motif|vations

THE NEWSLETTER OF ACTIVE MOTIF
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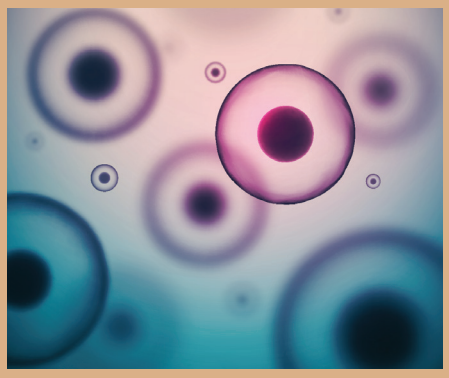
Special Cancer Epigenetics Edition

ACTIVE MOTIF®

Enabling Epigenetics Research



IN THIS ISSUE

Cancer Epigenetics

For over a decade, the number of known associations between epigenetic changes and cancer has been growing. During tumor initiation and progression, the epigenome goes through multiple alterations, including a genome-wide loss of DNA methylation, frequent increases in promoter methylation of CpG islands, changes in nucleosome occupancy and shifts in modification profiles. As researchers investigate the role of epigenetics in cancer, a growing number of tools and techniques are available to aid in their research.

Cancer-linked epigenetic modifications are found genome wide

Epigenetic alterations in cancer include changes in DNA methylation and associated histone modifications that influence the chromatin state and impact gene expression. The development of methods, such as chromatin immunoprecipitation (ChIP) and DNA methylation enrichment techniques, along with Next-Generation Sequencing tools, is enabling researchers to get a better picture of the genome-wide changes associated with cancer and other diseases.

Not every antibody is suitable for epigenetic techniques

One of the greatest challenges for epigenetic-based research has been the lack of antibodies displaying the proper specificity that have been validated for use in techniques such as ChIP and ChIP-Seq. The problem has been compounded by numerous antibody suppliers who do not manufacture or test the antibodies they sell, and who sell them to one another and then to researchers.

Developing and manufacturing histone-specific antibodies is challenging because histones have multiple post-translational modifications along their "tail" portion that are involved in gene regulation and disease. Effective antibodies must clearly differentiate between subtle variances in these modifications and also be able to perform in demanding epigenetic techniques.

What makes a good antibody?

Active Motif has established a validation program for its antibodies to qualify them for their intended use. This includes ChIP-Seq testing, ChIP validation, as well as testing the specificity of our histone antibodies with a unique MODified™ Histone Peptide Array. For a complete list of our epigenetics antibodies, please visit www.activemotif.com/antibodies.

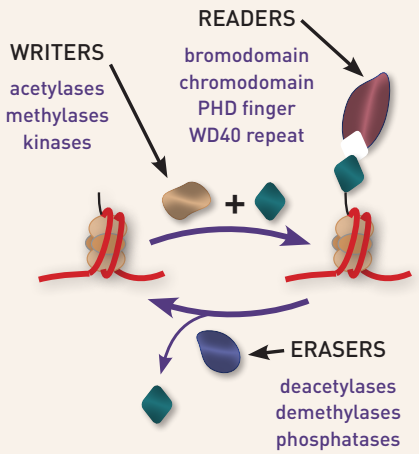
"The epigenetic state is controlled by the activity of proteins that add and remove small chemical modifications to histones and directly to DNA"

DNA methylation and hydroxymethylation

Aberrant changes in DNA methylation have been well characterized in a variety of cancers. In general, the cancer epigenome is marked by global DNA hypomethylation and promoter-specific DNA hypermethylation, which often leads to silencing of tumor suppressor genes. Also, the recently characterized DNA methylation variant 5-hydroxymethylcytosine (5-hmC) has also been linked to cancer.

Tools for DNA methylation analysis

Due to the roles it plays in development and disease, much research depends on the ability to accurately detect and quantify DNA methylation. Active Motif offers a number of products specific for this area of research, including kits and antibodies that enrich for DNA fragments that contain 5-mC and 5-hmC. Many of our DNA methylation antibodies have been validated for use in ChIP, methylated DNA immunoprecipitation, or MeDIP, and/or immunofluorescence. For complete details on our DNA methylation products, please visit www.activemotif.com/dnamt.



Regulating the histone code

The "histone code" is comprised of post-translational modifications that occur on the histone tails. These modifications are generated, interpreted and edited by proteins coined "Writers", "Readers" and "Erasers". Aberrant epigenetic regulation can lead to changes in gene expression and the development of cancer (Table 1).

	Histone H3					Histone H4				
Tumor Type	Multi-Kac	K4me1-3	K9ac	K9me1-3	K18ac	Multi-Kac	R3me2	K12ac	K16ac	K20me3
Breast		√	√		√		√	√	√	√
Colorectal									√	√
Esophageal					√		√			
Hematological									√	√
Kidney	√	√		√	√	√				
Lung		√			√					
Pancreatic		√		√	√					
Prostate		√	√*	√	√	√				√

Table 1: Histone modifications exhibiting altered levels in neoplastic tissue.

The central role of ChIP in epigenetic studies

ChIP is the principal technique used to map epigenetic marks to individual loci in the genome. By using ChIP, researchers can determine the relationships between DNA and histone modifications or proteins such as transcription factors, and gene expression.

Using the right tools for ChIP

ChIP is a technically challenging method, and researchers who are not experts are best served using well-validated and reliable kits to perform these assays. Active Motif has developed a number of kits and accessory reagents tailored to help researchers with their ChIP experiments. They provide all the critical components in a single kit along with easy-to-follow instructions. These kits and associated antibodies have been used in hundreds of labs and cited in thousands of papers in peer reviewed journals. For a complete list of available kits and reagents for ChIP, please visit us at www.activemotif.com/chip.

Let us do the work for you: Active Motif Epigenetic Services

Reproducibly generating high-quality, interpretable data from ChIP experiments can be challenging as it requires prior knowledge of working antibodies, optimized protocols for various cell types and knowledge of cell type-specific binding sites. Add in the technical and bioinformatics challenges associated with generating whole-genome data sets, and ChIP-Seq may literally be beyond your reach. That is why our Epigenetic Services team provides a wide variety of ChIP services, making it possible for you to utilize our expertise and research tools without having to be an expert in the techniques yourself. To find out more, or to get a quote, go to www.activemotif.com/services.

