MOTIF VATIONS

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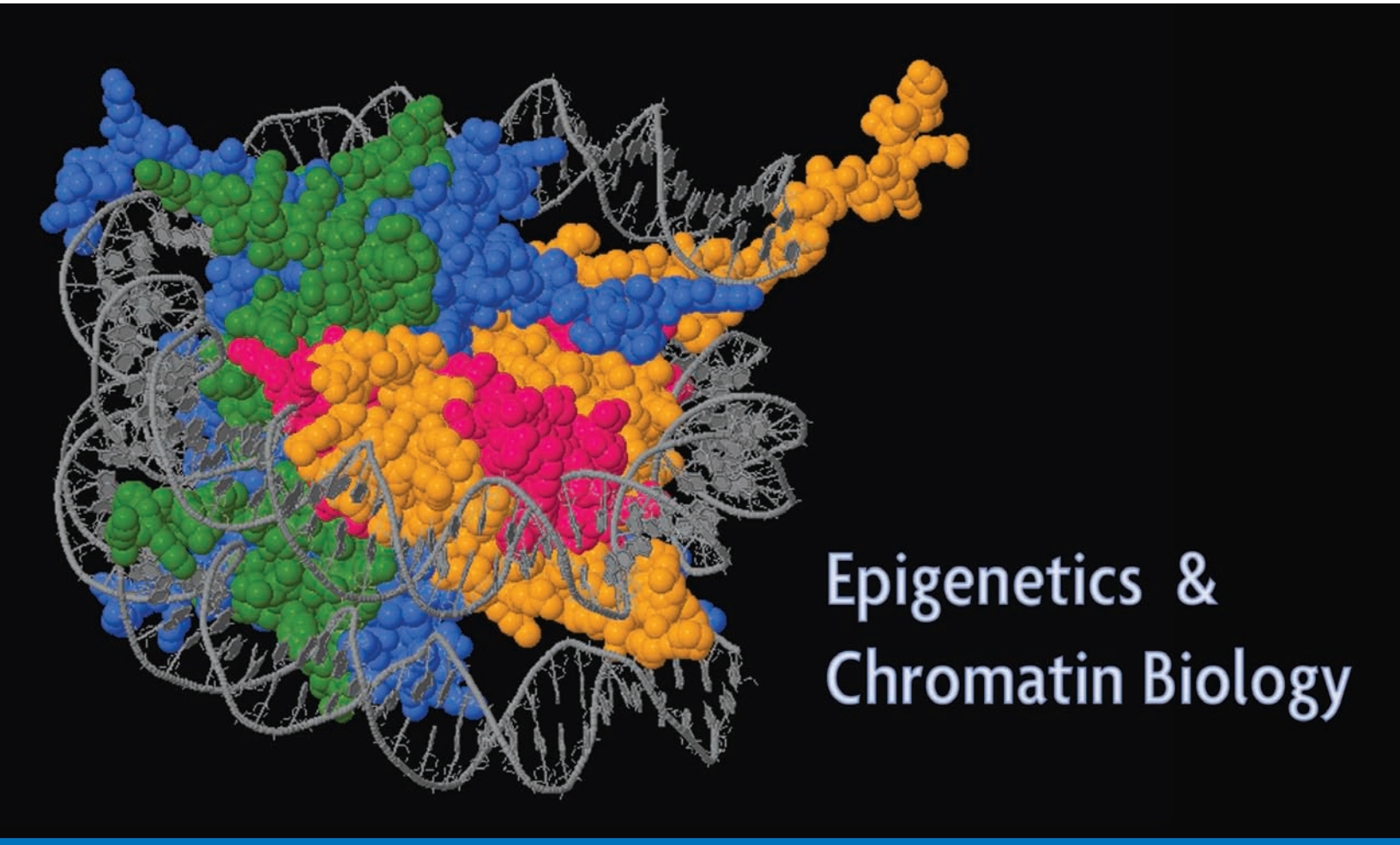
ACTIVE MOTIF®

Tools to Analyze Nuclear Function



Epigenetics & Chromatin Biology

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NEW: Recombinant Histones 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.

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.

Methylated Histone H3

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

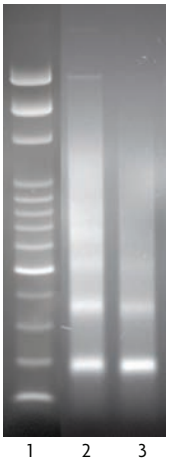

Figure 1: Ordered spacing of nucleosomes after enzymatic digestion of assembled chromatin.
Recombinant Histone H3 dimethyl Lys9 (4.5 µg) was used to generate chromatin *in vitro* using the Chromatin Assembly Kit (Cat. No. 53500). One µg of assembled chromatin was digested for 2 minutes (Lane 2) and 4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

How is the methylation state made?

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.

Order today!

Visit our website for more information on how to use these recombinant histones as a standard for your assays or as a building block for specific *in vitro* chromatin assays (Figure 1).

