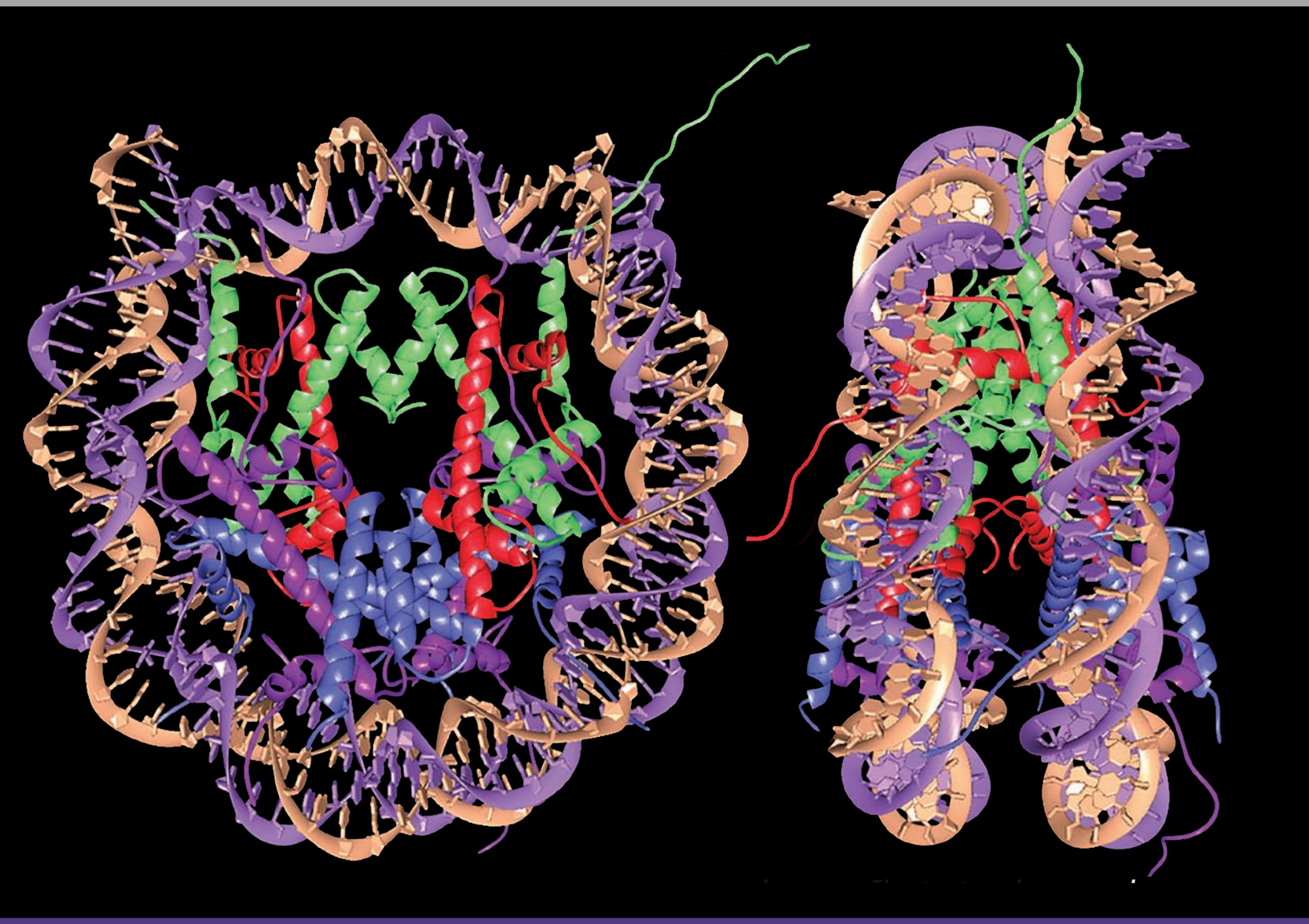

Histone Analysis

innovative tools designed to help unravel the histone code

Histone Antibodies
Histone Purification
Recombinant Histones
Histone Modifying Enzymes
Histone Modification ELISAs
Histone Demethylase Assay
Histone Peptide Arrays
Histone Binding Assay
HAT/HDAC Assays
Chromatin Assembly

Active Motif's unique portfolio of histone technologies provides researchers with a complete solution for the analysis of histones and their post-translational modifications, beginning with histone purification and continuing through to chromatin assembly.