"The link between epigenetics and cancer has been substantiated through the identification of mutations in, or altered expression of, epigenetic regulator proteins in many different types of cancer"

CRISPR / Cas9 Gene Editing

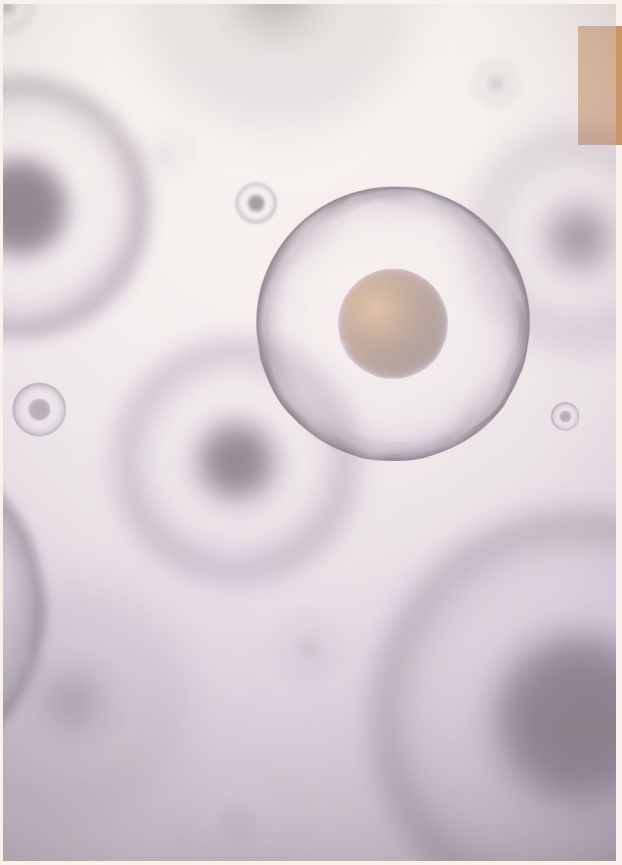
with Active Motif's Cas9 Antibody

Cas9 is part of the CRISPR/Cas9 system that has revolutionized genome engineering by providing a more efficient and robust method for targeted genome editing. Cas9 can be targeted to particular DNA sequences through a guide RNA. This RNA-guided system directs Cas9 to a specific location in the genome where it can edit sequences or alter epigenetic modifications & gene expression.

While Cas9 is a component of the CRISPR/Cas *Streptococcus pyogenes* prokaryotic immune system that provides protection against mobile genetic elements (viruses, transposable elements, plasmids, *etc.*), CRISPR/Cas9 has been adapted by scientists as a useful tool to insert, delete, or modify sequences at any specific location in a cell's genome.

Active Motif's new Cas9 Monoclonal Antibody enables you to monitor targeting efficiency and specificity of Cas9 endonuclease during your CRISPR/Cas9 experiments. Cas9 antibody is validated for use in immunoprecipitation, immunofluorescence and Western blot applications. This antibody can be used to verify expression of Cas9 and dCas9 based on its antigen design.

Product	Format	Catalog No.
Cas9 antibody (pAb)	100 µg	61577



ChIP-Seq Validated iPSC Factors

Active Motif now offers ChIP-Seq validated antibodies specific for stem cell pluripotency factors.

Name	Catalog No.
KLF5 antibody (pAb)	61099
Nanog antibody (pAb)	61419
Oct-4 antibody (pAb)	39811
Sox2 antibody (pAb)	39843
TECFL1/TCF3 antibody (pAb)	61125
Tet1 antibody (pAb)	61443

Stem cell biology is an important area of research, especially with regard to translational research and the huge potential of regenerative medicine to offer solutions for re-engineering tissue and organs and for treating and preventing disease. Epigenetics continues to guide much of this area of research given that stem cell renewal and differentiation are dependent on chromatin structure and gene transcription.

In the case of histone modifications, the repressive mark H3K27me3 forms a bivalent domain with the activating mark H3K4me3. This domain is critical to gene expression during embronic stem (ES) cell differentiation. Research suggests a link between these bivalent domains in ES cells and aberrant gene regulation in cancer. Being able to identify these links means having the right tools.

For this reason Active Motif has established a validation program for its antibodies to qualify them for their intended use. This includes ChIP-Seq testing, ChIP validation, as well as testing the specificity of our histone antibodies with our unique MODified™ Histone Peptide Array. For a complete up-to-date list of available epigenetics antibodies, please visit www.activemotif.com/antibodies.

Histone H3K27me3 Antibody

Catalog No. 39155
Introduction Date: 2007
Publications: 55

Modification role:
Transcription repressor

Active Motif Validated Applications:

ChIP | ChIP-Seq | ICC | IF | DB | WB

modENCODE validation

The modENCODE and NIH Roadmap Epigenomics Mapping Consortiums have implemented rigorous standardization criteria for all assays and reagents to be used. As part of this initiative, antibody specificity testing and the ability of the antibodies to work in ChIP-Seq were assessed in a large-scale study. Our Histone H3 trimethyl Lys27 antibody was validated for ChIP-Seq in the study (Figure 1).

Egelhofer T.A. *et al.* (2011) *Nat Struct Mol Biol.* 18, 91-3.

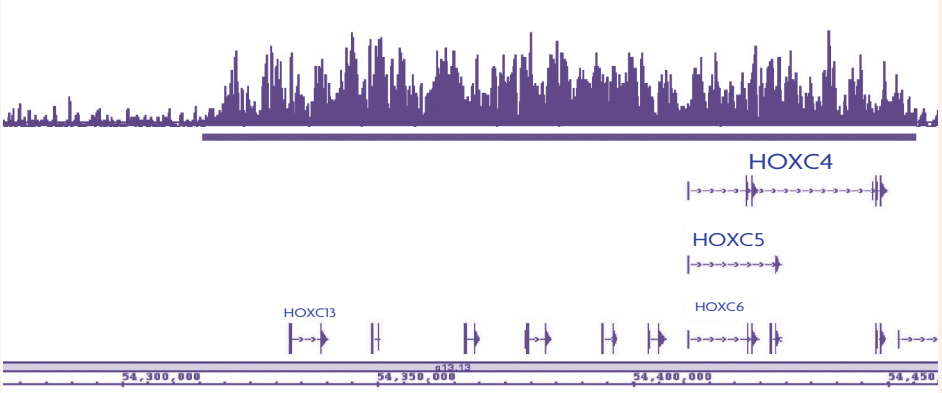


Figure 1: Histone H3 trimethyl Lys27 antibody tested in ChIP-Seq by Active Motif's Epigenetic Services.
ChIP was performed using chromatin from the human iPS 19.11 cell line. ChIP DNA was sequenced on the Illumina GA II and sequence tags were mapped to identify H3K27me3 binding. The image shows H3K27me3 binding across a 140,000 bp region of the HOX gene cluster on chromosome 12.

Recently Published

Epigenomic Analysis of Multilineage Differentiation of Human Embryonic Stem Cells.

"To investigate epigenetic regulation of embryonic development, we differentiated human embryonic stem cells into mesendoderm, neural progenitor cells, trophoblast-like cells, and mesenchymal stem cells and systematically characterized DNA methylation, chromatin modifications, and the transcriptome in each lineage."

Xie, W. *et al.* (2013) *Cell.* 153, 1134-48.

X Chromosome Reactivation Dynamics Reveal Stages of Reprogramming to Pluripotency.

"Reprogramming to iPSCs resets the epigenome of somatic cells, including the reversal of X chromosome inactivation. We sought to gain insight into the steps underlying the reprogramming process by examining the means by which reprogramming leads to X chromosome reactivation (XCR)."

Pasque, V. *et al.* (2014) *Cell.* 159, 1681-97.

Latent Enhancers Activated by Stimulation in Differentiated Cells.

"Here, we describe latent enhancers, defined as regions of the genome that in terminally differentiated cells are unbound by TFs and lack the histone marks characteristic of enhancers but acquire these features in response to stimulation."

Ostuni, R. *et al.* (2013) *Cell.* 152,157-71.

NEW

Tools to Assess Changes in DNA Methylation in Normal and Diseased Samples

The cancer epigenome is marked by alterations in a myriad of epigenetic features, including DNA methylation, nucleosome positioning, and histone post-translational modifications. These changes influence gene regulation and can serve as signatures of cancer. To better understand how changes in DNA modifications influence normal and diseased states, Active Motif offers reagents to perform comparative studies of global changes in methylcytosine (5-mC) levels across samples, as well as for mapping and profiling changes in hydroxymethylcytosine (5-hmC) across the genome.