*Patent pending.

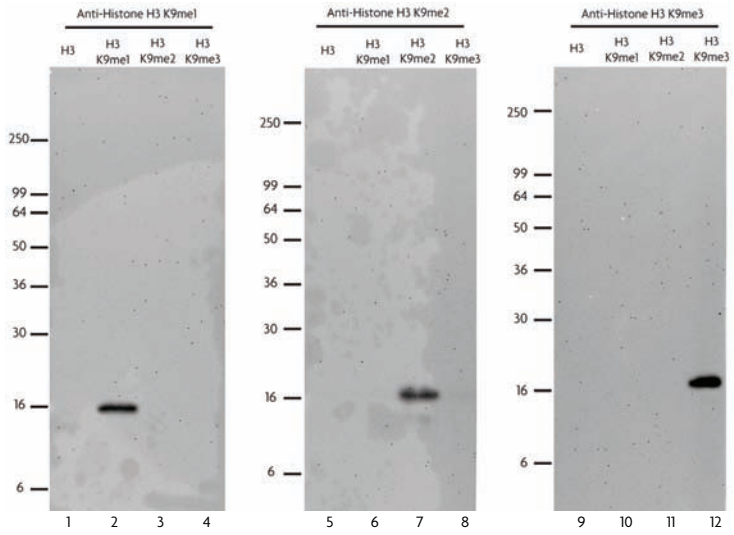

Figure 2: Western blot analysis of mono-, di- and tri-methylated Recombinant Histone H3.
One µg Recombinant Histone H3 (lanes 1, 5 & 9), 1 µg Recombinant Histone H3 monomethyl Lys9 (Lanes 2, 6 & 10), 1 µg Recombinant Histone H3 dimethyl Lys9 (Lanes 3, 7 & 11) and 1 µg Recombinant Histone H3 trimethyl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

Product	Format	Catalog 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

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).

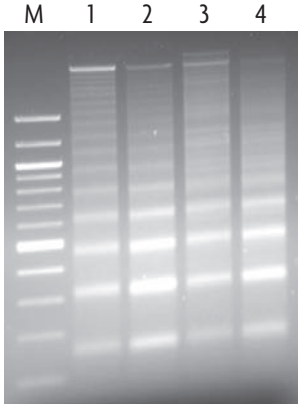

Figure 1: Enzymatic digestion of assembled chromatin. Chromatin assembled from 1 µg samples of circular DNA (Lanes 1 & 2) and linear DNA (Lanes 3 & 4) were digested for 2 and 4 minutes, respectively, deproteinated, phenol/chloroform extracted and run on an agarose gel. Each sample type resulted in regularly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

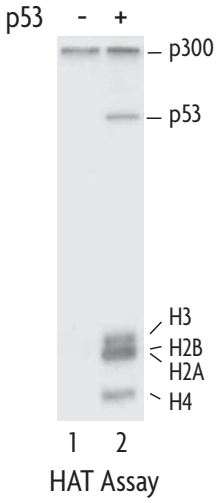

Figure 2: HAT assay using assembled chromatin. Chromatin was *in vitro* assembled with p300 and acetyl-CoA, in the presence (+) or absence (-) of p53 at 30°C for 30 minutes. The sample was then analyzed on an 18% SDS-PAGE gel and visualized following fluorography.

More natural for true results

Now you can use recombinant degree- and site-specific methylated histones (Page 2) 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

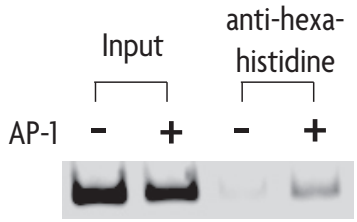

Figure 3: ChIP of *in vitro* assembled chromatin. *In vitro* ChIP was performed using *in vitro*-assembled chromatin containing an AP-1 binding site in the presence of p300 and acetyl-CoA, with or without hexa-histidine-tagged AP-1. The ChIP was performed with a hexahistidine antibody and PCR was conducted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

NEW: High-throughput ChIP with ChIP-IT™ Express HT

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 ChIP experiments and enables the processing of 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.

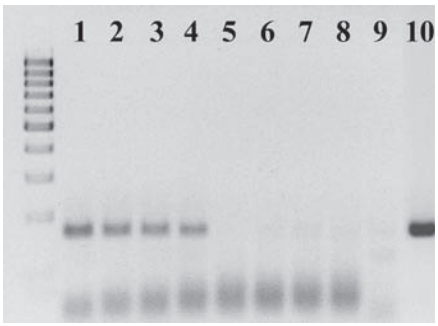

Figure 1: Chromatin IP performed on HeLa chromatin using ChIP-IT Express HT.
PCR carried out using primers specific for the GAPDH gene. Lanes 1-4, ChIP using 2 µg anti-RNA Pol II antibody. Lanes 5-8, ChIP using normal mouse IgG as a negative control. Lane 9, no DNA control. Lane 10, input DNA control.

FREE 96-well magnetic sorter!