Let Active Motif's antibodies, enzymes and modification-specific assays simplify your histone analysis.

ACTIVE MOTIF®

Tools to Analyze Nuclear Function

Histone Analysis – tools designed to help unravel the histone code

Histone modifications such as acetylation, phosphorylation and methylation at specific amino acid residues on the histone globular domain and the N-terminal tails have been shown to influence and regulate transcription, chromosome packaging and DNA damage repair. Due to the importance of histone modifications in regulating chromatin structure and disease, Active Motif has developed a variety of products that simplify histone analysis.

Histone Purification

Active Motif's Histone Purification and Histone Purification Mini Kits enable you to isolate core histones from any cell culture or tissue sample while preserving their post-translational modifications. The kits use a unique purification resin and a series of proprietary elution buffers to isolate very pure fractions of histones (Figure 1).

Unlike standard acid extraction techniques, it is possible to isolate core histones as either a single fraction, or to further separate them into H2A/H2B and H3/H4 fractions (Table 1). Additionally, our unique extraction buffer prevents unwanted enzymatic reactions from occurring, thereby preserving the existing histone modifications.

Purified histones are ready for downstream analysis with Active Motif's many different histone modification antibodies, or they can be used as substrates in functional assays, such as Active Motif's Histone Modification ELISAs and Chromatin Assembly Kits.

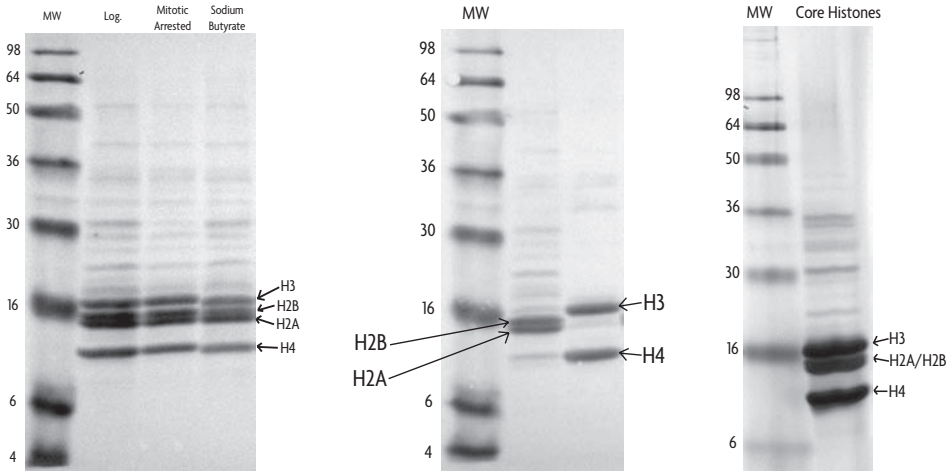

Figure 1: Core histone purified from HeLa cells and brain tissue.
Ten µg of sample were loaded per lane. **Left:** Core histones purified from logarithmically growing mitotic arrested or sodium butyrate-treated HeLa cells. **Center:** Separate H2A/H2B and H3/H4 fractions purified from HeLa cells. **Right:** Core histones isolated from rat brain tissue.

Kit	Format	Elution	Capacity
Histone Purification Kit	Gravity Flow	Separate H2A/H2B & H3/H4 fractions	05-2.5 mg
	Spin Column	H2A, H2B, H3 & H4 in a single fraction	0.5-2.5 mg
Histone Purification Mini Kit	Mini Spin Column	H2A, H2B, H3 & H4 in a single fraction	0.1-0.5 mg

Table 1: Comparison of the original Histone Purification Kit and the Histone Purification Mini Kit.