Quantify changes in global DNA methylation levels

In mammals, DNA methylation is characterized by the covalent addition of a methyl group within the cytosine of a CpG dinucleotide. These CpG sites are often found within repetitive regions and are normally methylated to repress transcription. Hypomethylation of these elements is observed in cancer.

Active Motif's Global DNA Methylation – LINE-1 Kit provides a quantitative method to screen for changes in 5-mC levels across samples for correlative studies to assess changes induced by variable treatments, environmental or social exposures, or other factors The assay uses a consensus sequence within the Long Interspersed Nucleotide Element 1 (LINE-1) as a surrogate readout for global DNA methylation. The kit includes methylated and non-methylated standards that can be utilized to generate a standard curve for determination of the % 5-mC.

Profile changes in 5-hmC in cancer

Hydroxymethylcytosine results from the oxidation of methylcytosine by the Ten Eleven Translocation (TET) enzymes. Hydroxymethylcytosine has been observed to be redistributed in cancer tissues and highly enriched at oncogenic promoters.

Active Motif's new Hydroxymethyl Collector™-Seq Kit is designed to specifically enrich genomic DNA fragments containing 5-hydroxymethylcytosine, or 5-hmC. This is accomplished by appending a glucose moiety to 5-hmC. Following biotin conjugation, the glucosyl-hydroxymethylcytosine residues are captured with streptavidin beads. High stringency washes are performed to reduce non-specific binding before the DNA is eluted. Enriched DNA can be used for genome-wide analysis to determine the localization of 5-hmC within the sample.

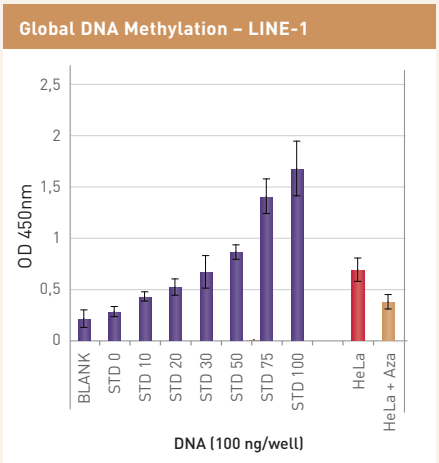


Figure 1: Global DNA Methylation Assay results showing a decrease in 5-methylcytosine levels resulting from treatment of HeLa cells with 5-azacytidine (Aza), a DNA methyltransferase inhibitor.

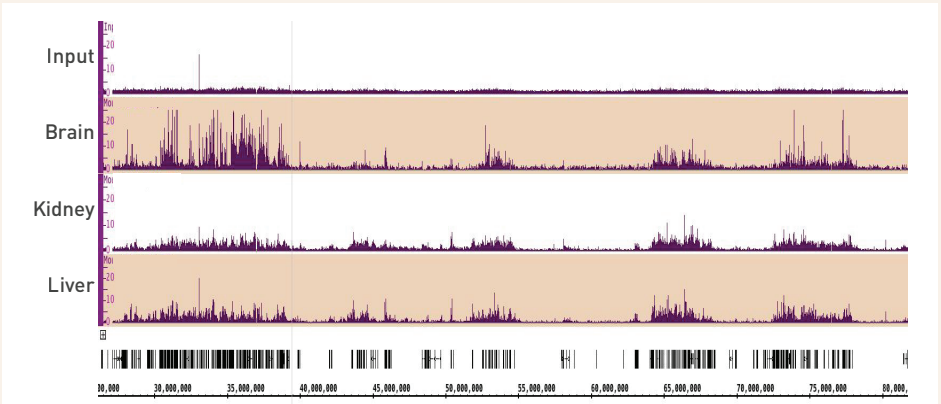


Figure 2: Hydroxymethyl Collector-Seq was performed on 5 µg each of mouse brain, mouse kidney and mouse liver genomic DNA and submitted for Next-Generation Sequencing to identify the localization of 5-hmC within each sample.

Product	Format	Catalog No.
Global DNA Methylation – LINE-1 Kit	1 x 96 rxns	55017
Hydroxymethyl Collector™-Seq	25 rxns	55019

NEW

ChIP Normalization Reagents Reduce Effects of Technical Variation and Reveal Subtle Biological Changes

ChIP is a multi-step process in which variations caused by sample loss during immunoprecipitation and library preparation, uneven sequencing read depth or hand-to-hand differences can lead to results that are difficult to interpret. To overcome this challenge, Active Motif has developed a spike-in strategy that utilizes *Drosophila* chromatin and a *Drosophila*-specific antibody for normalization of technical variation and sample processing bias. Additionally, the normalization strategy can be used for monitoring conditional effects, such as those induced by compounds or mutants, on your experiments.

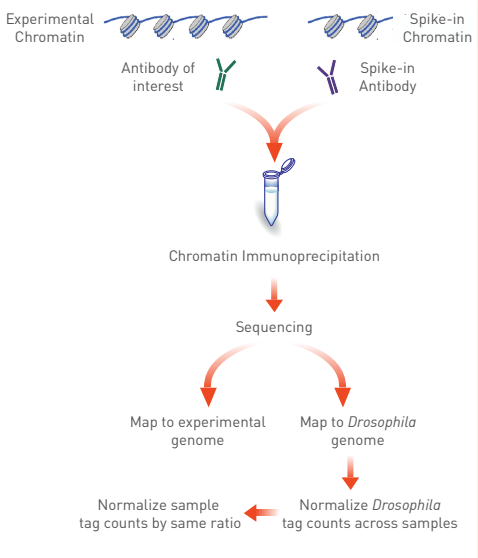
How does it work?

Active Motif's Spike-In Normalization Strategy works with both ChIP-qPCR and ChIP-Seq analysis to eliminate bias and reveal latent biological changes in your samples (Figure 1). ChIP normalization can easily be implemented simply by integrating our Spike-in reagents into your standard ChIP protocol.

A standard ChIP reaction is set up using experimental chromatin (*e.g.* human) and an antibody of interest. In addition, *Drosophila melanogaster* Spike-in Chromatin is added, or "spiked-in", to each reaction as a minor fraction of total chromatin. An antibody that recognizes the *Drosophila*-specific histone variant, H2Av, is also added to the reaction. The Spike-in Antibody provides a mechanism to reliably pull down a small fraction of *Drosophila* chromatin that is consistent across all samples (see Workflow below).

Since variation introduced during the ChIP procedure will also occur with the Spike-in Chromatin, a normalization factor can be created based on the *Drosophila* signal and applied to the sample genome.