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

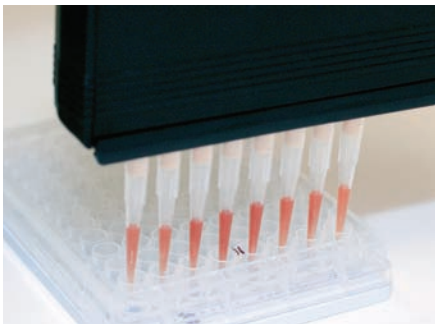

Figure 2: True High-throughput ChIP with ChIP-IT Express HT.
With the efficient plate-based protocol of ChIP-IT Express HT, you can process up to 96 ChIP reactions at a time.

Positive controls ensure success

Because interpreting ChIP data can be difficult, Active Motif has developed a complete line of control kits to help you troubleshoot your assays. To provide you with controls that are appropriate for your experimental model, we offer ChIP-IT control kits for human, mouse and rat.

Take ChIP to the next level

For additional information on the new ChIP-IT Express HT kit go to www.activemotif.com/htchip. Also, please browse our complete line of ChIP-validated antibodies at www.activemotif.com/chipabs.

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

ChIP Accessory Kits and Reagents

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 troubleshoot your ChIP experiments and make them more reproducible.

Superior ChIP-IT magnetic beads

Whether you are analyzing your ChIP results by PCR, ChIP-chip or ChIP-seq, having low non-specific background is always desirable. In the past, researchers have successfully used protein G agarose beads in their ChIP experiments. However, it is widely known that the agarose matrix itself non-specifically enriches for proteins due to the intrinsic electrostatic properties of agarose. The result is reduced assay sensitivity, increased data interpretation times or failed experiments. In an effort to avoid these problems, researchers have historically had to pre-block the agarose beads and increase the number of wash steps in their ChIP protocol. When you use Active Motif's ChIP-IT Protein G Magnetic Beads there's no need to waste your time and money performing extra steps. This is because the protein G is directly conjugated to a magnetic bead that will not bind non-specifically to proteins in your chromatin mix. Additionally, ChIP-IT Protein G Magnetic Beads are ready to use and will save you significant amounts of process time. If you're not looking for a complete solution like our ChIP-IT Express Kits, why not incorporate our ChIP-proven magnetic beads into your in-house ChIP method to improve results and save time?

ChIP-IT Control Kits

ChIP is an enrichment of DNA bound by a particular protein, not a complete purification of the protein-bound DNA. As a result, ChIPs are unavoidably contaminated with chromatin bound non-specifically. During PCR analysis, this contaminating DNA can lead to false positive PCR products, making data interpretation difficult. Active Motif offers a variety of control options to help eliminate false positives. You can order the original ChIP-IT Kits with or without human controls, or you can choose to order ChIP-IT Express and add on the control kit for your species of interest. The ChIP-IT Control Kits provide positive and negative control antibodies and positive control PCR primers, PCR buffer and a convenient DNA loading dye that makes your PCR reactions ready for loading on an agarose gel straight from the thermocycler (Figure 1).

Simplified chromatin shearing

For ChIP experiments to be successful, chromatin must first be sheared to 200-1000 bp fragments. Traditionally, shearing has been performed by sonication, which is effective, but it can be time consuming and difficult to optimize. To eliminate the problems associated with sonication, Active Motif has developed a more robust and user-friendly method to shear chromatin for ChIP. This method uses our proprietary Enzymatic Shearing Cocktail to quickly and easily shear chromatin. As enzymatic shearing is solely time and temperature dependent, the inconsistencies associated with sonication are eliminated, which helps to improve your ChIP results.

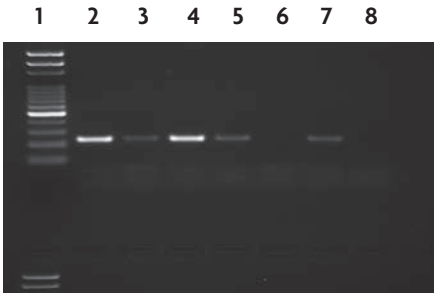

Figure 1: ChIP with the ChIP-IT™ Control Kit - Mouse
Chromatin IP using ChIP-IT Express (Cat. No. 53008) performed on mouse macrophage 4/4 cells, amplifying a region of the EF1α gene promoter. Lane 1: molecular weight ladder. Lanes 2 and 4: RNA pol II mAb (Cat. No. 39097). Lanes 3 and 5: negative IgG control. Lane 6: no primary antibody. Lane 7: input chromatin control. Lane 8: no DNA control.

Ready-to-ChIP Chromatin

For your convenience, Active Motif offers Ready-to-ChIP Chromatin. Ready-to-ChIP Chromatin has been optimally sheared by sonication and validated in ChIP. As a result, you can more easily validate your own antibodies and primer sets. It can be used in conjunction with all of the ChIP-IT Kits and controls, so you can be certain the only variable in testing an antibody for ChIP is the antibody itself.