Advantages

- Preserves modifications on histones, such as acetylation, phosphorylation and methylation
- Purifies total core histones (H2A, H2B, H3 & H4) or separate fractions of H2A/H2B dimers and H3/H4 tetramers
- Histone Purification Mini Kit can purify histones from budding yeast

To learn more about either the Histone Purification Kit or the Histone Purification Mini Kit, please call or visit us at www.activemotif.com/histonepur.

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

On the cover: Ribbon diagram of the nucleosome core particle structure (H2A.Z nucleosome, pdb entry 1F66) viewed down the superhelical axis (left) and rotated 90° (right). Original figure was prepared by Dr. Karolin Luger, Department of Biochemistry and Molecular Biology, Colorado State University.

MODified™ Histone Peptide Array & Analysis Software

The MODified™ Histone Peptide Array* is a valuable research tool that can be used to screen antibodies, proteins and enzymes for interactions with histones and their post-translational modifications. Each array contains 384 different histone modification combinations in duplicate. Modifications include acetylation, methylation, phosphorylation and citrullination on the N-terminal tails of histones H2A, H2B, H3 and H4.

This unique histone array contains up to four modifications per 19mer peptide to study not only individual modifications, but also to determine if neighboring modifications alter site recognition and binding. The MODified Histone Peptide Array can be used to screen antibodies for cross-reactivity or to study protein and enzyme interactions (Figure 3).

The simple array protocol works like a Western blot. Either ECL-based or colorimetric detection systems can be used. The image is captured using film or a CCD camera; no special equipment is needed.

The MODified Histone Peptide Arrays are available individually, or in packs of five. For a complete solution, the MODified Array Labeling Kit contains the necessary buffers and reagents for ECL-based detection.

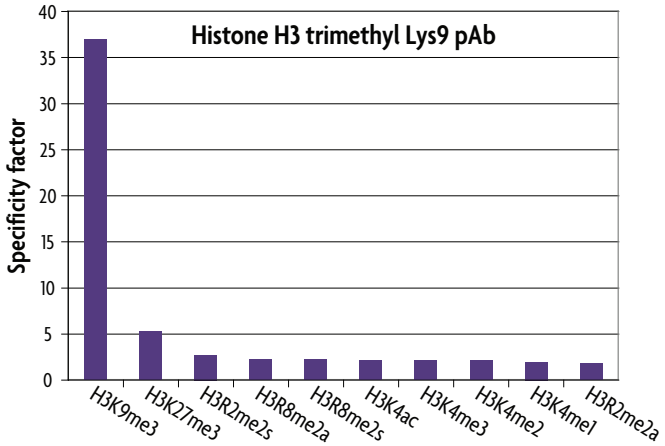

Figure 2: ECL detection and graphical analysis of the cross-reactivity of Histone H3 trimethyl Lys9 antibody. Active Motif's Histone H3 trimethyl Lys9 (H3K9me3) pAb (Catalog No. 39161) was used at a 1:2,000 dilution on the MODified Histone Peptide Array. Anti-rabbit HRP secondary antibody was used at a 1:2,500 dilution, followed by ECL detection and image capture with a CCD camera. Active Motif's Array Analyse Software was used to analyze spot intensity and generate a graphical analysis of decreasing specificity factors, which is the ratio of the average intensity of all spots containing H3K9me3 divided by the average intensity of all spots not containing H3K9me3. The results show this antibody is very specific for histone H3 trimethyl Lys9, with little cross-reactivity for other modifications.

