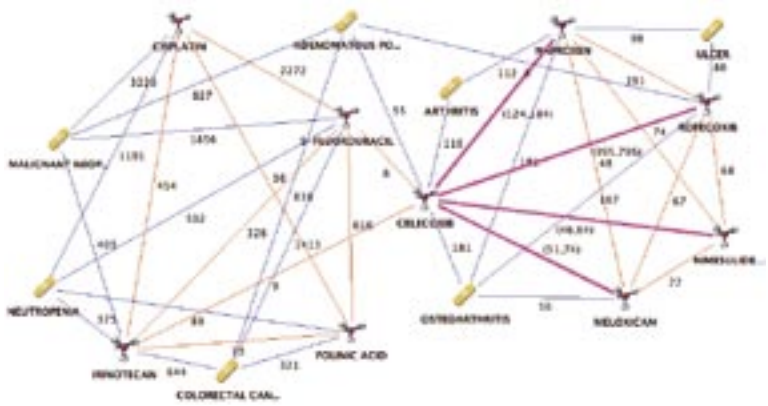


Figure 4: AKS includes a powerful visual environment to map relationships between bioentities.

Online Access to AKS v2.1 is Now Available at a Low Introductory Price!

With the introduction of AKS v2.1, Active Motif is pleased to announce that AKS is now available as an online subscription service. This is an ideal, low-cost solution that untethers AKS from your computer, making it available everywhere.

Through the end of 2007, an online subscription to AKS is available for just **$100/€99 per month**, a substantial discount from the normal price of $150/€150. To place your order, please contact your local Active Motif office.