Product	Format	Catalog No.
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014
ChIP-IT™ Control Kit – Human	5 rxns	53010
ChIP-IT™ Control Kit – Mouse	5 rxns	53011
ChIP-IT™ Control Kit – Rat	5 rxns	53012
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
ChIP-IT™ Shearing Kit	10 rxns	53002
Enzymatic Shearing Kit	10 rxns	53005

Double Destabilized Luciferase Provides Better Response & Sensitivity

RapidReporter®* is the only luciferase reporter gene assay that utilizes both mRNA and protein destabilizing elements. This greatly reduces background, increasing both the magnitude of the response that can be measured after stimulation or repression, and the speed in which the assay can measure changes in transcription (both increases and decreases).

Standard luciferase assays are limited by the fact that basal activity of the cloned promoter results in accumulation of both the luciferase mRNA and protein. The slow clearance rate of these pre-existing molecules substantially delays and dilutes the measurable response. Thus, a large proportion of the luciferase measured is derived from transcription that took place before the test agent was even added. As a result, in standard assays transient or relatively minor effects are hidden and kinetics are inaccurate. Consequently, drugs or treatments that are disregarded because they have little or no detectable effect may often elicit a 10-fold or more change in transcription.[1]

A good start, but not the end

In trying to address the problem of long half-life proteins, some vectors include protein-destabilizing elements, which cause the luciferase to degrade more rapidly. Destabilizing the protein, however, only partly addresses the problem, as clearance rates are also dependent on the half-life of the mRNA. As long as the reporter mRNA is intact, it continues to produce new reporter protein.

Eliminate both causes of background

To solve the problem, RapidReporter utilizes vectors that include both protein AND mRNA destabilizing elements. Such double destabilizing vectors have been shown to reduce luciferase half-life an additional 33% compared to vectors with only protein-destabilizing elements.[2]

Measure what's actually happening

The use of double destabilizing vectors makes RapidReporter more sensitive and responsive, so you can detect smaller changes and get more accurate real-time measurement than with non-destabilized reporter gene assays, or those that destabilize the protein only (Figure 1).

Pre-made vectors & complete kits

In addition to empty RapidReporter vectors in two different stringencies, Active Motif offers vectors that contain widely studied promoters. All are available separately or in complete assay kits, which also include a positive control vector and buffers & substrate optimized for *Gaussia* luciferase. To learn more about the only reporter assay with double destabilization, please give us a call or visit www.activemotif.com.

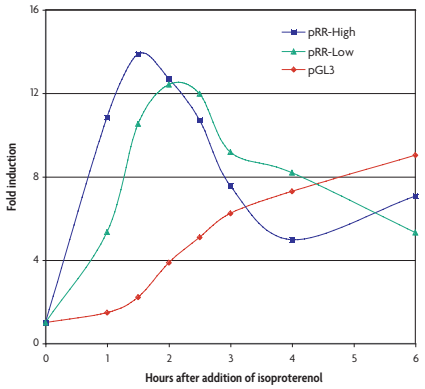

Figure 1: More accurate kinetics, higher fold induction. 293 cells transiently transfected with pRR-High-CRE, pRR-Low-CRE and a pGL3 vector containing CRE were plated onto 96-well plates. Twenty-four hours post-transfection, cells were stimulated with 4 µM isoproterenol, then measured for *Gaussia* (pRR vectors) and firefly luciferase activities (pGL3 vector).

Product	Format	Catalog No.
RapidReporter® Gaussia Luciferase Assay	100 rxns	33001
	1000 rxns	33002
RapidReporter® pRR-High vector	10 µg	33003
RapidReporter® pRR-High Assay	100 rxns	33004
RapidReporter® pRR-Low vector	10 µg	33005
RapidReporter® pRR-Low Assay	100 rxns	33006
RapidReporter® pRR-High-CRE vector	10 µg	33007
RapidReporter® pRR-High-CRE Assay	100 rxns	33008
RapidReporter® pRR-High-GR vector	10 µg	33011
RapidReporter® pRR-High-GR Assay	100 rxns	33012
RapidReporter® pRR-High-IRF-1 vector	10 µg	33017
RapidReporter® pRR-High-IRF-1 Assay	100 rxns	33018
RapidReporter® pRR-High-NFκB vector	10 µg	33009
RapidReporter® pRR-High-NFκB Assay	100 rxns	33010
RapidReporter® pRR-High-STAT1 vector	10 µg	33015
RapidReporter® pRR-High-STAT1 Assay	100 rxns	33016
RapidReporter® pRR-High-STAT3 vector	10 µg	33013
RapidReporter® pRR-High-STAT3 Assay	100 rxns	33014

* RapidReporter is covered under U.S. Patent No. 7,157,272 and various other patents worldwide and is sold under license granted by GeneStream Pty Ltd. Purchasers are subject to a Limited-use License; please contact Active Motif's Technical Services for details, or download a copy from our web site. RapidReporter is a registered trademark of GeneStream Pty Ltd.

