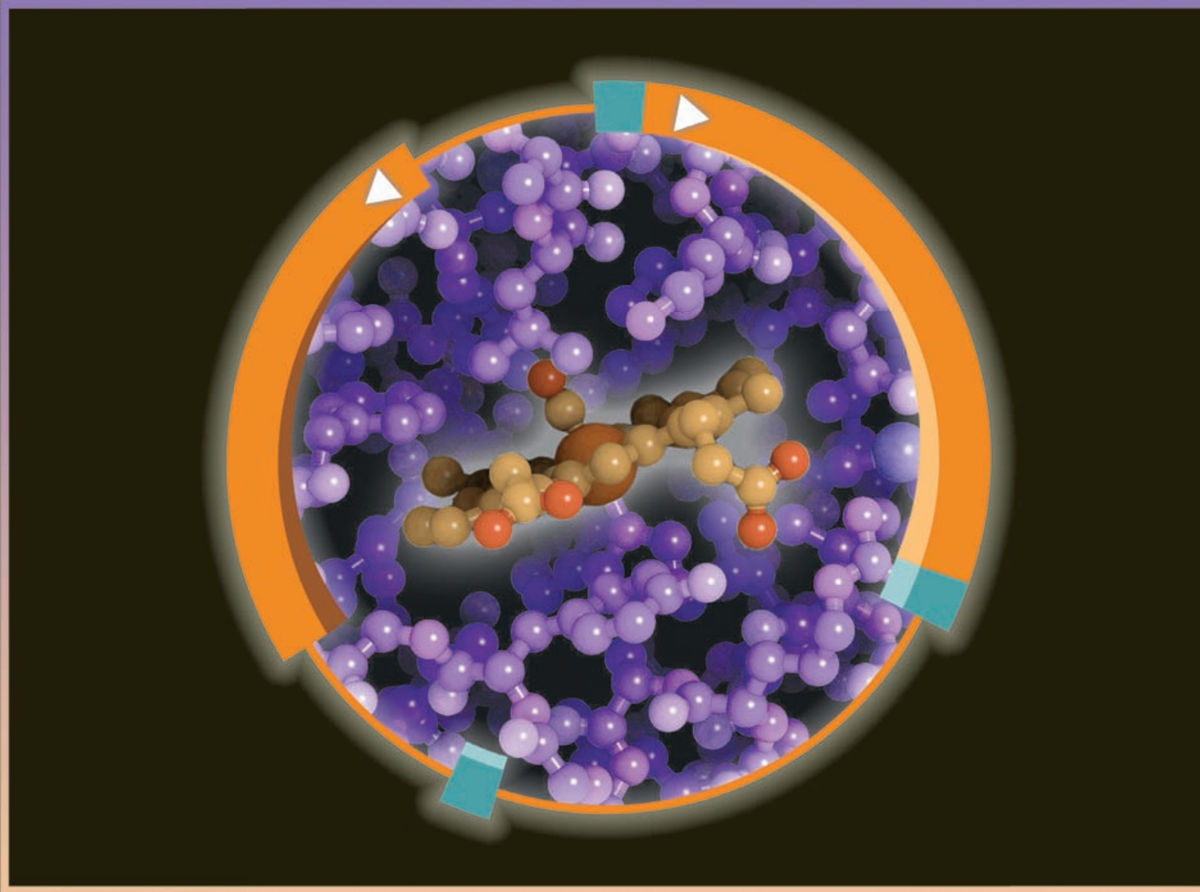
MOTIFvations

ACTIVE MOTIF®

The Newsletter of Active Motif June 2013 Volume 14 Number 2



IN THIS ISSUE

Reagents for Gene Regulation & Epigenetic Research

NEW: Study Gene Regulation with the LightSwitch™ Luciferase Assay System

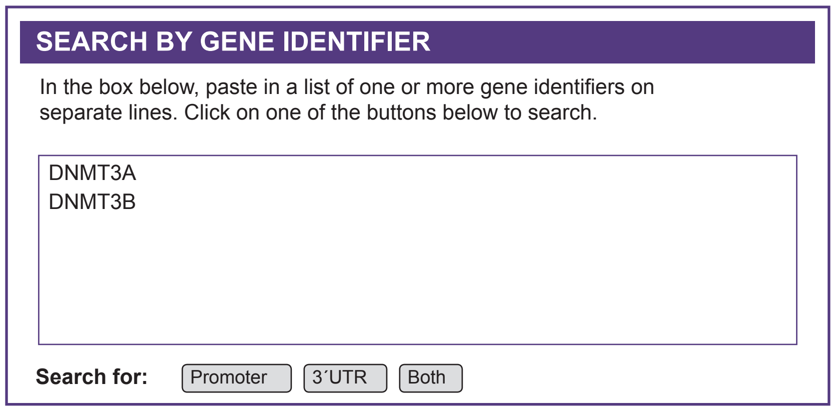
Active Motif's LightSwitch™ Luciferase Assay System is a complete solution for performing gene regulation studies in living mammalian cells, as well as for functional validation of protein binding events detected in ChIP assays. With GoClone™ Collections of over 18,000 human promoters and 12,000 3´UTRs available as transfection-ready LightSwitch luciferase reporter vectors, you can perform your reporter assay experiments immediately, without the need to clone or prepare DNA. Optimized assay & transfection reagents and protocols have been developed for every step of the process, making the entire procedure faster and simpler than with other methods. Also available are positive and negative control vectors, stable cell lines, miRNA mimics & inhibitors, and a collection of cloned long-range synthetic response elements. And, our services team can provide custom cloning, mutagenesis, pathway screening and sequence variant analysis. For more complete information, please visit www.activemotif.com/lightswitch.

LightSwitch GoClone Promoter and 3´UTR Reporter Collections

The LightSwitch GoClone Collections make it fast and easy for you to measure the transcriptional and post-transcriptional regulation of nearly any gene in the genome. To begin, simply search for your elements of interest using our online search tool (Figure 1), then you're ready to begin your gene regulation studies immediately.

The backbone of the LightSwitch System is the LightSwitch Promoter and 3´UTR reporter vectors (Figure 2). LightSwitch Promoter Reporter Constructs are created by isolating a promoter from the human genome and cloning it into the multiple cloning site that is upstream of the RenSP luciferase reporter gene on the pLightSwitch_Prom plasmid. The promoter activity can then be measured simply by assaying for the luminescence signal (Figures 3 & 4).

LightSwitch 3´UTR Reporter Constructs are created by cloning a human 3´UTR downstream of the RenSP gene in the pLightSwitch_3UTR plasmid. The vector's constitutive RPL10 promoter drives expression of hybrid luciferase-3´UTR transcripts. Assays are performed to determine how the miRNA-3´UTR interactions affect luciferase activity.



Prod ID	Gene Symbol	Type	Sequence of Cloned Elements	Format
S709833	DNMT3A	PROMOTER	Sequence and Clone Info	5 µg
S709808	DNMT3B	PROMOTER	Sequence and Clone Info	5 µg
S808608	DNMT3A	3´UTR	Sequence and Clone Info	5 µg
S809202	DNMT3B	3´UTR	Sequence and Clone Info	5 µg

Figure 1: Online search tool for finding LightSwitch Promoter and 3´UTR reporter vectors.
Finding cloned regulatory elements for your genes of interest is fast and easy. Simply enter your gene's name, accession number or other identifier in the search box and click a button to search. The search results detail which GoClones constructs are available for your elements, including links to view gene information at NCBI and the cloned sequence.

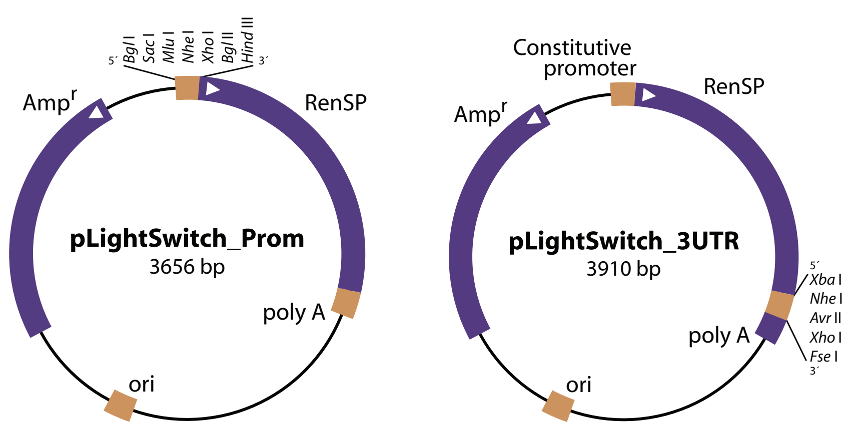


Figure 2: Maps of empty LightSwitch Promoter and 3´UTR reporter vectors.