ChIP-Seq Normalization Workflow



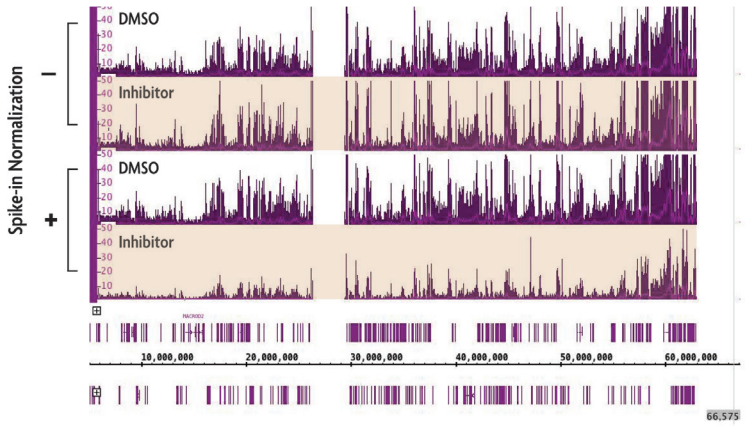


Figure 1: ChIP-Seq Spike-in Normalization Strategy reveals changes in H3K27me3 levels following treatment with EZH2 inhibitor compound. Cells treated with a small molecule inhibitor of EZH2 methyltransferase have dramatic reductions in global H3K27me3 levels. However, H3K27me3 ChIP-Seq using standard ChIP-Seq protocols (-) does not detect these differences. Incorporation of Active Motif's ChIP-Seq Spike-in Strategy (+) reveals the expected decrease in H3K27me3 ChIP-Seq signal.

Product	Format	Catalog No.
Spike-in Chromatin	10 µg	53083
Spike-in Antibody	10 µg	61686
Drosophila Positive Control Primer Set Pbgs	96 rxns	71037
Drosophila Negative Control Primer Set 1	96 rxns	71028

NEW

Tag-ChIP-IT® Enables ChIP Without Protein-specific Antibodies

Interested in studying transcription factor sequence variants, mutations or truncations? Can't find a suitable antibody for ChIP? Don't limit your ChIP experiments based on antibody availability. Active Motif's new Tag-ChIP-IT system enables you to create a fusion protein expressing Active Motif's unique AM-tag sequence that was specifically designed to work in ChIP. Simply clone your protein of interest in-frame with the C-terminal AM-tag. Transient or stable transfections of the fusion protein can be analyzed by ChIP with an antibody directed against the AM-tag.

No ChIP-validated antibody for your protein of interest? No problem!
Transcription factor ChIP is often challenging due to a lack of available antibodies that are capable of recognizing target-bound protein of interest post-fixation, or the inability of available antibodies to distinguish between protein isoforms. These limitations make it difficult to study the effects of sequence variants, mutations and truncations on gene regulation.

To overcome these challenges, Active Motif has developed the Tag-ChIP-IT Kit to enable ChIP without the use of a target-specific antibody. Tag-ChIP-IT utilizes a unique AM-tag specifically designed to minimize cross-reactivity with mammalian samples for reduced background signal. Additionally, the design maximizes exposure of the AM-tag during the immunoprecipitation reaction to increase the enrichment efficiency of low abundance transcription factors for more reliable and consistent ChIP results (Figure 1).

Tag-ChIP-IT advantages

- Ideal for targets lacking ChIP Abs
- Distinguish between protein isoforms and mutants
- High specificity due to low cross-reactivity of AM-tag with mammalian samples

Tag-ChIP-IT is Used to Identify Estrogen Receptor (ER) Binding Motifs

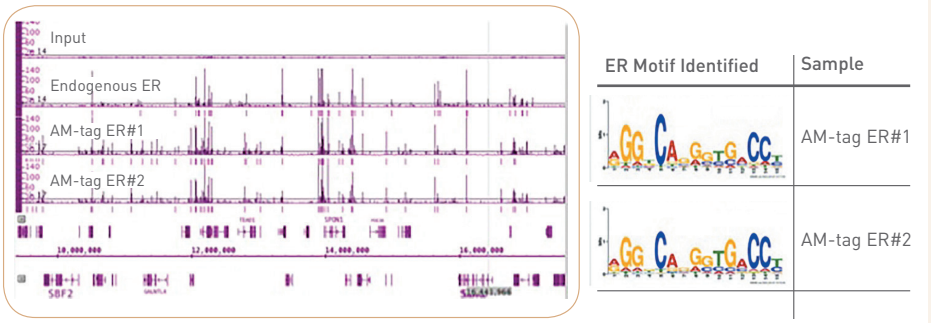


Figure 1: ER cDNA was cloned into pAM_1C Empty Vector and transiently transfected into cells. Cells were induced with estradiol and chromatin was harvested and Tag-ChIP performed using the Tag-ChIP-IT Kit. Following cross-link reversal, enriched DNA was submitted for Next-Generation Sequencing. Data was compared to published ChIP-Seq results using an anti-ER antibody in the same cell line and induction conditions. ChIP-Seq data shows the same ER peak profile with the AM-tag ChIP as endogenous ER. Detected binding sites were further evaluated for binding motifs and results show that the ER motif was identified in both Tag-ChIP-IT samples.

How does it work?
Use the pAM_1C Empty Vector to clone your protein of interest in-frame with the C-terminal AM-tag. Alternatively, the AM-tag sequence can be cloned into your expression vector of choice. Following transfection and expression of your tagged protein, the Tag-ChIP-IT Kit can be used to isolate chromatin and perform immunoprecipitations using AM-Tag antibody specific for the AM-tag.

What's in the box?
The Tag-ChIP-IT Kit contains the same optimized reagents as our ChIP-IT® High Sensitivity Kit to enable specific detection of low abundance proteins. The kit includes buffers, protease inhibitors, Protein G agarose beads, DNA purification columns and the AM-Tag antibody. For cloning, the pAM_1C Empty Vector is available separately. For more information, visit www.activemotif.com/tagchip.

Product	Format	Catalog No.
Tag-ChIP-IT® Kit	16 rxns	53022
pAM_1C Empty Vector	20 µg	53023
pAM_1C_JunD Vector	50 µg	53044
AM-Tag antibody	100 µg	61677
FuGENE® HD Transfection Reagent	0.2 ml	32042

Histone Acetylation Analysis – Finding the Right Tools For Your Research

Histone acetylation plays an important role in chromatin decondensation. Decondensation is a prerequisite of gene activity as it enables regulatory proteins, such as transcription factors and DNA repair elements, to access the underlying DNA. Histone acetylation is regulated by the "Writers", "Erasers" and "Readers" of the Histone Code. These include histone acetyltransferases (HATs) and deacetylases (HDACs), and bromodomain-containing proteins that bind acetylated lysine residues and recruit other regulatory factors to the acetylated sites. Because of their role in transcriptional control, these regulators are often the focus of drug targets and inhibitor screens. Active Motif offers a broad portfolio of kits and reagents for the study of histone acetylation.

Quantify histone acetylation levels

The Histone Modification ELISA Kits offer a quick and easy way to screen and quantify specific histone modifications within your sample (Figure 1). The kits are designed as sandwich ELISAs that utilize a histone H3 capture antibody and a detecting antibody that is specific for the modification of interest. A recombinant protein standard is included in each kit for quantitation, and the Total Histone H3 ELISA can be used to normalize histone levels across samples. In addition to Active Motif's new Histone H3 acetyl Lys27 ELISA, which marks active enhancers, assays are also available to study acetylation of Lys9, Lys14, methylation and phosphorylation. For available kits and more information, please visit www.activemotif.com/hiselisa.

Multiplex histone modification analysis

To enable screening of multiple histone modifications in a single sample, Active Motif's Histone H3 PTM Multiplex Kit offers the first-of-its-kind multiplex epigenetic assay for use with Luminex® instruments. The kit enables you to simultaneously measure changes in the relative levels of multiple histone modifications in a single well using only nanogram amounts of sample material. Antibody-conjugated bead sets are available to study Histone H3 pan-acetyl, K9ac, K27ac and K56ac. A Total H3 bead can be used for normalization. To learn more, and to see the complete list of available antibody-conjugated bead sets, visit www.activemotif.com/luminex.