REFERENCES

1. Voon, C. *et al.* (2005) *Nucleic Acids Research* 33(3): e27.
2. Almond, A. *et al.* (2004) *Promega Notes* 87: 18-22.

Easily Compare CpG Methylation in Various DNA 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, greatly improving assay sensitivity.

MethylCollector advantages

- **Rapid** – simple protocol is completed in less than 4 hours
- **Adaptable** – enables detection of samples from 5 ng to 1 µg of DNA
- **Verified** – positive control DNA and PCR primers ensure success

The MethylCollector method

In MethylCollector, His-tagged MBD2b specifically binds to CpG-methylated DNA fragments prepared by sonication or enzymatic digestion. 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 (Figure 1). Because MethylCollector is highly efficient, you can analyze the methylation state of any specific locus on genomic DNA isolated from less than 800 cells (~5 ng DNA).

MethylCollector applications

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.

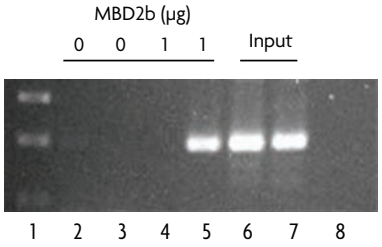

Figure 1: Isolation of Fully Methylated Jurkat DNA. MethylCollector was used to isolate methylated DNA from 100 ng genomic DNA (lanes 2 & 4) and Fully Methylated Jurkat DNA (lanes 3 & 5), with (lanes 4 & 5) or without (lanes 2 & 3) inclusion of 1 µg of the kit's His-tagged MBD2b protein. These samples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

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

Reproducible Bisulfite Conversion for Accurate Analysis of Methylated DNA

The MethylDetector™ Bisulfite Modification Kit makes DNA methylation analysis fast and efficient by providing optimized reagents, time-saving DNA purification columns and positive control PCR primers for assay validation.

Proven controls verify your success

DNA methylation analysis often uses bisulfite to convert unmethylated cytosines to uracils, leaving methylated cytosines unchanged. The DNA is then PCR amplified and analyzed by sequencing or restriction digest, which can be costly and time-consuming. Thus, confirming that conversion was successful before analyzing the samples is a big benefit. To that end, the MethylDetector Kit provides positive control PCR primers specific for bisulfite-converted DNA, so you can confirm the conversion worked before starting the analysis (Figure 1).

Advantages

- **Reproducible** – 99% conversion efficiency
- **Flexible** – use high G/C content sequences and uncut DNA
- **Easy** – DNA purification columns mean no more precipitations

Try MethylDetector today

MethylDetector will speed and simplify your methylation analysis. Learn more at www.activemotif.com.

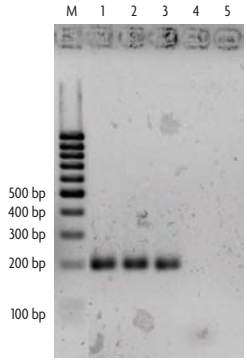

Figure 1: Reproducible conversion by MethylDetector. MethylDetector was used for bisulfite conversion of 3 different DNA samples (Lanes: 1-3) and a control with no DNA (Lane: 4). PCR was performed on these samples and an unconverted DNA control (Lane: 5) using the kit's control PCR primers. The presence of PCR product in only the converted samples demonstrates the efficiency and reproducibility of the MethylDetector Kit.

Product	Format	Catalog No.
MethylDetector™	50 rxns	55001

NEW: Assays for IRF-3 & -7 and SREBP1 Activity

TransAM™ Kits are fast and simple DNA-binding ELISAs in a convenient format with high sensitivity and specificity for over 40 targets. Now TransAM, the most published alternative to EMSA, is available for IRF-3 & IRF-7 and SREBP1.

The TransAM method

TransAM Kits use a unique plate-based format to capture activated transcription factors, which are analyzed using an antibody specific to your isoform of interest. TransAM Kits are non-radioactive and offer up to 100-fold more sensitivity than traditional gelshift techniques, so even the smallest changes in transcription factor levels can be detected.

How it works

TransAM Kits assay for transcription factor activity by capturing the activated factor with a double-stranded oligonucleotide containing the target-specific consensus sequence that is bound to a 96 strip-well plate. When nuclear extracts from tissue or cell samples are added to the plate, the activated transcription factor binds the consensus sequence on the plate. Next, a primary antibody specific to the transcription factor is added which is subsequently detected by an HRP-conjugated secondary antibody and developed to give a colorimetric readout.

TransAM IRF-3 & 7

Studying Interferon Regulatory Factors (IRFs) and their involvement in host defense through regulation of immune responses, cell growth and hematopoietic development can be difficult. Accurate monitoring of IRF activation is now easier with TransAM Kits. Kits are available for mouse and human IRF-3 and human IRF-7.