Optimized luciferase and assay reagents for the best results possible

All LightSwitch vectors utilize RenSP, an optimized *Renilla* luciferase gene that has been engineered for maximum brightness and minimal background. Starting with the native *Renilla* gene, thousands of synthetic gene sequence variants were functionally screened for increased enzymatic activity (light output). A protein destabilization domain was added to decrease the half-life of the RenSP protein. And, transcription factor binding sites were removed as these might confound expression measurements. In addition, the LightSwitch Assay Kit features a novel, proprietary substrate and an optimized lysis buffer that were formulated to provide high sensitivity over a broad dynamic range when used with our engineered RenSP.

Transcriptional vs. Post-transcriptional Regulation

Studying transcription factor activity with promoter reporter assays

Transcription factors interact with promoters to modulate when, where and how a gene's mRNA is made. Transcription factor activity can be affected by the cell's environment including exposure to signaling molecules like hormones and growth factors. With the LightSwitch System, researchers can monitor changes in any promoter's activity in response to variations in transcription factor function that may be modulated by techniques like over-expression, knock-down or treatment with a known agonist or antagonist. LightSwitch Promoter Reporter constructs are also useful for assessing the functional consequences of transcription factor binding detected through ChIP experiments, as well as for sequence variant assays that measure the effects of mutagenizing a transcription factor's binding motif.

Post-transcriptional regulation through miRNA-3´UTR interactions

Gene regulation studies have historically focused on events that influence transcription, such as the binding of transcription factors to promoters. More recently, much research is centered on the presence or absence of histone modifications that influence DNA accessibility by altering chromatin structure. However, post-transcriptional regulation plays an equally important role. The interaction of microRNAs (miRNAs) with the 3´UTRs on transcribed mRNAs affect both a transcript's stability and its rate of translation. Interestingly, miRNAs seem to play a role in regulating proteins involved in regulation of chromatin structure (*e.g.* EZH2, HDAC1 and DNAMT3A & 3B).

For miRNA-3´UTR interaction studies, utilize our 12,000 LightSwitch 3´UTR Reporter constructs, 900 synthetic miRNA target constructs, and 1000 miRNA mimics and inhibitors, which are for co-transfection with the 3´UTR & miRNA target constructs.

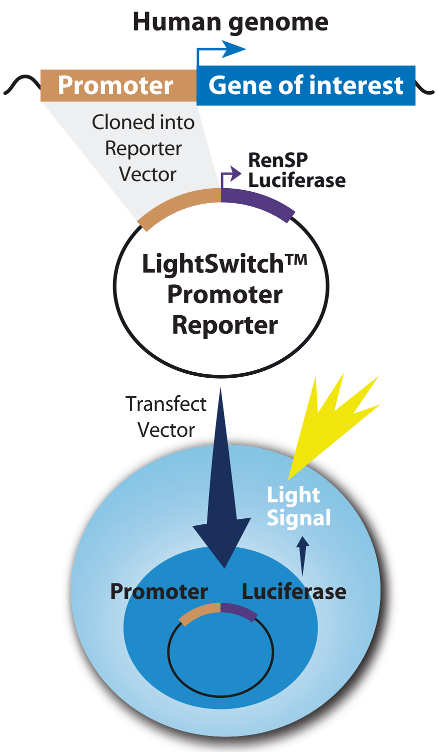


Figure 3: The LightSwitch Luciferase Assay work flow. After selecting a pre-cloned LightSwitch Promoter (shown above) or 3´UTR Luciferase Reporter Vector, it is transfected into an appropriate cell line. The cells are stimulated, if required, to induce transcription of RenSP luciferase. Because RenSP catalyzes oxidation of its substrate in a reaction that produces light, promoter activity is quantified using a LightSwitch Assay Kit and a luminometer to measure the luminescence signal.

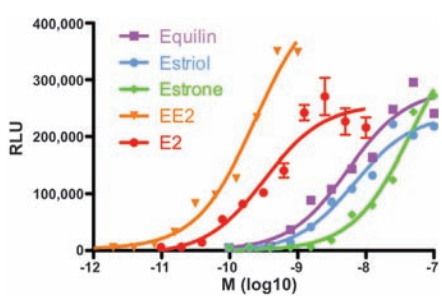


Figure 4: Response of LightSwitch SYT8 Promoter Reporter vector to various estrogen compounds. Dose response data is shown for the SYT8 promoter (Product ID S714388) for five different estrogenic compounds. The graph summarizes the luciferase activity of this promoter in HT1080 human fibrosarcoma cells that were co-transfected with an ER cDNA expression plasmid, then treated with various estrogen compounds for 24 hours before the luminescence was measured using the LightSwitch Luciferase Assay Kit. The Y-axis shows relative luminescent values; the X-axis shows the molar concentration of compound on a log10 scale. The Coefficients of Variance (CVs) ranged from 10% to 12%

Complete solution for gene regulation

LightSwitch is the most comprehensive set of reagents for the study of gene regulation. For details, please visit us at www.activemotif.com/lightswitch:

- 18,000 human promoter and 12,000 human 3´UTR constructs
- Positive and negative controls
- Synthetic TF response elements
- Empty Vector Suite
- FuGene® HD & DharmaFECT® Duo Transfection Reagents
- Synthetic miRNA target constructs
- miRNA mimics and inhibitors
- Stable cell lines
- LightSwitch™ Luciferase Assay Kit
- Custom services

Antibody Use in Drug Discovery Research

Epigenetic targets are the most promising class of drugable targets to emerge in a decade as they are not only relevant to oncology research, but also have potential in metabolic, neurological, inflammatory and cardiovascular disorders. Antibodies are an important tool used in drug development to better understand the epigenetic changes and potential off-target effects. Active Motif's extensive portfolio of antibodies against DNA methylation, histone modifications and histone modifying enzymes will help advance research in all phases of drug discovery.

Target ID → Target Validation → Screen → Preclinical Development

Minimize risks

Active Motif specializes in manufacturing antibodies against histone modifications and chromatin proteins. Because we manufacture and test our own antibodies, we can deliver the high-quality antibodies that your research requires.

Assay compatible formulations

Antibody formulations containing glycerol, BSA, amine-containing azides, Tris or glycine can wreak havoc on established assays. Active Motif offers antibodies in PBS, so that they can be seamlessly integrated into existing pipelines.

Large lot sizes

We produce large lots to ensure consistency in all phases of the drug discovery process, including antibody-based HTS.

Specificity is rigorously tested

Active Motif histone antibodies are tested for cross-reactivity to multiple histone tail modifications.

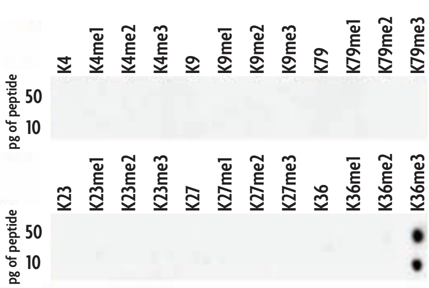


Figure 1: Dot blot analysis of Histone H3K36me3 pAb. The reactivity of Histone H3K36me3 pAb (Cat. No. 61101) to various H3 lysine modifications was tested by dot blot.

Tools to build assays

Active Motif antibodies are compatible with many applications and can be combined with our recombinant modified histones and recombinant histone modifying enzymes to build assays quickly. Antibodies against modified histone and histone modifying enzyme pairs include: EZH2 & H3K27me3; MMSET & H3K36me2; LSD1 & H3K4me2; DOTIL & H3K79me2.