Advantages

- **Histone specific** – unique array panel tests for specific histone modifications
- **Study neighboring effects** – each peptide contains up to four modification combinations, enabling analysis of the effects of neighboring modifications
- **Detects like a Western blot** – fast and easy to use; works with either ECL-based or colorimetric detection

Free Software for Analysis

Active Motif's Array Analyse Software is a free program designed for use with the MODified Histone Peptide Arrays. This PC compatible software will analyze the spot intensities from the MODified array and generate a graphical analysis of the histone modification interactions (Figure 2). Information about spot intensity, averages and errors can be saved in Excel-compatible files. For added convenience, up to three individual modifications can be displayed in superposition to the experimental data, enabling better visualization of neighboring effects.

To learn more about the MODified™ Histone Peptide Arrays, or to download the free Array Analyse Software, please visit www.activemotif.com/modified.

Histone Antibodies

Active Motif offers a variety of antibodies to histones and biologically relevant histone modifications. Each antibody has been rigorously tested and validated for use in important applications such as immunofluorescence (IF), Western blotting and chromatin immunoprecipitation (ChIP). Our years of expertise in antibody development ensures that only the highest quality antibodies are offered for use in your research.

- **Immunogen selection** – ensures the antibody recognizes the modification of interest, and does not cross-react with related proteins
- **Specificity screening** – every antibody must have a greater than 25-fold selectivity for the desired modification
- **Application validation** – important applications are tested, giving you confidence when using them in your research

To see our extensive list of histone modification antibodies, including quality control data for IF, Western blot, ChIP or MODified Histone Peptide Array, please visit www.activemotif.com/histoneabs.

To learn about our fluorescent antibodies for IF, or our antibody labeling kits, visit www.activemotif.com/chromeo.

*CelluSpots™ arrays are manufactured under license by INTAVIS Bioanalytical Instruments AG and sold through Active Motif as MODified™ Histone Peptide Arrays

Histone Modifying Enzymes

Histones are subject to a variety of post-translational modifications that influence a number of nuclear processes. To better investigate some of the complex functional questions about chromatin-associated proteins, nucleosome remodeling, transcriptional regulation, replication and DNA repair, Active Motif offers a range of histone modifying enzymes for the following protein types:

- **Methyltransferases**
- **Demethylases**
- **Acetyltransferases**
- **Deacetylases**

These modifying enzymes can be used in conjunction with Active Motif's antibodies, recombinant histones, ELISAs and MODified™ Histone Peptide Arrays (Figure 3).

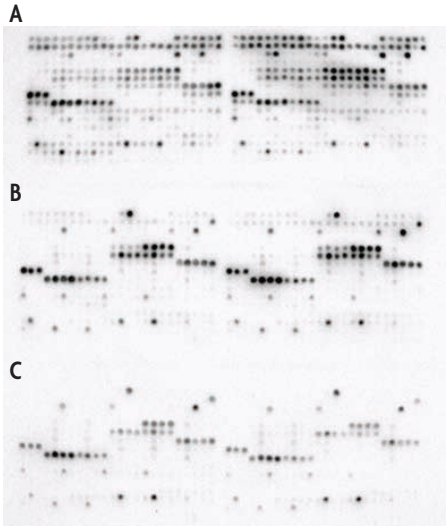

Figure 3: Images of ECL detection of MODified Histone Peptide Arrays treated with G9a. MODified Histone Peptide Arrays were treated with A) 25 µM G9a methyltransferase (Catalog No. 31327), B) 25 µM G9a mutant H904K (Catalog No. 31328), or C) no enzyme control, overnight in the presence of 1 mM AdoMet. The arrays were detected using a Histone H3 dimethyl Lys9 antibody. Novel methylation sites were observed on the array treated with wild-type G9a histone methyltransferase, showing the activity of this histone modifying enzyme on the peptide substrates.

To view our full listing of histone modifying enzymes and their associated technical data sheets, please visit www.activemotif.com/hismodenz.