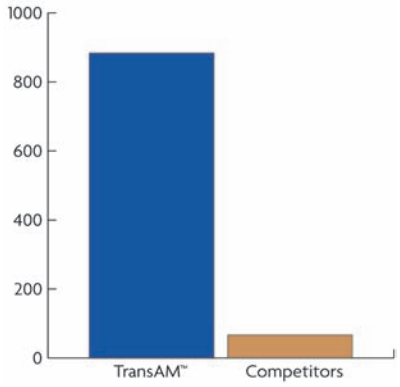

Figure 1: TransAM Kit citations.
Using HighWire Press, http://highwire.stanford.edu, a comparison was made by searching for citations of TransAM from Active Motif versus the tradenames of all competitor kits combined. TransAM is clearly the leader.

TransAM citations

TransAM is the most published ELISA assay for transcription factor activation (Figure 1). The combination of a fast, user-friendly format with high sensitivity and specificity makes it easy to see why over 800 citations are available for TransAM Kits.

TransAM SREBP1

Sterol-regulatory element binding proteins (SREBPs) can be difficult to study since they are synthesized as inactive precursor proteins bound to the endoplasmic reticulum and nuclear envelope, yet once they are activated by sterol deprivation, the precursor

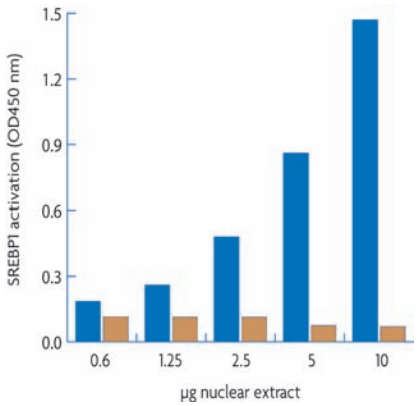

Figure 2: TransAM SREBP1 activation.
0.6 to 10 µg of Cos-7 (SREBP1 transfected) nuclear extract (black bars) and mock-transfected Cos-7 nuclear extracts (gray bars) were assayed per well. .

protein is cleaved to release the N-terminal portion to enter the nucleus and activate transcription of genes involved in cholesterol and fatty acid synthesis. TransAM SREBP1 simplifies studying SREBP1 activation, and detects SREBP-1a and -1c isoforms in nuclear extracts from human, mouse and rat samples (Figure 2).

Widest selection of targets and formats

Active Motif offers the broadest selection of targets, including kits for NFκB, AP-1, STAT, PPARγ, HIF-1, NFATc1, C/EBP and many others. Please visit our website for a complete product listing, and to check for new releases!

Product	Format	Catalog No.
TransAM™ IRF-3 (Human)	1 x 96 rxns	48396
	5 x 96 rxns	48896
TransAM™ IRF-3 (Mouse)	1 x 96 rxns	48496
	5 x 96 rxns	48996
TransAM™ IRF-7	1 x 96 rxns	50196
	5 x 96 rxn	50696
TransAM™ SREBP1	1 x 96 rxns	50496
	5 x 96 rxn	50996

Lower Background Co-IP of Both Nuclear & Whole-cell Complexes

The Universal Magnetic Co-IP Kit includes protein G-coated magnetic beads that simplify the IP and wash steps while providing lower background, which improves your Co-IP results. The kit also includes reagents to prepare both nuclear and whole-cell extracts, so you can study intact protein complexes whether they were originally bound to DNA or in the cytoplasm.

Protein/protein interactions are often studied using Co-Immunoprecipitation (Co-IP), in which a single antibody is used to precipitate an entire protein complex. Additional antibodies are then used in Western blot to identify/verify other complex members (Figure 1).

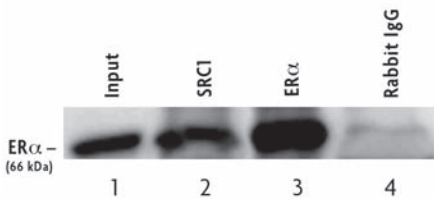

Figure 1: Nuclear Co-IP of SRC-1 and ERα.
The Universal Magnetic Co-IP Kit was used to make nuclear extract from MCF-7 cells induced 1 hour with 10 nM Estradiol. IP was performed on 300 µg samples using 2 µg of SRC-1 pAb, ERα pAb and rabbit IgG (as a negative control). Western blot was then performed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

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.

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 was designed to help maintain weaker complexes.