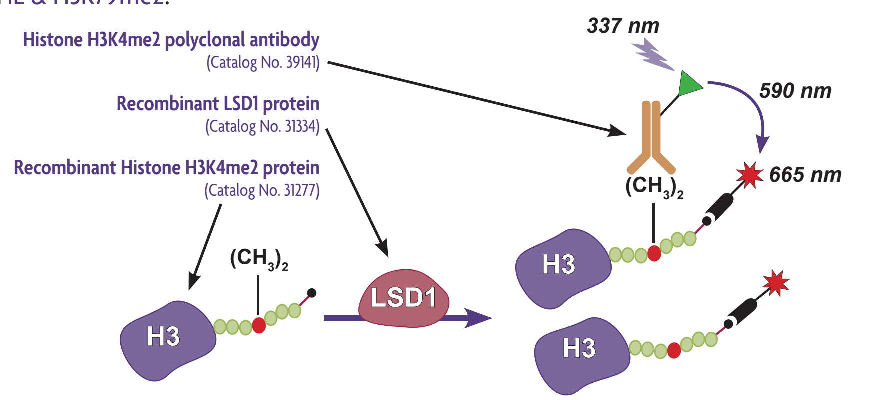


Figure 2: Time-Resolved FRET assay to monitor demethylation of H3K4 by LSD1.

DRUG TARGET ANTIBODIES

Target	Antibody	Format	Catalog No.
HMTs	DOTIL antibody (pAb)	100 µl	39953
	EZH2 antibody (pAb)	100 µl	39901
	MLL / HRX antibody (pAb)	100 µg	61295
HDMs	JARID1C / KDM5C antibody (pAb)	200 µl	39229
	JMJD2A antibody (mAb)	100 µg	39815
	JMJD2B / KDM4B antibody (pAb)	100 µl	61221
HATs	GCN5 antibody (mAb)	100 µg	39975
	MOF / MYST1 antibody (pAb)	100 µl	61245
HDACs	HDAC1 antibody (pAb)	100 µg	40967
	HDAC2 antibody (mAb)	200 µl	39533
	SIRT1 antibody (mAb)	200 µg	39353
Readers	BRD4 antibody (pAb)	100 µl	39909
	PELP1 antibody (pAb)	100 µl	61263
DNMTs	DNMT1 antibody (mAb)	100 µg	39204
	DNMT2 antibody (pAb)	100 µg	39205
	DNMT3A antibody (mAb)	100 µg	39206

Expanding Our Offering of New, Cutting-edge Epigenetic Assays

Active Motif's Epigenetic Services is world-renowned as the leading provider of end-to-end ChIP-Seq services. With over 10 years of experience providing ChIP services, our team has the experience and know-how that allows us to push the envelope and offer the latest technological advances in ChIP and MeDIP.

NEW: Active Motif will perform ChIP-Seq from your FFPE samples!

Breakthroughs at Active Motif's Epigenetic Services lab have resulted in technology that enables us to generate genome-wide profiles of histone modifications using formalin-fixed, paraffin-embedded (FFPE) samples.

FFPE tissue blocks and histology slides are a valuable resource for retrospective research on clinical samples. Clinical information, treatments and outcomes are often available for these sample types. This provides researchers with an opportunity to link data from FFPE samples to disease, diagnosis and biomarker discovery. In the field of epigenetics, until now, the benefits of this archived material have not been fully realized due to the limited size of the samples and the loss of antigenicity due to the harsh conditions used to prepare these samples.

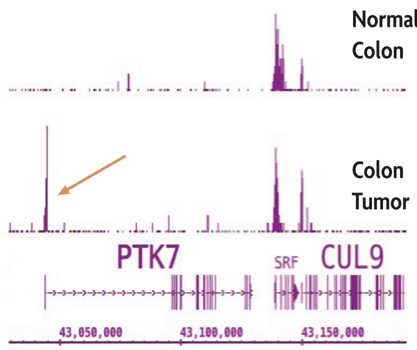


Figure 1: H3K4me3 ChIP-Seq on normal and tumor human colon FFPE samples.
Chromatin was extracted from 10-year-old histological sections of a colon tumor and matched normal colon. The chromatin was used in H3K4me3 ChIP-Seq to generate genome-wide profiles of this histone modification. The expected promoter enrichment at > 12,000 genes was detected. A portion of that data is presented in the figure. It shows nearly equal H3K4me3 occupancy at the SRF and CUL9 promoters. However, H3K4me3 is present at the promoter of the PTK7 gene, a gene known to be up-regulated in colon cancer, only in the tumor sample.

NEW: Genome-wide profiles of all cytosine variants now available

Active Motif's Epigenetic Services team has expanded our DNA analysis portfolio to include genome-wide hydroxymethylcytosine (hmC), formylcytosine (fC) and carboxylcytosine (caC) profiling. Simply send us your DNA, cells or tissues and we will deliver the data.

Advances in DNA methylation research have led to the discovery of multiple cytosine variants that are derived from the oxidation of 5-methylcytosine through the activity of the Tet family of enzymes. These modifications include 5-hmC, 5-fC and 5-caC. Given that these are relatively recent discoveries, the biological relevance of these modifications is not fully understood. Are these simply by-products along the way to DNA demethylation? Do they mark specific regions of the genome? Do they recruit other important proteins? Let us help you be the first to know!

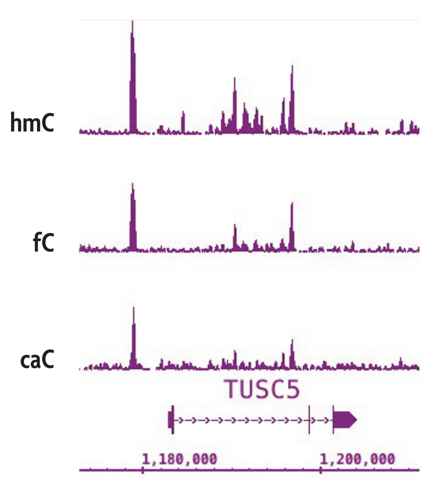


Figure 2: Genome-wide profiling of DNA variants.
DNA was extracted from a human tumor and enrichment was performed using antibodies against hmC, fC and caC. The resulting profiles identified over 100,000 genomic locations marked by these DNA variants. The figure shows one gene, TUSC5, that is marked in the promoter and across the gene body by all three variants. The intensity of the peaks reflects the expected abundance of each DNA variant.

To obtain information on all of our Epigenetic Services, please contact us at services@activemotif.com.

NEW: Perform ChIP on FFPE Samples with the ChIP-IT® FFPE Kit

Active Motif has a long history of innovation in chromatin immunoprecipitation assays. We develop technologies and utilize quality reagents to make the fastest, easiest, most sensitive ChIP kits available. Because we are able to achieve such a high level of sensitivity, Active Motif has been able to develop a new ChIP-IT® FFPE Chromatin Preparation Kit and a new ChIP-IT® FFPE Kit. These first-of-their-kind products enable extraction of chromatin from FFPE slides or tissue blocks for use in ChIP analysis.

First of its kind assay

Formalin-fixed paraffin-embedded (FFPE) tissue serves as the gold standard for the preservation of pathology samples. As a result, large collections of FFPE samples are commercially available. These samples have the potential to provide valuable information about the epigenetic variances between normal and diseased tissue states.

Traditionally, FFPE samples have not been useful in chromatin immunoprecipitation because of the difficulty in obtaining high-quality chromatin from slides or tissue blocks. The formalin fixation process often causes degradation and loss of antigenicity. With Active Motif's new ChIP-IT® FFPE Chromatin Preparation Kit, high-quality chromatin can be extracted from FFPE samples and, when used in combination with the ChIP-IT FFPE Kit, the assay is sensitive enough to obtain ChIP-enriched DNA for analysis by qPCR or Next-Generation sequencing.

What's in the box?

The ChIP-IT FFPE Chromatin Preparation Kit provides specially formulated reagents and protocol guidelines to extract chromatin from preserved samples, including human FFPE tissues. While the quality of the extracted chromatin is dependent upon sample fixation and storage conditions, we have successfully extracted ChIP-grade chromatin from normal and tumor human colon FFPE blocks that were stored for more than 10 years under less than ideal conditions (Figure 1).