Formaldehyde Detection of Histone Demethylase Activity

The fluorescent Histone Demethylase Assay is designed to detect the formaldehyde released from the reaction of lysine specific demethylase 1 (LSD1, also known as KDM1) with a methylated substrate. The recombinant histone H3K4me2 substrate used in the assay mimics a native histone substrate, generating results that more closely resemble *in vivo* conditions. As the LSD1 enzyme demethylates the recombinant histone substrate, formaldehyde is released as a by-product. The Detection Reagent reacts with each formaldehyde molecule to generate a fluorescent signal equivalent to the overall production of formaldehyde.

The Histone Demethylase Assay Kit can be used to analyze the overall conversion efficiency of an LSD1 sample, or to screen compounds that cause changes in histone demethylation activity.

As shown in Figure 4, the LSD1 enzyme is able to more efficiently demethylate the included recombinant histone H3K4me2 protein than a histone H3K4me2 peptide substrate. Because the recombinant histone more closely resembles a native histone, the Histone Demethylase Assay enables more accurate analysis of histone demethylation activity.

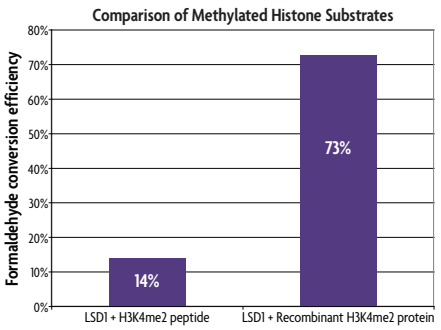

Figure 4: Comparison of different histone substrates and their effect on LSD1 demethylase efficiency. The positive control LSD1 enzyme from the Histone Demethylase Assay was used to evaluate demethylase activity using either a histone H3K4me2 peptide or the kit's recombinant histone H3K4me2 protein. One µg of LSD1 was tested with either 70 µM H3K4me2 peptide or with 13 µM recombinant histone H3K4me2 protein. LSD1 was able to convert 73% of the recombinant histone substrate into a formaldehyde by-product, yet it was only able to convert 14% of the peptide substrate into a formaldehyde by-product, even though there was 5-fold more peptide available than recombinant protein for the same amount of LSD1 enzyme.

Product	Format	Catalog No.
Histone Demethylase Assay (Fluorescent)	48 rxns	53200
Recombinant LSD1 protein, active	50 µg	31334

HeLa Acid Extracts

Active Motif's HeLa acid extracts are a reliable control for studying histone modifications. Extracts are available either untreated, or treated with chemicals known to affect epigenetic events, such as sodium butyrate, paclitaxel, etoposide and anacardic acid.

To learn more, or to see a complete list of available extracts, please visit us at www.activemotif.com/acidextract.

Recombinant Histone Proteins

Active Motif is the first company to offer recombinant histones with acetylation and site-specific mono-, di- and trimethylation. These recombinant histones can be used as controls for histone antibodies, substrates for histone modification enzymes, or to generate chromatin *in vitro*, using Active Motif's Chromatin Assembly Kit (Catalog No. 53500).

Recombinant methylated lysine residues are created using a patented approach in which an analog of methyl lysine is installed in the histone via chemical alkylation. This enables the site and degree of methylation to be carefully controlled. Each methylation reaction is over 99% complete and is verified by high-resolution mass spectrometry. The recombinant histones are also analyzed by dot blot to confirm identity (Figure 5).

Acetylated histones are created with our patent pending technology that enables us to acetylate the histone tail, without altering the native peptide bonds. The ability of the acetylated and methylated histones to mimic their native counterparts enables these substrates to yield more "natural" results than histone peptides.

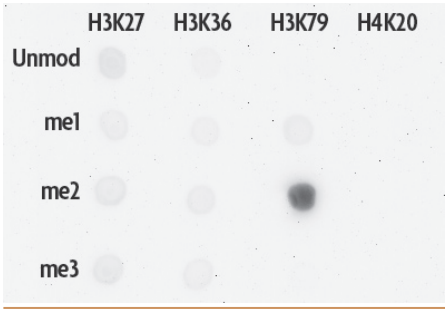

Figure 5: Dot blot of recombinant histones. One µg of unmodified, mono-, di- or trimethylated recombinant proteins for H3K27, H3K36, H3K79 and H4K20 were spotted onto a PVDF membrane and probed with Histone H3 dimethyl Lys79 pAb (Catalog No. 39143) at a 1:1000 dilution. The dot blot confirms the identity of the recombinant H3K79me2 protein.