Universal Magnetic Co-IP Kit advantages

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

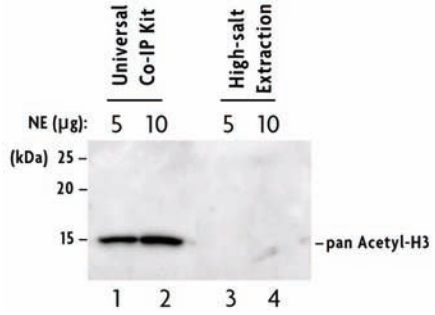

Figure 2: Detection of acetylated Histone H3.
HeLa nuclear extracts were made using the Universal Magnetic Co-IP Kit and a traditional high-salt extraction protocol, each supplemented with the 1 µM trichostatin A, a deacetylase inhibitor. Five and ten µg samples of each extract were used in Western blot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

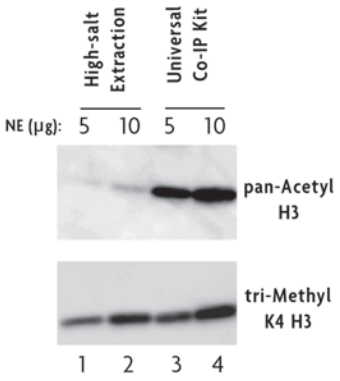

Figure 3: Preservation of acetylation and methylation.
Nuclear extracts were made from HeLa cells treated with 500 nM trichostatin A for 20 hours using either the Universal Magnetic Co-IP Kit (with its deacetylase inhibitor) or traditional high-salt extraction. Five and ten µg samples of these extracts 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. To find out more, please give us a call or visit us on the web at www.activemotif.com.

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

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 & 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.

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 is provided as a control for use with your samples; enough is provided so that it could also be used as a HAT to screen an entire 96-well plate of inhibitors. Anacardic acid is provided for use as a control, as it is a potent HAT inhibitor (Figure 1).

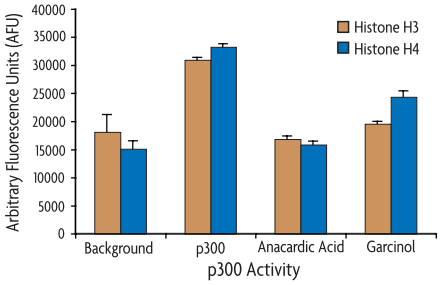

Figure 1: HAT inhibitor effects on p300 activity.
50 ng p300 were assayed per well with 50 µM acetyl-CoA and 50 µM histone H3 or H4 peptide substrates in the absence or presence of 15 µM anacardic acid or 25 µM garcinol, known HAT inhibitor compounds.

How 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 to result in 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).

Assay for HDAC activity or inhibition

With these simple HDAC Assay Kits, you can easily assay for HDAC activity or you can screen compounds for their inhibitory effects. The provided deacetylated assay standard enables you to quantify HDAC effects with great accuracy.

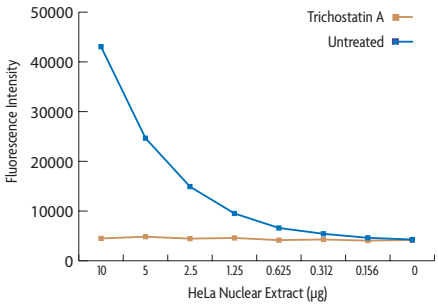

Figure 2: HDAC activity in HeLa cells.
HeLa Nuclear Extracts were assayed at 0 to 10 µg per well using the fluorescent version of the HDAC Assay Kit. Untreated extract results are shown with a purple line, and extracts inhibited with 1 mM Trichostatin A are shown with a copper line.

Try them today!

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

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

NEW: Antibodies to Study Transcription, Histones and Chromatin Biology

Active Motif offers high-quality antibodies that are manufactured in-house, rigorously tested and validated in the applications that you need them for.

Validated, specific antibodies

Active Motif specializes in providing researchers with a wide variety of tools to study the biology of the nucleus. If you are studying chromatin biology, transcriptional regulation or cell function, we specialize in making antibodies to protein targets involved in those processes. As you can see from our new product releases, we also specialize in manufacturing antibodies against histones and histone modifications, many of which have been validated for use in chromatin immunoprecipitation (ChIP) and immunofluorescence (IF). We also offer a wide range of antibodies against transcription factors, chromatin modifiers and cell-cycle regulators.

Superior characterization

Your choice of antibody supplier is crucial and can have a dramatic impact on the success or failure of your experiments. At Active Motif, we are committed to providing researchers with the highest quality antibodies for studying chromatin and the biology of the nucleus. Every antibody we sell is rigorously tested to ensure that you are not wasting your precious time and research dollars on antibodies that don't perform as advertised. We are not interested in just reselling products from other companies, a very common practice these days. We manufacture the antibodies to histones and histone modifications in-house, so we control antibody quality and performance. Anyone can just SELL antibodies, but Active Motif actually makes and characterizes our antibodies so our antibody performance is guaranteed to work for you.