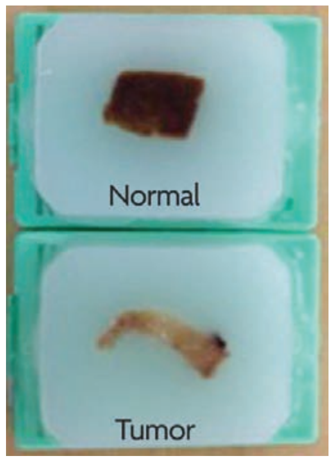


Figure 1: FFPE tissue blocks of normal and tumor samples from human colon stored for over 10 years. Images of the normal and tumor human colon FFPE blocks used with the ChIP-IT FFPE Chromatin Preparation Kit. These samples had been stored in an uncontrolled environment for more than 10 years. Five 20 µm sections from each sample were pooled and processed to obtain enough chromatin for use in the ChIP-IT FFPE Kit.

Important considerations

Optimization of extraction conditions will depend on the size and tissue type. Our detailed protocol offers guidelines and troubleshooting tips for processing samples to obtain sufficient chromatin for use in downstream ChIP analysis. The kit also includes positive control DNA and primers to help validate chromatin quality by qPCR analysis prior to proceeding with the ChIP reactions. Table 1 lists examples of the sample material we have successfully validated with the ChIP-IT FFPE Chromatin Preparation Kit.

FFPE sample	Sample used per chromatin prep
Human Colon	Tissue block – five 20 µm sections
Human Kidney	Tissue block – twenty-five 20 µm sections
Human Lung	Tissue block – two 20 µm sections
Rat whole brain	5 slides – two 5 µm sections per slide
Rat hippocampus	25 slides – two 5 µm sections per slide

Table 1: Examples of FFPE samples successfully used with the ChIP-IT FFPE Kit.

Enrich for histone or transcription factor targets with the ChIP-IT FFPE Kit

To perform ChIP analysis on the chromatin extracted from FFPE samples it is necessary to use the new ChIP-IT FFPE Kit. This is the only ChIP Kit available that has the sensitivity required to work with extremely limited starting material while producing minimal background signal, thereby enabling specific detection of the target protein of interest. Alternative ChIP kits are unable to provide the level of sensitivity required for specific enrichment. The ChIP-IT FFPE Kit includes a positive control antibody to validate successful ChIP reactions. For downstream applications such as Next-Generation sequencing, the ChIP-IT qPCR Analysis Kit is recommended to evaluate the quality of the ChIP-enriched DNA.

ChIP-IT FFPE advantages

- **Obtain quality chromatin** – use slides or tissue blocks as input
- **Sample versatility** – works with human, mouse or rat samples
- **Positive controls** – included in both the chromatin preparation and ChIP kits to validate results at each step of the process
- **Validated** – ChIP-enriched DNA was tested using both qPCR & ChIP-Seq to confirm the enrichment of histone and transcription factor targets

For more complete details, or to download a copy of our product manuals, please visit www.activemotif.com/ffpechip.

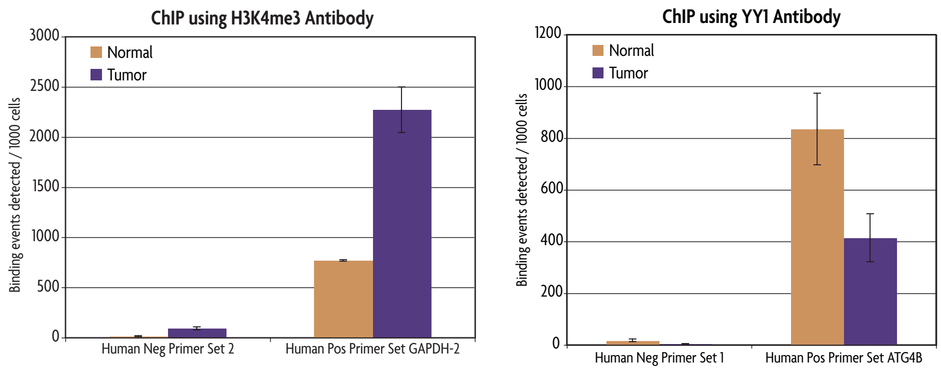


Figure 2: qPCR analysis of normal and tumor human colon samples assayed using ChIP-IT FFPE.
The normal and tumor human colon samples shown in Figure 1 were sliced into 20 µm sections; five sections per sample were pooled and used to prepare one chromatin preparation each with the ChIP-IT FFPE Chromatin Preparation Kit. The chromatin quality was validated using the included positive control DNA and primer sets. 300 ng and 200 ng of normal and tumor chromatin, respectively, were used per ChIP reaction using the ChIP-IT FFPE Kit. Antibodies for histone H3K4me3 or transcription factor YY1 were used for enrichment according to the recommendations in the manual. The quality of the ChIP-enriched DNA was then validated using the ChIP-IT qPCR Analysis Kit, which enables normalization of the data to account for differences in chromatin amounts, primer efficiency and ChIP elution volumes. The qPCR results for each ChIP antibody are shown above. The H3K4me3 results match observed data in which GAPDH is upregulated in certain cancers.

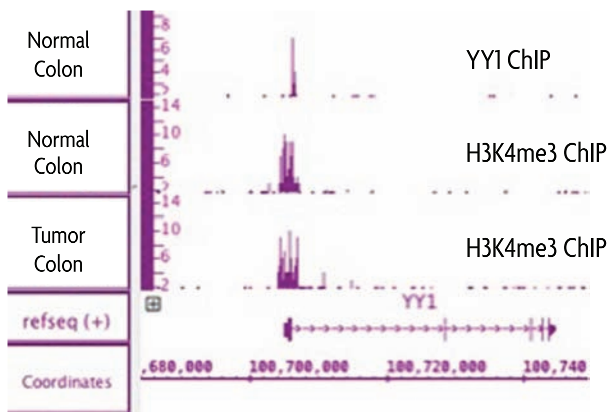


Figure 3: ChIP-IT FFPE ChIP-Seq results using normal and tumor human colon samples.
H3K4me3 ChIP-Seq results from tumor and normal FFPE colon samples and YY1 ChIP-Seq from normal colon are displayed above. Only the portion of the genome-wide data surrounding the YY1 gene is shown. The top panel shows YY1 binding at the promoter of the YY1 gene, thus illustrating the known autoregulation of YY1. The middle and bottom sequence represent H3K4me3 binding at the YY1 gene in normal and tumor, respectively, indicating YY1 is expressed in both the normal and tumor samples.

Product	Format	Catalog No.
ChIP-IT® FFPE Chromatin Preparation Kit	5 rxns	53030
ChIP-IT® FFPE	16 rxns	53045
ChIP-IT® qPCR Analysis Kit	10 rxns	53029

NEW: Ready-to-use ChIP Columns and Fixation Buffer for Your ChIP Protocols

For labs that routinely perform ChIP, Active Motif is making it faster and easier to obtain consistency in chromatin preparations and improve the quality of the ChIP reactions. The addition of ChIP-IT® Fixation Buffer during chromatin extraction improves both the consistency of sample preparation and also enhances ChIP efficiency. With Active Motif's ready-to-use Protein G Agarose Columns, simply add the ChIP reaction directly into the column to perform incubation and washing steps. The columns provide a faster alternative to centrifugation methods and ensure complete sample recovery for better reproducibility.

Ready-to-use ChIP Columns

Active Motif's new Protein G Agarose Columns provide the perfect solution for labs that routinely perform ChIP. The columns contain high-affinity protein G agarose beads that strongly bind IgG. The beads have been specifically engineered to eliminate non-specific binding, making them a better option than traditional agarose or protein G magnetic beads. The protein G agarose supplied in each column has a binding capacity of 10 µg IgG/µl bead, and can be adapted to work with any ChIP protocol.

The beads have been pre-washed and loaded into filtration columns. Simply add the ChIP reaction to the columns and use the cap to seal. The incubation and wash steps are all performed within the column to streamline the process and prevent the loss of any material. This provides a faster solution to centrifugation and magnetic separation methods, and results in better reproducibility between samples because no material is lost.

In addition to ChIP, the Protein G Agarose Columns can also be used for co-immunoprecipitation (Co-IP) experiments. The columns have been validated using Active Motif's Nuclear Complex Co-IP Kit (Catalog No. 54001), where the strong binding affinity and reduced background of the protein G agarose make these columns an ideal tool for antibody capture during Co-IP.