Histone purification

Active Motif's Histone Purification Kits are the only commercially available assays for isolation of purified core histones from cell or tissue samples. Unlike standard acid extraction techniques, purification better preserves post-translational modifications, and histones can be isolated either as a single fraction or separate H2A/H2B, H3/H4 fractions. The kits are available in three different formats to support low, medium or high throughput sample processing. Purified histones are compatible for use in Western blot, mass spectrometry or with Active Motif's Histone Modification ELISAs and Histone H3 PTM Multiplex Assay.

For more information, please visit www.activemotif.com/histonepur.

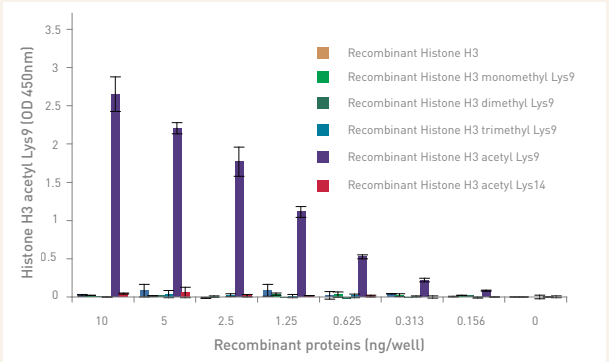


Figure 1: Histone H3 acetyl Lys9 ELISA. Histone H3 acetyl Lys9 ELISA includes a recombinant histone for quantitation of H3K9ac in acid extracted or purified histone samples. When tested in the presence of other histone modifications, the assay shows specificity for H3K9ac.

Product	Web page
Histone Purification Kits	www.activemotif.com/histonepur
Histone Modification ELISAs	www.activemotif.com/hiselisa
Histone H3 PTM Multiplex Kit	www.activemotif.com/luminex
Histone Peptide Array	www.activemotif.com/modified
HAT & HDAC Assays	www.activemotif.com/histone
Recomb. HATs, HDACs, BRDs, Histones & Nucleosomes	www.activemotif.com/hisprot
Epigenetic Activators / Inhibitors	www.activemotif.com/smallmol
Histone Antibodies	www.activemotif.com/histoneabs

Tools to Enable Insight into the Cancer Epigenome

Epigenetic changes are hallmarks of human cancers. Changes in DNA methylation, as well as histone modifications, have been found in every tumor type studied to date, both benign and malignant, and have been associated with the progression of colorectal cancer. Thus, gaining further insight into these complex regulatory mechanisms is crucial to understanding disease susceptibility, initiation and progression. In a recent study performed by the laboratory of Dr. Manuel Perucho at the IMPPC in Barcelona, numerous assays from the Active Motif product line were utilized in colorectal cancer studies to analyze the role of epigenetic aberrations, in particular global hypomethylation, in the development of cancer.

Because changes in global methylation are a hallmark of many human diseases, including cancer, simpler methods than HPLC or bisulfite sequencing are warranted for correlative studies that analyze variances in genome-wide DNA methylation status.

Active Motif's Global DNA Methylation – LINE-1 Assay uses a unique hybridization approach that quantitates 5-methylcytosine (5-mC) levels at LINE-1 repeats as a surrogate measure of global methylation. This approach offers better specificity and reproducibility than other available methods that utilize non-specific passive adsorption (Figure 1).

Quantify changes in global DNA methylation levels

The global hypomethylation observed in cancer cells primarily reflects the somatic demethylation of DNA repetitive elements. Hypomethylation predisposes normally compacted DNA to decondensation which can lead to chromosomal instability and tumor development and/or progression.

Through a genome-wide analysis of DNA methylation alterations, a specific family of non-coding pericentromeric DNA repetitive elements, SST1, was found to exhibit a wide degree of demethylation in colorectal tumors.

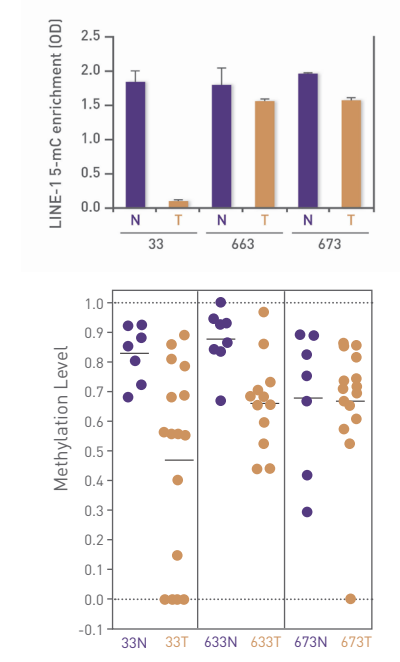


Figure 1: LINE-1 methylation levels of four human colon cancer cases, determined by the Global DNA Methylation – LINE-1 assay (top) and bisulfite sequencing (bottom).

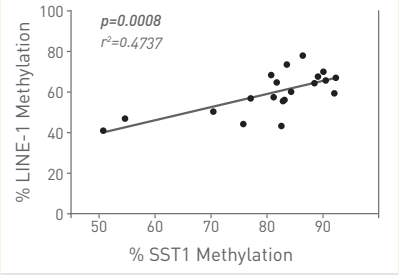


Figure 2: Demethylation of SST1 correlates with LINE-1 demethylation (p=0.0008), a commonly accepted marker for global DNA methylation.

As LINE-1 demethylation is a commonly used marker of global hypomethylation, the LINE-1 methylation levels were determined by the Active Motif Global DNA Methylation – LINE-1 kit (Figure 1, top) and bisulfite sequencing (Figure 1, bottom) in a subset of colorectal cancer patients in which SST1 methylation levels had been determined. The data reveal both assays yield similar estimated levels of LINE-1 hypomethylation in tumors compared to corresponding normal tissue.

Further, the analyses reveal that demethylation occurs in both repeats, especially in LINE-1s, in a high proportion of the patients. The results also demonstrate that demethylation of SST1 positively correlates with demethylation of LINE-1 (Figure 2).

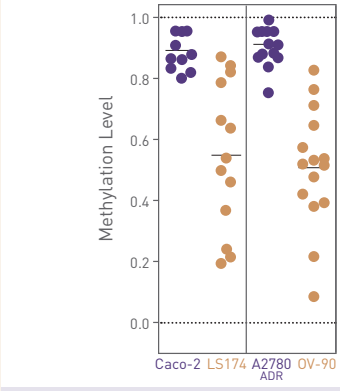


Figure 3: SST1 methylation levels of individual clones analyzed by bisulfite sequencing in colon and ovarian cancer cell lines. One clone represents one single SST1 element.

Assess chromatin features associated with DNA methylation in normal & diseased cells

Investigation into the chromatin changes that occur during loss of methylation at SST1 repeat elements in colon and ovarian cancer cell lines revealed that, while the cell lines with retained SST1 methylation (Figure 3) exhibit enrichment of Histone H3 lysine 9 trimethylation (H3K9me3), SST1 demethylation is associated with Histone H3 lysine 27 trimethylation (H3K27me3), an epigenetic repressive mark of facultative heterochromatin (Figure 4).