For a complete list of our over 20 unique recombinant histones, please visit us at www.activemotif.com/recombhis.

Histone Modification ELISAs

To better understand the effects of histone modifications on chromatin remodeling and transcriptional regulation, Active Motif has developed over 10 different assays for important histone modification sites, such as lysine methylation at K4, K9 and K27 or serine phosphorylation at S10 and S28. These modification sites serve as key targets of histone methyltransferase and histone demethylase enzymes, or act as markers for mitosis.

The Histone Modification ELISA Kits provide a sensitive method for detecting changes in the level of specific histone modifications from purified core histones, or histones isolated by acid extraction. These easy-to-use kits are sandwich ELISAs that utilize a capture antibody against histone H3 and a detecting antibody specific to the modification of interest. An HRP-conjugated secondary antibody and developing solutions provide a colorimetric readout in less than 3 hours (Figure 6).

Each kit includes validated modification specific controls. The included methylated recombinant histone proteins can be used to generate a standard curve, enabling quantification of the amount of site- and degree-specific methylated histone in each sample. The acid extracts provided in the phosphorylated ELISAs serve as a qualitative control. The recombinant histones and acid extracts are also available separately (see above and on previous page).

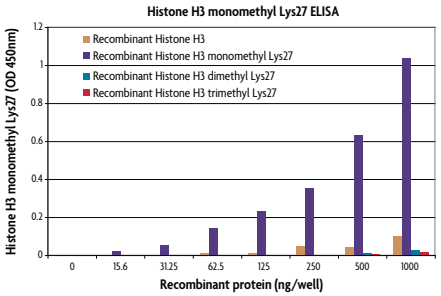

Figure 6: Histone H3 monomethyl Lys27 specificity. Recombinant Histone H3, mono-, di- and trimethyl Lys27 proteins were assayed from 15 ng - 1 µg per well using the Histone H3 monomethyl Lys27 ELISA. The results show the specificity of the assay for the monomethyl modification. The monomethyl protein is included in the assay for sample quantification.

To see an up-to-date list of the more than 10 Histone Modification ELISAs available, please visit www.activemotif.com/hiselisa.

Chromatin Assembly

Design your own chromatin with Active Motif's Chromatin Assembly Kit. Using either purified core histones, or Active Motif's recombinant histones, combine histones with the kit components and incubate with DNA to generate assembled chromatin that functions in a context that closely resembles *in vivo* chromatin.

Product	Format	Catalog No.
Chromatin Assembly Kit	10 rxns	53500

The Chromatin Assembly Kit is an ATP-dependent method that utilizes purified recombinant human chromatin assembly complex ACF and histone chaperone NAP-1 with core histones for *in vitro* assembly of extended, regularly ordered, periodic arrays of nucleosomes. For details, please visit www.activemotif.com/chromassembly.

Histone Acetyltransferase (HAT) Activity

The Histone Acetyltransferase (HAT) Assay Kit is a quick and sensitive method to determine the activity of your own source of purified histone acetyltransferases, or to screen for potential inhibitors of HAT activity.

This fluorescent 96-well plate assay includes N-terminal histone H3 and H4 substrate peptides for screening HAT enzymes and a positive control p300 catalytic domain protein to screen inhibitors. HATs will catalyze the transfer of acetyl groups from the provided acetyl-CoA to generate an acetylated peptide and CoA-SH. After stopping the reaction with stop solution, a developer is added that reacts with the free sulfhydryl groups on the CoA-SH to give a fluorescent signal (Figure 7).

A standard curve can be generated with either β-mercaptoethanol or acetyl-CoA in order to relate the fluorescence of your HAT to pmol/min/µg specific activity.