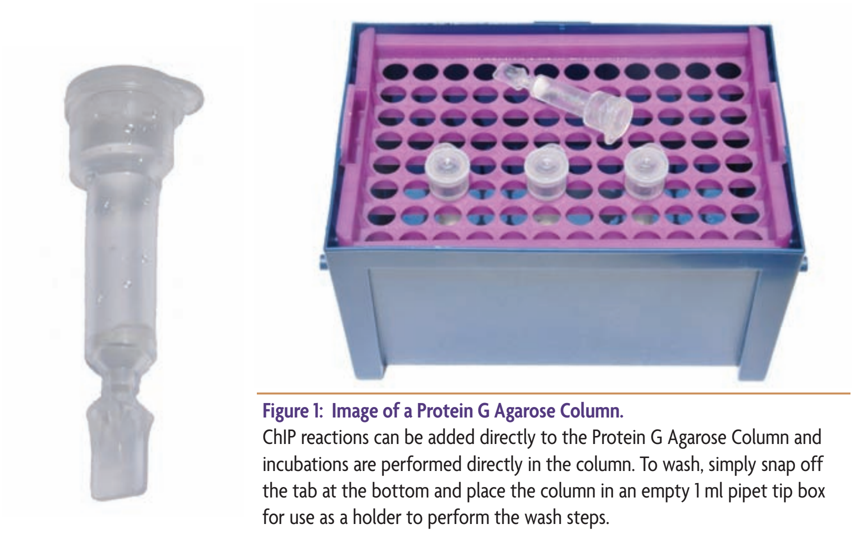


Figure 1: Image of a Protein G Agarose Column.
ChIP reactions can be added directly to the Protein G Agarose Column and incubations are performed directly in the column. To wash, simply snap off the tab at the bottom and place the column in an empty 1 ml pipet tip box for use as a holder to perform the wash steps.

ChIP-IT Fixation Buffer

The unique properties of the ChIP-IT Fixation Buffer provide consistency between chromatin preparations for better reproducibility across experiments. This specially formulated buffer is combined with formaldehyde and added directly to the cell culture growth medium (with or without serum) or to tissue material. The buffer helps eliminate pH effects during chromatin preparation for more consistent ChIP results.

Protein G Column advantages

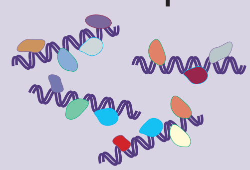
- **Ready-to-use** – the beads have been pre-washed and loaded into the column
- **No pre-blocking needed** – the protein G agarose beads have been engineered to eliminate non-specific binding
- **Avoid sample loss** – the column ensures complete retention of the beads to prevent sample loss
- **Versatility** – use them for both ChIP and Co-IP experiments

Product	Format	Catalog No.
ChIP-IT® Fixation Buffer	3 ml	53038
Protein G Agarose Columns	30 rxns	53039

ChIP Products Overview

Active Motif has consistently introduced innovations that make ChIP faster, simpler and more reproducible. We were the first to introduce magnetic beads into a ChIP kit, which lead to the first kits for sequential ChIP, HT and RNA ChIP. Our latest kits make it possible to perform ChIP with low-binding affinity antibodies and from FFPE samples (page 6). We also offer analysis products to validate your ChIP results, including 18,000 cloned promoters for functional validation of protein binding (page 2). The diagram below highlights some of our products for each step of the ChIP process.

Chromatin Preparation



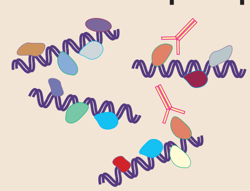
Chromatin Shearing

- EpiShear™ Sonicators
- ChIP-IT® Sonication & Enzymatic Shearing Kits
- Dounce Homogenizers
- FFPE Chromatin Prep Kit

Pre-Made Chromatin

- Ready-to-ChIP Chromatin

Chromatin Immunoprecipitation



ChIP Antibodies

- ChIP & ChIP-Seq validated antibodies
- ChIP Antibody Validation Services
- MODified™ Histone Peptide Array

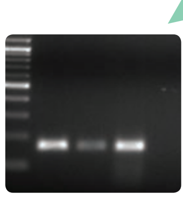
ChIP Kits

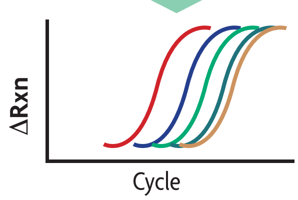
- ChIP-IT® Express
- ChIP-IT® High Sensitivity
- ChIP-IT® ChIP-Seq
- Re-ChIP-IT®
- RNA ChIP-IT®
- ChIP-IT® FFPE

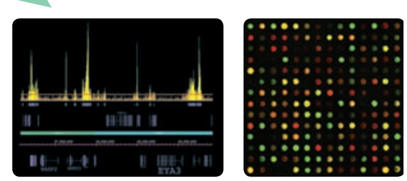
DNA Clean-up

- Chromatin IP DNA Purification Kit

Downstream Analysis







PCR Analysis

- ChIP-IT® Control Kits

qPCR Analysis

- ChIP-IT® qPCR Analysis Kit
- ChIP-IT® qPCR Control Kits
- ChIP Control qPCR Primer Sets

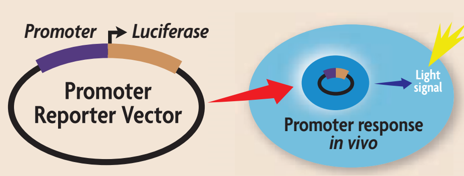
Epigenetic Services

- FactorPath™ ChIP-Seq & ChIP-chip
- TranscriptionPath™ ChIP-Seq & ChIP-chip
- HistonePath™ ChIP-Seq & ChIP-chip

Luciferase Reporter Assays

- LightSwitch™ Promoter Reporter GoClone™ Collections
- LightSwitch™ Stable Cell Lines
- LightSwitch™ Luciferase Assay Reagents

Functional Validation



Gene Regulation Services

- Custom Cloning and Mutagenesis
- Pathway Screening
- Sequence Variant Assays

NEW: Histone PTM Multiplex Assays for Use on Luminex® Instruments

Active Motif is the first company to provide epigenetic assays for use on the Luminex platform. We have developed a collection of histone post-translational modification (PTM) antibody-conjugated bead sets. These beads can be combined together with a single lysate sample for multiplexing the interrogation of the level of histone PTMs. A total Histone H3 antibody-conjugated bead is available for normalization of histone levels between samples.

The first multiplex epigenetic assay

Histones are responsible for packaging DNA into nucleosomes, the basic structural unit of chromatin. They are subject to a variety of post-translational modifications on the N-terminal tails of the histone proteins, including acetylation (Ac), methylation (Me) and phosphorylation (P), that function to regulate gene expression and the chromatin structure.

The importance of studying histone modifications has implications for human health and disease because a strong correlations have been shown to exist between specific histone modifications and human pathologies. Current methods to study histone PTMs include Western blot, immunohistochemistry and genome-wide mapping. All of these methods are time-consuming and lack high throughput capabilities. Active Motif's Histone Modification ELISAs offer a solution to the issues of time and throughput, but are limited to the analysis of a single histone PTM per ELISA.

Active Motif has partnered with Luminex®, the industry leader in multiplexing technology, to develop the first multiplex epigenetic assay for the study of histone PTMs. This assay enables high throughput processing using low sample amounts (nanogram quantities) to interrogate multiple histone modifications within a single well. The assay format also offers the ability to normalize PTM data against total histone H3 levels to evaluate relative histone PTM levels.

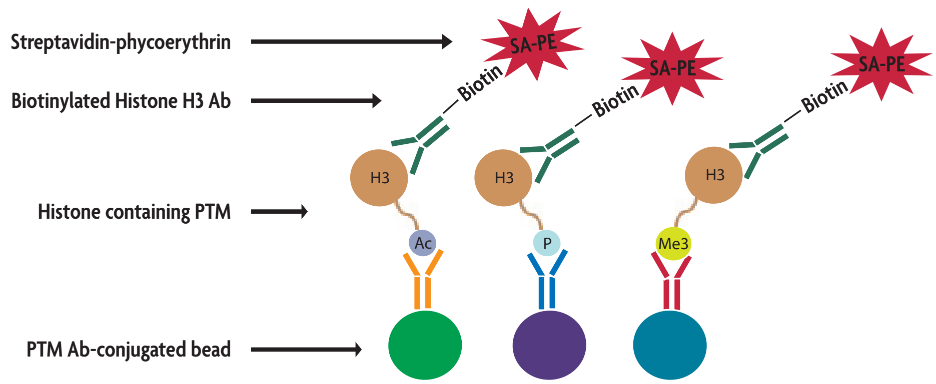


Figure 1: Schematic of the Histone PTM Multiplex Assay.