Heterogeneity of SST1 methylation levels was observed in the LS174 T colon cancer cell line employed to study the putative effects of SST1 demethylation on chromatin structure. Therefore, Active Motif's ChIP-Bis-Seq Kit, which offers a method to directly assess DNA methylation patterns associated with chromatin modifications (or chromatin-associated factors), was utilized to test whether SST1 H3K27me3 actually co-occurs in association with demethylated SST1 elements. LS174 and Caco-2 chromatin was immunoprecipitated with an anti-H3K27me3 antibody followed by bisulfite treatment of isolated DNA. The SST1 methylation status of H3K27me3-associated DNA was then determined by bisulfite sequencing (Figure 5). The ChIP-Bis-Seq results confirmed that the H3K27me3-associated SST1 elements are actually demethylated.

For more information, please visit www.activemotif.com/chip-bis-seq.

Validate results obtained from cell models in primary tissues

Cell model systems often do not reflect true biology. Thus, it is important to confirm results in primary tissues. The Active Motif ChIP-IT® FFPE Chromatin Preparation Kit and ChIP-IT® FFPE Kit were used on colon primary FFPE samples and confirmed the shift from high levels of DNA methylation and H3K9me3 in normal tissues to SST1 hypomethylation and increased levels of H3K27me3 in tumor cells (Figure 6).These findings suggest that demethylation of SST1 elements may be a marker of an epigenetic reprogramming event associated with changes in chromatin structure that may ultimately affect chromosomal integrity. In addition to ChIP-IT FFPE products, we also offer the ChIP-IT PBMC Kit to perform ChIP on difficult-to-lyse peripheral blood mononuclear cells (PBMCs), including lymphocytes (T cells, B cells & NK cells) and monocytes.

For more complete information on ChIP-IT products for primary cells, visit us at www.activemotif.com/chip.

Data was provided courtesy of Dr. Johanna Samuelsson and Dr. Manuel Perucho. Samuelsson *et al.*, in preparation.

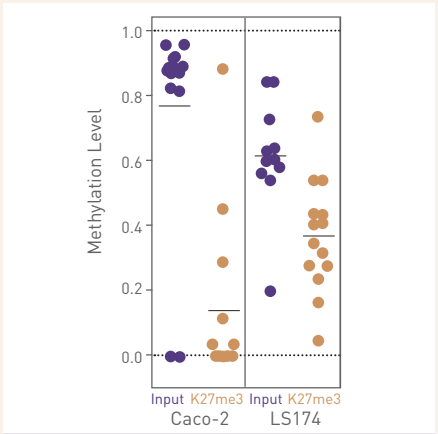


Figure 5: SST1 elements associated to H3K27me3 are highly demethylated.

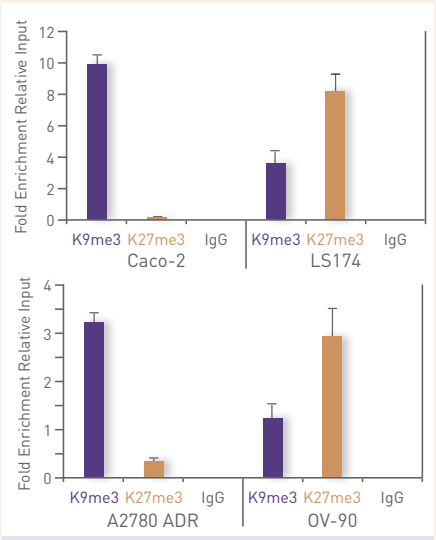


Figure 4: SST1 methylation correlates positively with H3K9me3 levels and negatively with H3K27me3. ChIP analysis corresponding to data sets in Figure 3 show demethylation of SST1 is associated with increased H3K27 trimethylation and a reduction in H3K9me3. Fold change calculated relative to input.

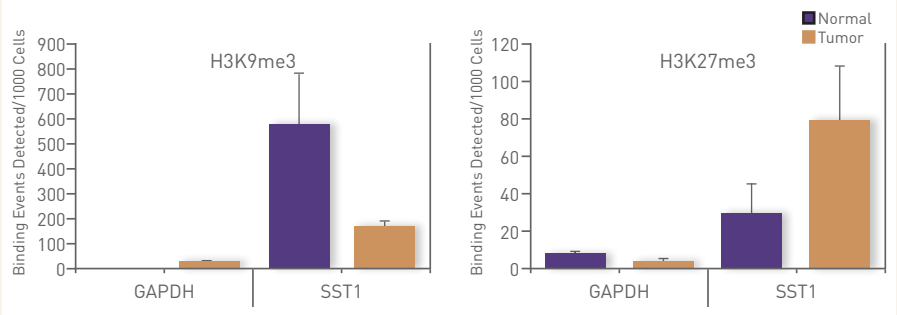


Figure 6: ChIP analysis of histone modifications associated with SST1 repetitive elements in colon cancer FFPE primary tissue samples confirms the shift from high levels of SST1 methylation and H3K9me3 enrichment in the normal tissue (purple) to SST1 demethylation, a reduction of H3K9me3 and increase in H3K27me3 levels in the tumor (copper).

Product	Format	Catalog No.
Global DNA Methylation - LINE-1 Kit	1 x 96 rxns	55017
ChIP-Bis-Seq Kit	10 libraries	53048
ChIP-IT® FFPE Chromatin Preparation Kit	5 rxns	53030
ChIP-IT® FFPE Kit	16 rxns	53045
ChIP-IT® PBMC Kit	16 rxns	53042

NEW

Study Regulation of lncRNA Promoters

It has been estimated that while only 1% of the mammalian genome codes for protein, 70% to 90% is transcribed at some point during development. The vast majority of this transcriptome is thought to be long noncoding RNAs (lncRNAs). Previously thought of as simply "junk DNA", the relatively recent revelations that lncRNA regulation is distinct from protein-coding genes and that they are important regulators of both transcription and chromatin organization has elevated their status to that of "dark matter", reflecting that their mechanisms of action are still poorly understood. Active Motif's LightSwitch™ Assay System offers a simple solution for unraveling lncRNA regulation.

lncRNA promoter regulation

As compared to protein-coding genes, lncRNA genes appear to be regulated in a more condition-specific manner. Evidence suggests the possible existence of distinct modes of regulation for the two different gene groups, making functional assays imperative.

The LightSwitch Assay System was designed to make it fast & easy to study promoter regulation. The backbone of the LightSwitch System is the LightSwitch Promoter Reporter vector. After a promoter of interest is cloned upstream of RenSP in this vector, it is transfected into live cells. The cells can be stimulated, if desired, and the promoter activity can then be determined simply by assaying for the luminescence signal, which is measured quickly and accurately as light output (Figures 1 & 2).

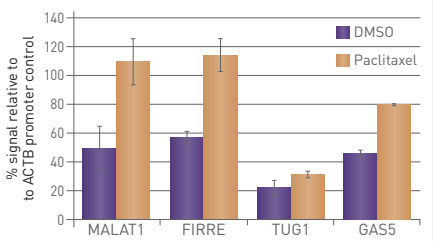


Figure 1: Measuring lncRNA promoter activity.
The promoters of 4 different lncRNA genes were cloned into the LightSwitch Promoter Reporter vectors and transfected into MCF-7 cells. Following stimulation with DMSO or paclitaxel, luciferase assays were performed and the data normalized using an ACTB positive control.

Select pre-made lncRNA promoter constructs, or clone your own

For your convenience, we have cloned a number of widely studied lncRNA promoters, including FIRRE, GAS5, HOTAIR, MALAT1, MEG3 and TUG1, into the LightSwitch Promoter Reporter vector. These are available as sequence-verified, transfection-ready reporter constructs, so you can begin assays to study how these promoters are regulated immediately, without the need to clone, sequence or prepare the DNA. Alternatively, you can clone your own sequence into the empty reporter vector or utilize our economical Custom Cloning Services.