Product	Format	Catalog No.
HAT Assay Kit (Fluorescent)	1 x 96 rxns	56100
Recombinant p300 protein, catalytic domain	5 µg	31205

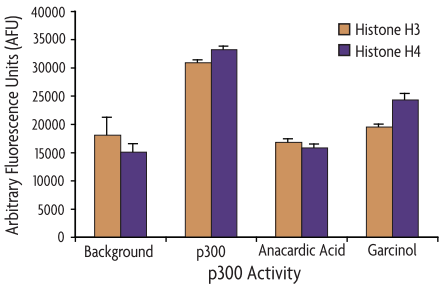

Figure 7: HAT inhibitor effects on p300 activity. The HAT activity of 50 ng p300 catalytic domain was assayed using 50 µM acetyl-CoA and either 50 µM Histone H3 or Histone H4 peptides. The addition of 15 µM anacardic acid or 25 µM garcinol inhibited the HAT activity down to background levels. The background signal indicates the level of autoacetylation present from the p300 acetyltransferase.

Histone Deacetylase (HDAC) Activity

To measure HDAC activity in your nuclear extracts, immunoprecipitates, column fractions or purified proteins, Active Motif offers your choice of fluorescent or colorimetric detection. Both HDAC kits utilize a short peptide substrate containing an acetylated lysine residue that can be deacetylated by Class I, II and IV HDAC enzymes. (Class III & Sirtuin HDACs require the addition of NAD^+ cofactor to the assay.) Once the substrate is deacetylated, the lysine reacts with the developing solution and releases either a chromophore or a fluorophore, which is then measured (Figure 8). A deacetylated assay standard is provided in each kit to enable calculation of HDAC activity in pmol/min/mg. Additionally, the HDAC Assay Kits can be used to screen inhibitor compounds.

Product	Format	Catalog No.
HDAC Assay Kit (Fluorescent)	1 x 96 rxns	56200
HDAC Assay Kit (Colorimetric)	1 x 96 rxns	56210

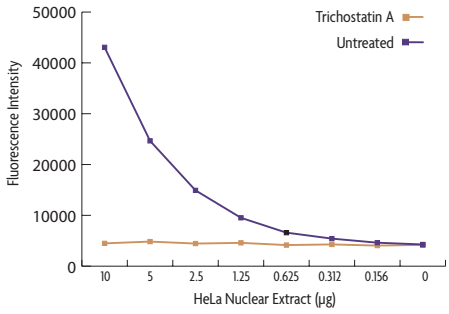

Figure 8: Fluorescent HDAC assay results. HeLa nuclear extracts were assayed for HDAC activity in duplicate from 0 to 10 µg per well in the presence (copper line) or absence (purple line) of 1 µM Trichostatin A inhibitor.

Histone Binding Assay to Identify Chromatin-modifying Proteins

Active Motif's HiLite™ Binding Assay is an innovative tool for identifying chromatin-modifying proteins that bind to the histone tails of methylated H3K9 and H3K27. This fast, homogeneous assay uses fluorescence polarization (FP) to measure the affinity of the binding interactions between your protein of interest and specific histone methylation states, which in turn enables fast and efficient inhibitor screening studies.

Each kit contains 8 fluorescently labeled peptides that correspond to unmodified, mono-, di- and trimethylated histone H3 lysine 9 and lysine 27 regions, as well as a positive control HP1 protein, assay buffer, calibration dye and five 96-well half area black polystyrene plates. When the protein of interest binds the modified peptide, it slows the rotation of the peptide, causing the amount of polarized light that is emitted to be greater than an unbound peptide. This provides a quantitative measure of the histone binding protein's affinity for the peptide's histone modification.

Product	Format	Catalog No.
HiLite™ Histone H3 Methyl-Lys9 / Lys27 Binding Assay	1 kit	57001

To learn more about how this FP assay works, visit www.activemotif.com/hilite.