How does the assay work?

The Histone PTM Multiplex assays work as solution-based sandwich ELISAs to interrogate the levels of histone modifications within acid extracted cell lysates or purified histones. Fluorescently labeled magnetic beads, which have been conjugated to antibodies that target a specific histone PTM, are used to bind the histone within the sample. A biotinylated antibody specific for the C-terminus of Histone H3 is then added to bind the captured histone to form a "sandwich". Streptavidin-phycoerythrin is introduced into the reaction to bind the biotinylated antibody and determine the signal intensity (Figure 1).

Because each antibody-conjugated bead set emits a unique fluorescence, antibodies for multiple histone modifications can be added to the same sample for multiplexing. Inside the Luminex 200™ or MAGPIX instruments, the identity of each bead (and it's associated histone PTM) is determined based on the emitted fluorescent signal. A second light source is then applied to the same bead to determine the magnitude of the streptavidin-phycoerythrin signal. The Luminex xPONENT software program provides a real-time readout of signal as median fluorescent intensity (MFI). The readout from the streptavidin-phycoerythrin is proportional to the amount of captured histone. When the Histone H3 Total bead set is included in the reaction, values across samples can be normalized for histone H3 levels to show relative amounts of each specific histone modification (Figure 2).

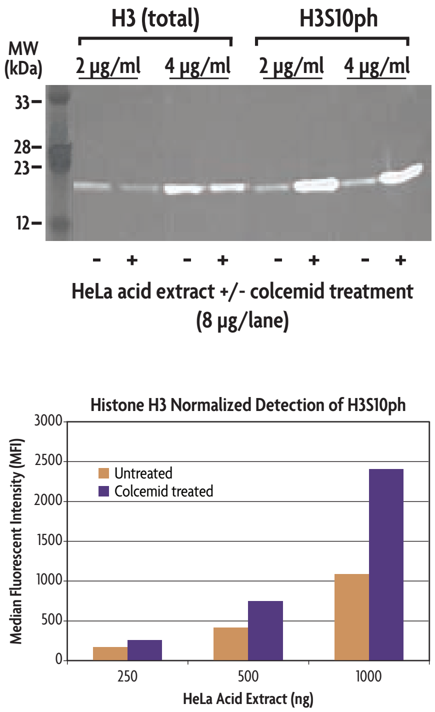


Figure 2: Comparison of data obtained from a Western blot or Histone PTM Assay for amounts of H3S10ph.
To evaluate changes in total histone H3 and H3S10ph levels from untreated or colcemid-treated HeLa acid extract, a Western blot (top image) was compared with a 2-plex Histone PTM assay using Total H3 and H3S10ph beads (bottom image). The Western blot lacks through-put capabilities, requires microgram sample quantities to study each antibody and does not provide normalized information. The Histone PTM assay requires only nanogram quantities of sample from which multiple antibodies can be evaluated simultaneously in a single well. Data can then be normalized against the total H3 values to demonstrate the relative amounts of H3S10ph in each sample.

Histone PTM Multiplex advantages

- **First-of-its kind** – only commercially available kit to perform multiplex histone analysis
- **Sensitivity** – works on crude acid extracts or purified histones using only nanogram quantities
- **Multiplexing** – enables analysis of multiple histone PTM levels within the same sample
- **Normalization** – relative levels of histone PTMs can be determined by normalizing against the Histone H3 Total bead set

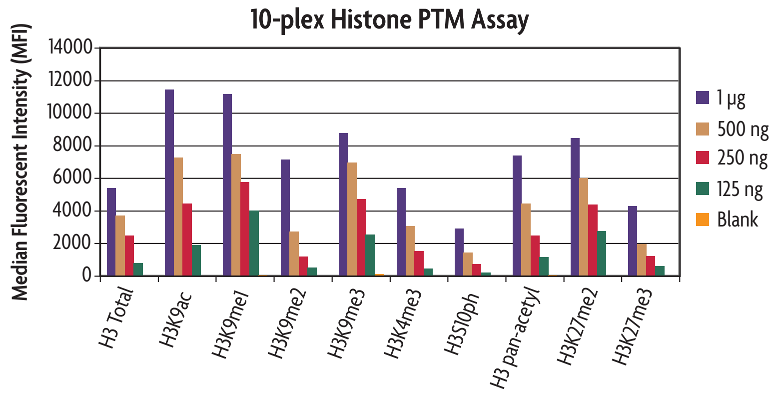


Figure 3: Histone PTM Assay using all 10 bead sets in multiplex.
HeLa acid extract was tested at varying concentrations using all 10 available antibody-conjugated bead sets in multiplex. Raw MFI values are displayed. The combination of all 10 bead sets within a single well had no discernible effects on the assay.

What's in the box?

The **Histone PTM Multiplex Kit** includes a 96-well assay plate, a biotinylated Histone H3 reporter antibody, streptavidin-phycoerythrin, and assay buffers.

The **Histone PTM antibody-conjugated beads** are purchased separately. This enables the assay to be customized to fit the individual needs of each researcher. For your convenience, a positive control for the PTM of interest is included with each histone PTM bead set.

All antibody-conjugated beads have been confirmed to work when multiplexed, without any negative effects on assay results (Figure 3). Simply use the bead sets of interest in combination with the Histone PTM Multiplex Kit and you will have all the reagents needed to perform the assay. For normalization of PTM data, the Histone H3 Total-conjugated bead set is required.

To learn more about our Luminex assays, visit www.activemotif.com/luminex.

Product	Format	Catalog No.
Histone PTM Multiplex Kit	96 rxns	33115
Histone H3 Total Ab-conjugated beads	48 rxns	33116
Histone H3K9ac Ab-conjugated beads	48 rxns	33117
Histone H3K9me1 Ab-conjugated beads	48 rxns	33118
Histone H3K9me2 Ab-conjugated beads	48 rxns	33119
Histone H3K9me3 Ab-conjugated beads	48 rxns	33120
Histone H3K4me3 Ab-conjugated beads	48 rxns	33121
Histone H3S10ph Ab-conjugated beads	48 rxns	33122
Histone H3 pan-acetyl Ab-conjugated beads	48 rxns	33123
Histone H3K27me2 Ab-conjugated beads	48 rxns	33124
Histone H3K27me3 Ab-conjugated beads	48 rxns	33125

NEW: Screen Protein Domains for Histone Binding Partners

Active Motif was the first company to introduce an array capable of analyzing antibody and protein interactions with histone post-translational modifications (PTMs). Our unique MODified™ Histone Peptide array offers the ability to screen 384 different modification combinations to evaluate cross-reactivity between individual modifications as well as investigating the effects of adjacent modifications on binding affinity. Active Motif has expanded our product offering with our new MODified™ Protein Domain Binding Kit. This assay is designed for researchers interested in screening their protein domains (*e.g.* bromodomains, chromodomains, *etc.*) for reactivity with specific histone PTMs.

What are protein domains?

The epigenetic information that exists in the form of post-translational modifications on histone tails is generated, interpreted and edited by proteins that are coined "writers", "readers" and "erasers". There are several classes of protein domains that influence gene regulation and chromatin remodeling by interacting with specific histone PTMs. See Table 1 for a list of some common chromatin remodeling protein domains.

Our current understanding of these protein domains has been limited by the lack of appropriate tools to fully interrogate their binding specificity. Active Motif's MODified™ Histone Peptide Array provides a solution to this problem as it enables researchers to screen a large panel of histone modifications in a single experiment. The results provide information on protein domain interactions with specific histone modifications, and also reveals the influence that adjacent modifications may play on protein binding.

How does it work?

Active Motif's MODified Histone Peptide Array contains modifications to study histone acetylation, methylation, phosphorylation or citrullination on histones H2A, H2B, H3 and H4. Each peptide is spotted in duplicate on the array to analyze the reproducibility of the binding events.