Optimized luciferase and assay reagents for the best results possible

All LightSwitch vectors utilize an optimized *Renilla* luciferase gene (RenSP) that has been engineered for maximum brightness and minimal background when reacted with the proprietary substrate found in LightSwitch Luciferase Assay Kits. In addition, the LightSwitch reagents were developed to enable one-step addition directly to cultured cells, eliminating the need for a separate lysis step. Collectively, these improvements ensure that LightSwitch delivers superior results with simple and robust protocols. For more information, please visit us at www.activemotif.com/ls-lncrna.

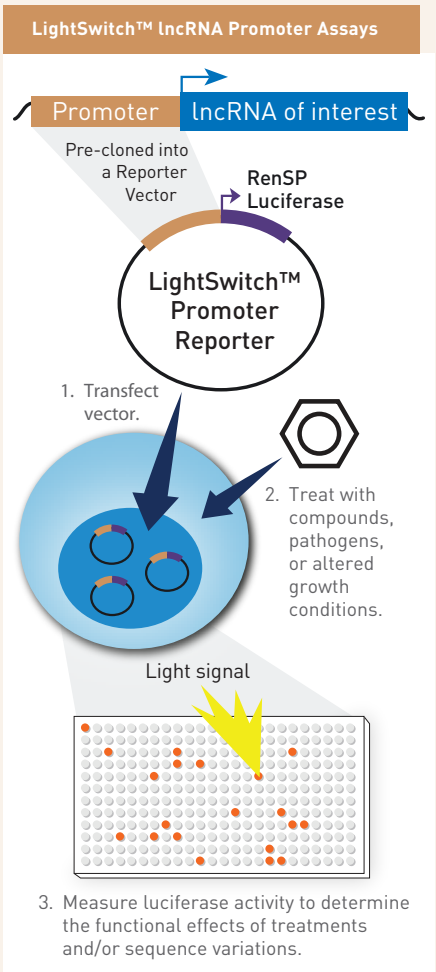


Figure 2: LightSwitch lncRNA Promoter Reporter Assays.
To study a lncRNA promoter's regulation, it is cloned into a LightSwitch Promoter Reporter vector and transfected. The cells are treated, if desired, and then assayed. The luciferase produced oxidizes the assay substrate in a reaction that also produces light. The amount of light is proportional to the activity of the cloned lncRNA promoter.

Dysregulation of miRNAs in Cancer

MicroRNAs (miRNAs) regulate many different biological processes, including development, differentiation, proliferation and apoptosis. Expression levels of specific miRNAs have been observed to either be upregulated or downregulated in a variety of cancers. An estimated 50% of miRNA genes are located at fragile sites in the genome that are deleted, amplified or translocated in human cancer, making miRNAs useful as biomarkers to identify patients at risk of developing a cancer, as well as predicting those that may respond to therapy. For a list of miRNAs implicated in specific cancer types, visit www.activemotif.com/mirna-cancer.

miRNA-mediated regulation by RISC complexes

miRNAs act as post-transcriptional regulators of gene expression by binding to the 3´ Untranslated Region (3´UTR) of messenger RNA transcripts (mRNAs). The interaction of a miRNA with a 3´UTR results in either the repression of translation or the degradation of the transcript.

The targeting of a given miRNA to a specific mRNA is mediated through the formation of an RNA Induced Silencing Complex (RISC). While RISCs can contain a combination of different RNA-binding proteins, at a minimum a RISC is comprised of an Argonaute protein (Ago) and a miRNA. The Ago protein binds the miRNA in a manner that enables the miRNA-loaded RISC complex to base-pair with a mRNA transcript (Figure 1).

Validate miRNA/mRNA interactions using Ago IP

Active Motif's miRNA Target IP Kit utilizes protein G-coupled magnetic beads and a pan-Ago antibody that recognizes Ago1, Ago2 and Ago3 proteins to immunoprecipitate miRNA/mRNA complexes that have associated with Ago1, Ago2 or Ago3. Following their immunoprecipitation as part of the RISC complex, the mRNA targets can be purified and then amplified by RT-PCR using gene-specific primers.

By comparing cells transfected with or without a miRNA mimic, one can validate the mRNA targets of a particular miRNA based on over-expression of that miRNA. It is also possible to profile the immunoprecipitated mRNA molecules using conventional expression microarrays or RNA-Seq. For more information, please visit us at www.activemotif.com/mirna-ip.

Study how sequence variation alters miRNA-3´UTR interactions

Single Nucleotide Polymorphisms (SNPs) in an mRNA's 3´UTR have been shown to play a role in the miRNA dysregulation seen in cancers. Active Motif's LightSwitch™ System is ideal for studying miRNA-3´UTR interactions and for assessing the functional impact of variations in the sequence of the target 3´UTR. The system includes a collection of over 12,000 human 3´UTRs that can be purchased as ready-to-transfect LightSwitch luciferase reporter vectors. These constructs can easily be mutagenized to determine how sequence variations impact function. Combined with our large collections of miRNA Mimics & Inhibitors, you have everything needed to study miRNA-3´UTR interactions, validate miRNA targets, and measure the functional impact of miRNAs on a gene-by-gene basis. For information, visit www.activemotif.com/ls-3utr.

Translational Repression by a miRNA-directed, RNA Induced Silencing Complex (RISC)

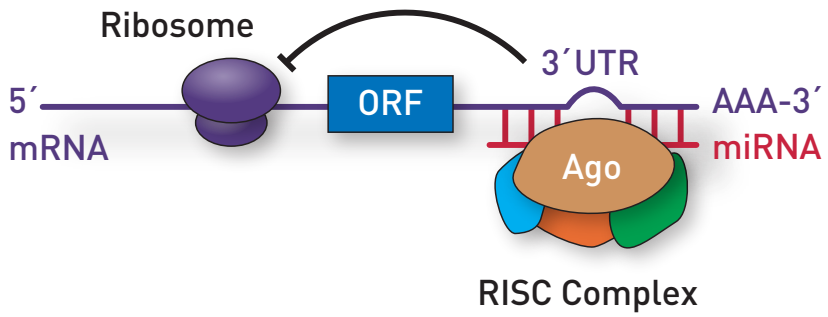


Figure 1: The miRNA contained in a RISC complex enables precise silencing of specific mRNA transcripts.
The key components in a RISC complex are an Argonaute protein (Ago) and a miRNA. The Ago protein binds the miRNA, positioning it in a conformation that enables the RISC to base-pair in a Watson-Crick manner with a mRNA transcript. This leads to either inhibition of translation (shown) or increased degradation of the targeted transcript.

Enzymes & Substrates for Drug Discovery

Recombinant proteins are a critical component of epigenetic drug discovery programs. Robust enzymatic assays are critical for screening, structural and mechanistic studies that guide chemical design of epigenetic modulators. To achieve robust enzymatic assays requires high quality epigenetic recombinant proteins. Active Motif's protein biochemistry team has produced hundreds of histone and nucleosome substrates and highly potent enzymes to many of the hot drug targets, such as NSD2, Dot1L, LSD1, KDM5A, KDM5B, KDM6A, as well as PRC2 and MLL complexes, that are ready to incorporate into your assay design.