New Antibody Releases

Cell Biology Antibodies	Applications	Catalog No.
Aurora B Rabbit pAb	IF, WB	39261
INCENP mouse mAb	IF	39259
Lamin A/C Mouse mAb	IF, IP, WB	39287
LAP2 alpha mouse mAb	WB	39267

Chromatin Modifier Antibodies	Applications	Catalog No.
CARM1 Rabbit pAb	WB	39251
Dimethyl-Arginine, asymmetric Rabbit pAb	IF, WB	39231
HP1 alpha Rabbit pAb	WB	39295
JARID1C Rabbit pAb	WB	39229
JMJD2D Rabbit pAb	WB	39247
JMJD2F Rabbit pAb	WB	39257
Mi2 beta Rabbit pAb	WB	39289

Histone and Histone Modification Antibodies	Applications	Catalog No.
Histone H2A phospho Ser129 Rabbit pAb (yeast)	ChIP, IF, IP, WB	39271
Histone H2A Rabbit pAb (yeast)	ChIP, WB	39235
Histone H2B Rabbit pAb (yeast)	ChIP, WB	39237
Histone H3 pan-methyl Lys9 Rabbit pAb	IF, WB	39241
Histone H3 monomethyl Lys9 Rabbit pAb	IF, WB	39249
Histone H3 dimethyl Lys9 Rabbit pAb	IF, WB	39239
Histone H3 trimethyl Lys9 Mouse mAb	ChIP, IF, WB	39285
Histone H3 phospho Ser10 Rabbit pAb	IF, WB	39253
Histone H3 dimethyl Lys27 Rabbit pAb	IF, WB	39245
Histone H3 dimethyl Lys36 Rabbit pAb	IF, WB	39255
Histone H3 acetyl Lys56 Rabbit pAb	WB	39281
Histone H3 monomethyl Lys56 Rabbit pAb	WB	39273
Histone H3 dimethyl Lys56 Rabbit pAb	WB	39277
Histone H4 Rabbit pAb	WB	39269
Histone H4 pan-acetyl Rabbit pAb	IF, WB	39243
Histone H4 dimethyl Arg3, symmetric Rabbit pAb	IF, WB	39275

Transcription Factor Antibodies	Applications	Catalog No.
Aiolos Rabbit pAb	IF, IP, WB	39293
Ikaros Rabbit pAb	IF, IP, WB	39291
NFκB p65 Rabbit pAb	WB	39283
RNA pol II CTD phospho Ser5 Rabbit pAb	IF, WB	39233

Epitope Tag Antibodies	Applications	Catalog No.
Myc Tag mouse mAb	WB	39279

Products for Chromatin & Transcriptional Regulation

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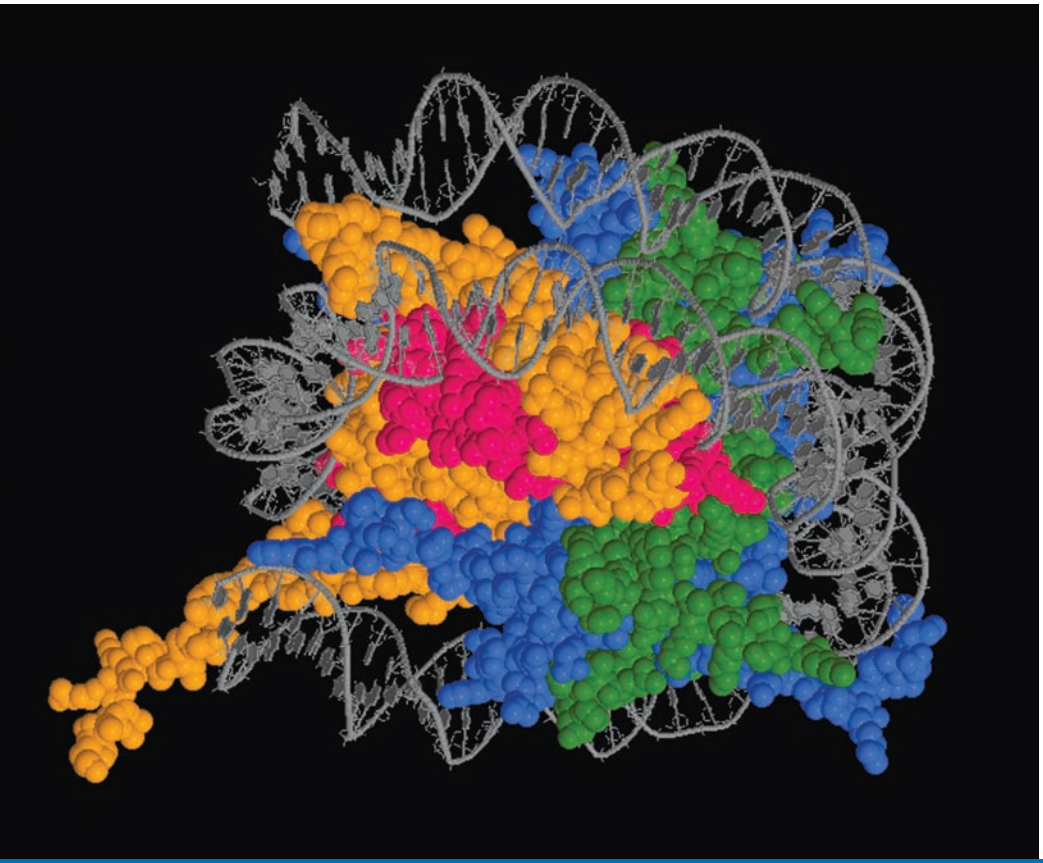

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