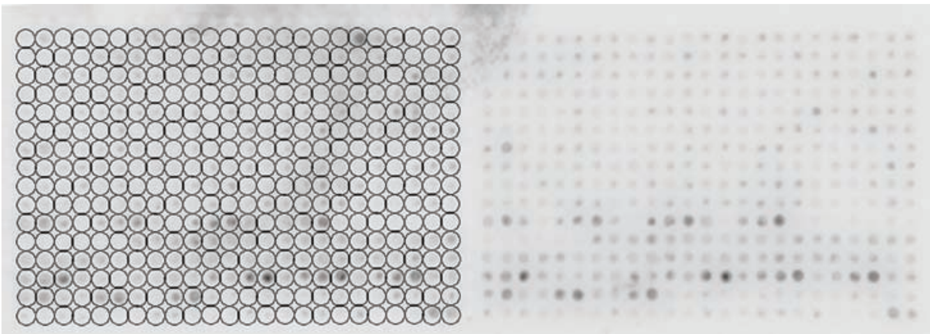
The MODified Protein Domain Binding Kit was designed to be used in conjunction with the peptide arrays. The simple assay works like a Western blot. The array is first incubated in blocking buffer. Then, the His-tagged protein domain of interest is added to the array and detected via an anti-His tag antibody and an HRP-conjugated secondary antibody. ECL detection is used to produce a signal. An image of the array is captured using a luminescent imaging system.

Free Array Analyze Software

Active Motif offers free Array Analyze software that can be downloaded from our website to analyze the intensity of the spots. Information about binding partners can be interrogated through the software program to help identify the histone modifications associated with binding of the protein domain (Figure 1). We have recently updated our Array Analyze software package to include new features that make the software easier to use and simplify the way the results are displayed. Download a copy of our new software program today at www.activemotif.com/modified.

Domain	Binding Site	Function	Examples
Bromo domain	Acetylated lysine residues on histones or other proteins	Regulates chromatin structure and gene expression as part of histone acetyltransferases or chromatin remodeling factors	TAF_{II}250, PCAF, GCN5
Chromo domain	Methylated lysine residues on Histone H3	Associated with the assembly of protein complexes on chromatin	HP1β, MPP8, CHD1
Tudor domain	Methylated lysine or arginine residues on Histone H3 and H4	It may be involved in RNA binding, DNA damage response and chromatin modification	JMJD2A, 53BP1, SMN
MBT domain	Methylated lysine residues on Histone H3 and H4	The exact function is unknown, but MBT often appears as repeats in proteins associated with transcriptional repression	L(3)MBTL, CGI-72

Table 1: Examples of some common protein domain classes and their associated binding specificity and function.



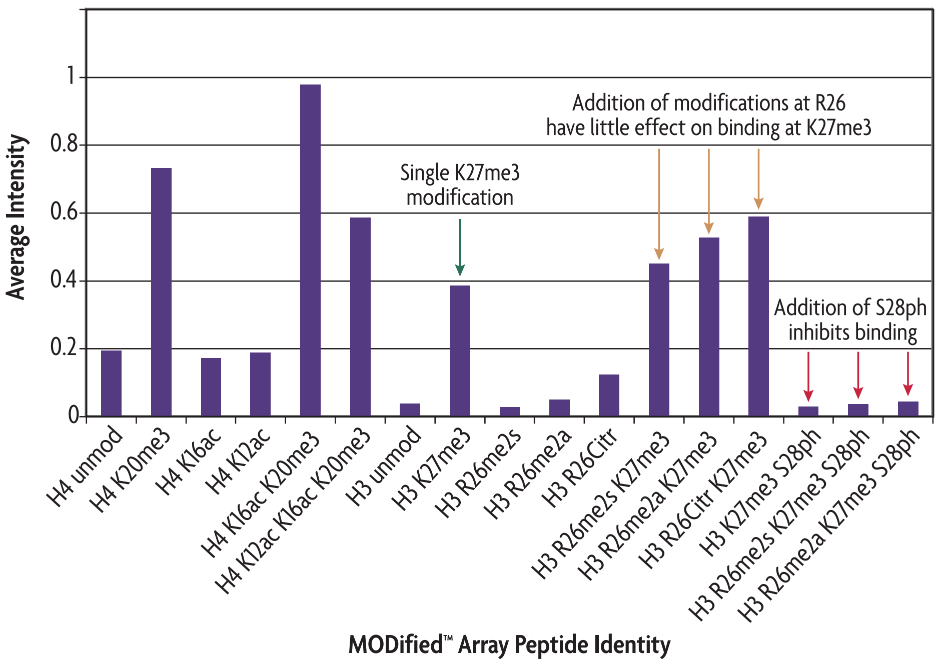


Figure 1: Analysis of the JMJD2A protein domain using the MODified Protein Domain Binding Kit and the Modified Histone Array. The His-tagged Tudor domain protein, JMJD2A, was used at a 50 nM concentration with the MODified Protein Domain Binding Kit and the MODified Histone Peptide Array. An image of the array was captured using a chemiluminescent imaging system.

Top: JMJD2A array image in which the grid overlay on the left half demonstrates the alignment performed by the Array Analyze software program for spot identification.

Bottom: Graphical analysis of selected data from the Array Analyze software program which was used to calculate the spot intensity of binding events for the JMJD2A protein domain. The average intensity was calculated for each duplicate on the left and right side of the array and graphed for the peptides listed.

The data shows the specificity of the JMJD2A domain for Histone H4K20me3 and H3K27me3 (green arrow). The presence of additional modifications within the same peptide had varying influences on spot intensity. There was relatively little change when the H3K27me3 modification was present together with modifications at R26 (copper arrows). But, when the S28ph modification was adjacent to K27me3, binding of the JMJD2A protein domain was inhibited greatly (red arrows).

MODified Array Labeling Kit

In addition to the new Protein Domain Binding Kit, the MODified Histone Peptide Arrays can also be used to screen antibodies for cross-reactivity. The MODified™ Array Labeling Kit provides all the reagents needed for the blocking, washing and chemiluminescent detection of the array. HRP-conjugated secondary antibodies for use with either rabbit or mouse primary antibodies, and a positive control antibody that recognizes the c-Myc control spot printed on the peptide array are also included. The c-Myc control antibody can be combined with the antibody you are screening to assist with spot identification within the Array Analyze software.

Product	Format	Catalog No.
MODified™ Histone Peptide Array	1 array 5 arrays	13001 13005
MODified™ Protein Domain Binding Kit	5 rxns	13007
MODified™ Array Labeling Kit	5 rxns	13006

MethylCollector™ Ultra for the Most Sensitive Enrichment of Methylated DNA

Active Motif's popular MethylCollector™ Ultra Kit is the fastest, most sensitive and specific kit available for enrichment of CpG methylated dinucleotides from fragmented genomic DNA. The patented MIRA (Methylated CpG Island Recovery Assay) technology combined with a magnetic bead-based protocol enables the most efficient methylated DNA enrichment method possible, with results in less than half the time required for MeDIP.

MethylCollector Ultra for the fastest enrichment of methylated DNA

Active Motif's MethylCollector Ultra Kit provides the most rapid and sensitive method for enrichment of methylated DNA. The kit's design is based on the patented MIRA technology that uses a purified MBD2b/MBD3L1 protein complex to bind methylated DNA. This high-affinity binding method provides greater enrichment than other MBD capture or antibody immunoprecipitation (MeDIP) methods (Table 1). MethylCollector Ultra requires as little as 1 ng (~200 cells) of input DNA and enables detection of methylation from DNA fragments containing as few as 5 methylated CpGs. Magnetic capture allows more rapid and efficient recovery of methylated DNA. Recovered dsDNA is compatible with various downstream applications including Next-Gen Sequencing (Figure 1).