Available Products

- Histone modifying enzymes - HMTs / HDMs, HATs / HDACs
- Binding domains - Bromodomains, SET domains
- Substrates - Unmodified & modified histones, histone octamers & nucleosomes
- DNA modifying enzymes - DNMTs, TET
- Transcription Factors - NFκB, AP-1, p53, and more...

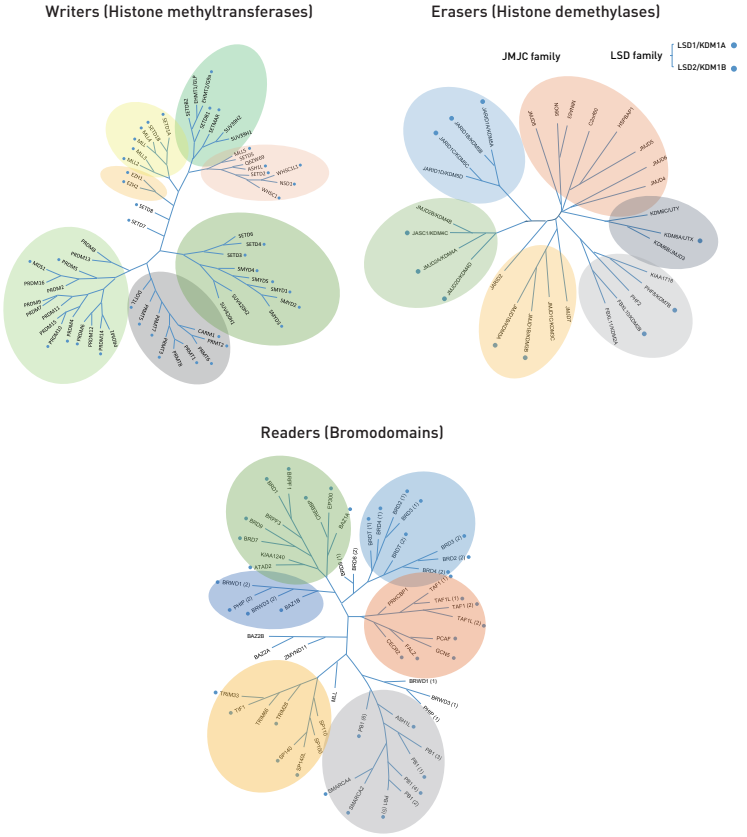
Assay-ready 'Writers', 'Readers' & 'Erasers'

There is no need to waste valuable time and resources generating proteins for screening, drug discovery and target validation programs. With Active Motif's comprehensive portfolio of purified assay-ready recombinant proteins – including methyltransferases and demethylases, acetyltransferases and deacetylases, as well as binding domains – you are sure to find what you need to develop more efficient epigenetic assays.

Bulk sizes available

Call us at 877-222-9543 to inquire about custom or bulk orders.

Writers, Readers and Erasers



The epigenetic state of the cell is controlled by the activity of three major classes of epigenetic proteins: the "Writers" (enzymes that deposit modifications), the "Erasers" (enzymes that remove modifications) and the "Readers" (proteins that recognize and bind epigenetic modifications). Recent drug development strategies that target these enzymes have proved highly successful, resulting in many FDA approved cancer drugs, with more promising leads on the horizon.

To learn more, go to www.activemotif.com/proteins or download a copy of our Tools for Drug Discovery product brochure.

NEW

Epigenetic Services: RIME for Identification of Transcriptional Networks Associated with Disease

Active Motif's newest addition to it's suite of epigenetic and gene regulation focused services, RIME (Rapid Immunoprecipitation Mass Spectrometry of Endogenous Proteins), sheds light on the complex process of gene regulation by enabling the capture and identification of interactomes, or the associated protein networks, of an endogenous protein of interest. The RIME service includes immunoprecipitation of a target protein from cross-linked cell extracts followed by ChIP-Seq, mass spectrometry and data analysis.

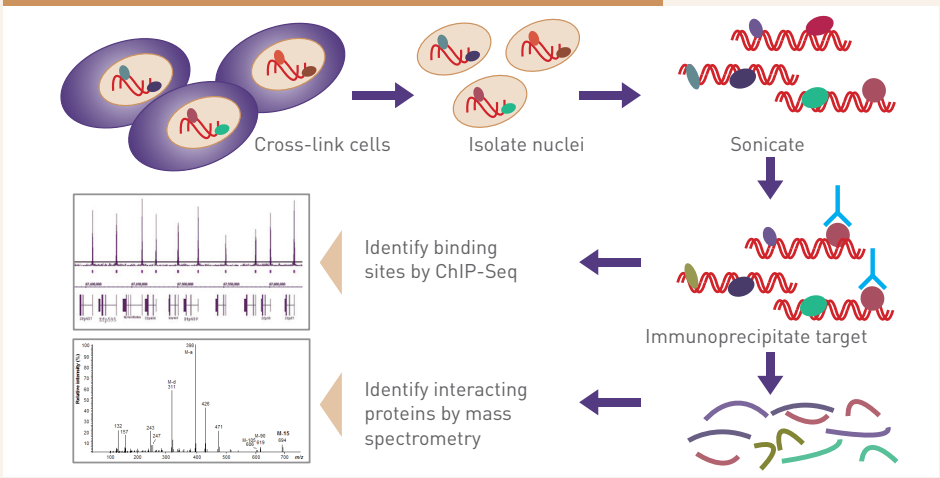
Identify interactomes regulating gene expression

Gene regulation is complex but tends to be oversimplified by researchers focused on one particular transcription factor (TF) in any given cellular model. In reality, differential gene expression is greatly influenced by cofactors and other protein interactions with chromatin. For example, gene activation by the Estrogen Receptor is thought to be modulated through ligand-induced conformational changes in the receptor that mediate cofactor recruitment. Additionally, ChIP-Seq data sets often show transcription factor binding at the same promoters in multiple cells although they display differentially expressed target genes, suggesting protein networks are responsible for such differential gene activation. RIME sorts out this complexity by providing a means to identify the protein interactions that are important for gene regulation.

RIME advantages

- Identify transcriptional cofactors
- Identify co-occurance of TFs bound to DNA at sites adjacent to your target TF
- Identify important targets for ChIP-Seq studies
- Detect low affinity interacting proteins

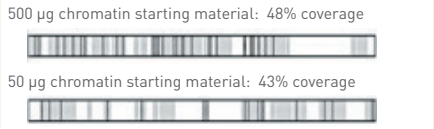
RIME Service Workflow for Identification of Endogenous Interactomes



Why RIME?

Immunoprecipitation (IP) followed by mass spectrometry has traditionally been the approach used to identify proteins interactions with a target of interest. RIME is an adaptation of the ChIP-Seq method that, in addition to enabling the mapping of transcription factor binding sites, enables the identification unknown interacting proteins. RIME differs from traditional IP approaches because the starting material has been cross-linked, which offers the advantage of 1) enabling capture of low affinity interactions which are lost in IP/mass spectrometry, 2) allowing more stringent wash conditions resulting in less non-specific interactions and better preservation of protein complexes, and 3) stabilizing protein/DNA interactions to enable the capture of proteins that are bound at adjacent sites in the DNA and are independent of protein/protein interactions.

POLR2A Coverage



Target protein detection in the mass spectrometry data is a measure of success in a RIME experiment.

To learn more, please visit us at www.activemotif.com/rime.

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Enabling Epigenetics Research