MethylCollector™ Ultra (Cat. No. 55005)	MeDIP Kit (Cat. No. 55009)
Input material: 1 ng - 1 µg	Input material: 100 ng - 1 µg
–	Denature DNA for 10 minutes at 95°C
Combine DNA with MBD2b/MBD3L1 protein mix and nickel-coated magnetic beads for 1 hour at 4°C	Combine DNA, antibody, bridging antibody and magnetic beads, incubate overnight at 4°C
Wash beads 4 times using the included magnet	Wash beads 5 times
Elute methylated DNA at 50°C for 30 minutes	Elute methylated DNA at 4°C for 15 minutes
Add Proteinase K Stop Solution	Add Neutralization Buffer
DNA clean up by phenol/chloroform and ethanol precipitation, or with purification columns	DNA clean up by phenol/chloroform and ethanol precipitation, or with purification columns
DNA is ready for analysis	DNA is ready for analysis
Total time required: < 3 hours	**Total time required:** > 7 hours

Table 1: Comparison of the MethylCollector™ Ultra process to MeDIP.

NEW: MethylCollector Ultra-Seq Services Now Available

Active Motif has now expanded its offerings to include end-to-end MethylCollector™ Ultra-Seq Epigenetic Services. Simply submit your cells or DNA and receive fully analyzed genome-wide methylation data in just 6-8 weeks. For complete details on this and other services offered, please visit us at www.activemotif.com/services.

MIRA-Seq Service includes:

- DNA isolation from cells or tissues
- Methylated DNA enrichment using MethylCollector Ultra
- qPCR analysis of positive and negative control sites
- Next-Gen Library generation
- Sequencing of at least 30 million tags using the Illumina HiSeq platform
- Analysis: mapping, peak calling, visualization files and Excel output

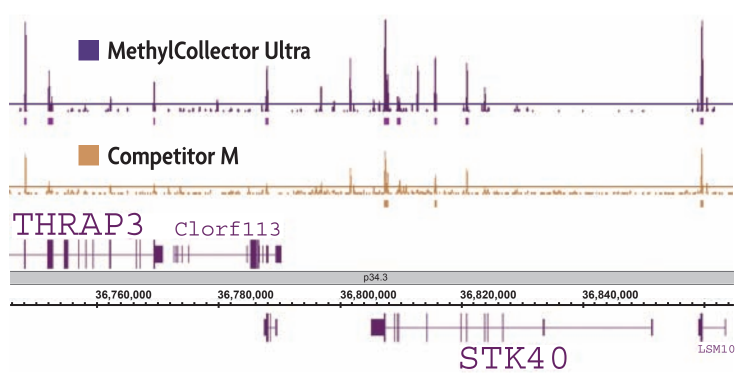


Figure 1: MethylCollector Ultra is more sensitive than other MBD-based enrichment methods.
The performance of MethylCollector Ultra was compared to that of an alternative MBD capture kit. Methylated DNA was isolated from 1 µg of human PBMC DNA using both enrichment kits. The enriched DNA was sequenced using the Illumina HiSeq platform. Regions of enrichment from a 1.2 Mb region of the genome are shown. The MethylCollector Ultra technology detected more methylated regions and the peaks were of greater intensity than those detected by the competitor's kit.

NEW: CDy1 Fluorescent Dye for Identification and Imaging of Live Stem Cells

Active Motif's new Stem Cell CDy1 Dye was developed in a collaboration with scientists at the Agency for Science, Technology and Research (A*STAR) in Singapore as a live cell imaging probe that is selective for stem cells. CDy1 offers a faster, higher throughput, more cost-effective way to identify and isolate stem cells compared to conventional techniques, such as alkaline phosphatase or immunostaining methods. Furthermore, unlike techniques that require cell manipulation or fixation, CDy1 does not impact cell growth, morphology or differentiation, making it ideal for FACS sorting and isolation of purified stem cell populations. Because the integrity of cells is preserved, additional experiments and immunostaining can be performed following CDy1 staining.

Novel stem cell dye for live cells

Active Motif's Stem Cell CDy1 Dye is an easy-to-use live cell dye with a fast and simple cell staining protocol for stem cell identification and isolation from feeder cells or mixed cultures. The Stem Cell Dye is simply added to the culture media. Following a 1 hour incubation, the cells are washed and prepared for imaging by fluorescence microscopy or FACS analysis. The spectral properties of the CDy1 dye enable the detection of stained cells using common fluorescent filter sets. For microscopy, TRITC/Cy3 filter sets can be used, while a 488 nm laser and a PE-Texas Red filter are used for flow cytometry.

Why use the Stem Cell CDy1 dye?

Aside from ease of use and higher throughput, CDy1 offers other advantages over conventional methods for stem cell identification. CDy1 staining does not affect the differentiation potential of stem cells. As this staining method does not require the sacrifice of cells, additional experiments or immunostaining can be performed following CDy1 staining. Another advantage of this novel staining method is that it eliminates the need to generate a reporter system to screen for stem cell marker expression. For reprogrammed cells, CDy1 is known to identify iPS cells earlier than genetic reporter systems (Kang *et al.* (2011) *Nature Protocols* 6: 1044-1052).

How are researchers using CDy1?

Recent publications highlight the advantages of using CDy1 for identification, enrichment and characterization of various stem cell types including embryonic, reprogrammed, neural and cancer stem cells. For an up-to-date list of publications, information and data on the CDy1 Dye, visit www.activemotif.com/cdy1.

Recent CDy1 Publications:

- Hawley *et al.* (2013) *Am J Hematol.* 88: 265-272.
- Vukovic *et al.* (2013) *Stem Cells Dev.* Apr 27.
- Vendrell *et al.* (2012) *Stem Cell Res.* 9: 185-191.
- Kang *et al.* (2011) *Nature Protocols* 6: 1044-1052.
- Im *et al.* (2010) *Angew. Chem. Int. Ed. Engl.* 49: 7497-7500.

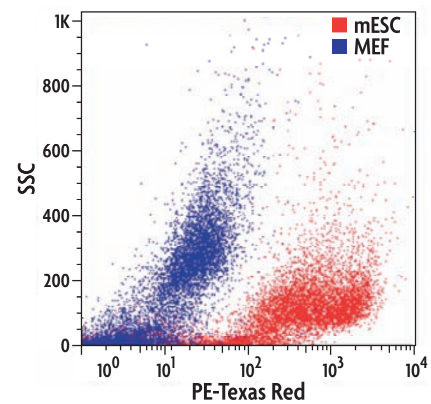


Figure 1: FACs data of CDy1 staining on mESCs and MEFs. Flow cytometry dot-plot image overlay of pure populations of Stem Cell CDy1 stained mouse ESCs (mESC) in red and mouse embryonic fibroblasts (MEF) in blue. The cells were analyzed using side scatter (SSC) and a PE-Texas Red channel. CDy1 enables clear identification and isolation of embryonic stem cells.

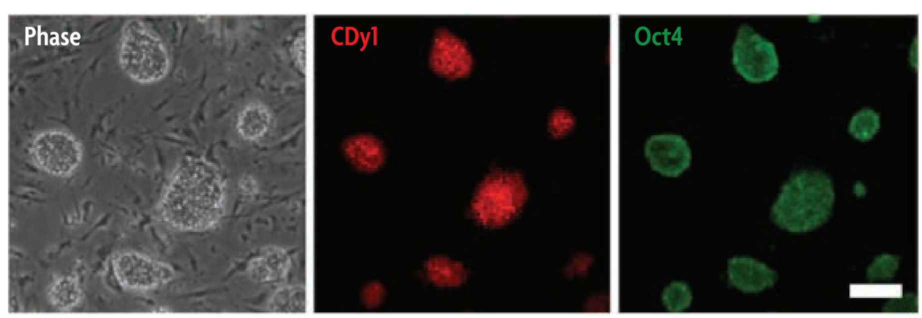


Figure 2: CDy1 Staining Coincides with Expression of Stem Cell Markers. Mouse embryonic stem cells (mESCs) grown on a mouse embryonic fibroblast (MEF) feeder layer were stained without fixation with Stem Cell CDy1 dye. CDy1 staining was visualized by fluorescent microscopy. The cells were then fixed in 4% paraformaldehyde, stained with Oct4 antibody and visualized with FITC-conjugated secondary antibody. The images show that mESC aggregates, but not MEF feeder cells, stain positive for both CDy1 (red) and Oct4 (green). Scale bar, 100 µm. The images were kindly provided courtesy of Dr. Y-T Chang at the National University of Singapore, Republic of Singapore.

Product	Format	Catalog No.
Stem Cell CDy1 Dye	50 µl	14001