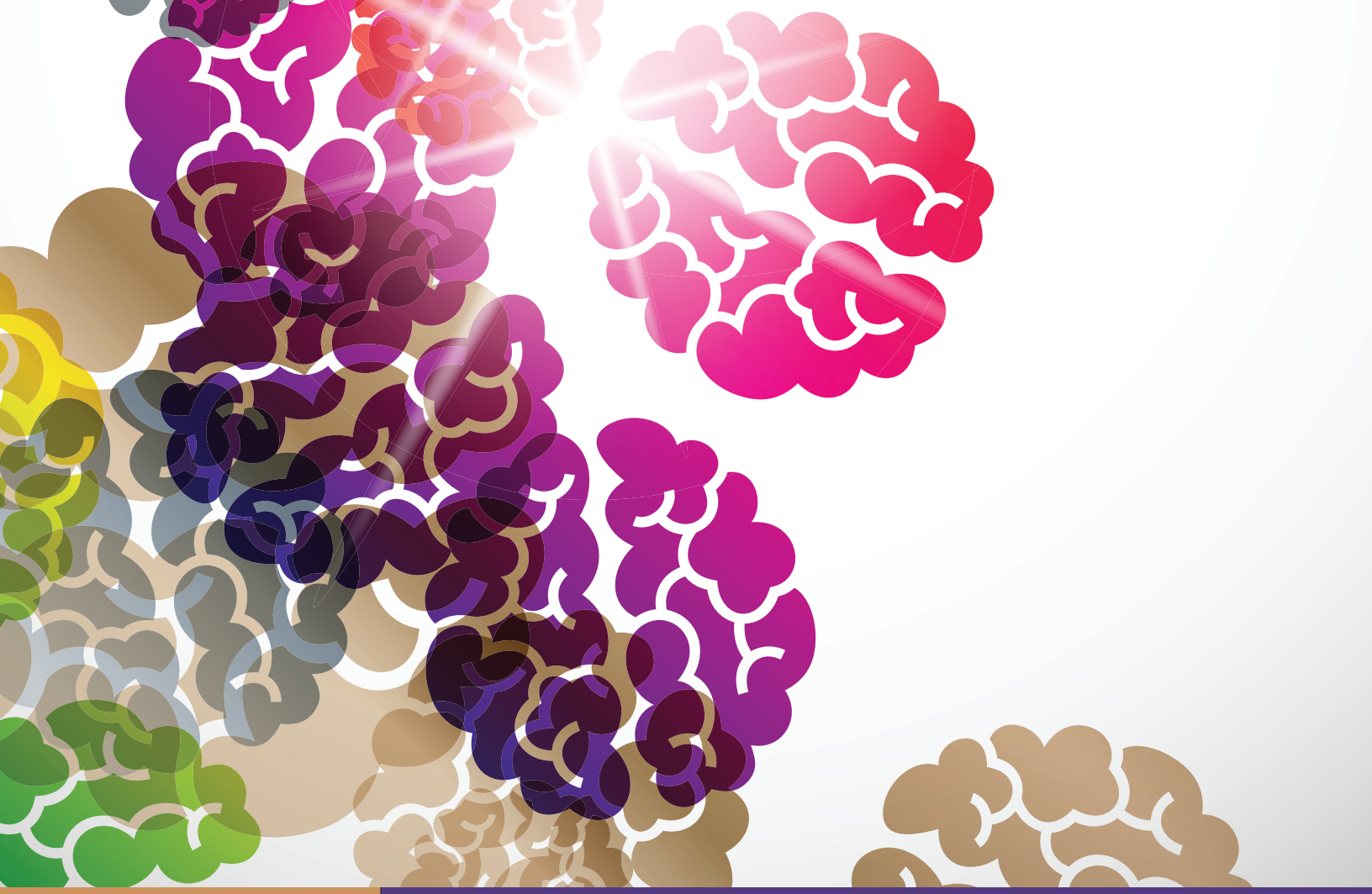


motif|vations

THE NEWSLETTER OF ACTIVE MOTIF
OCTOBER 2014 | VOLUME 15 NUMBER 2

Special Neuroepigenetics Edition

ACTIVE MOTIF®

Enabling Epigenetics Research

IN THIS ISSUE

New to Neuroepigenetics? We Can Help.

There is increasing evidence that several neurodevelopmental, neurodegenerative and psychiatric disorders are, in part, caused by aberrant epigenetic modifications. As a result, researchers in neurogenetics are now beginning to investigate the mechanisms underlying epigenetic regulation in neuronal gene expression and how this impacts normal brain function. The transition into epigenetics research does not have to be intimidating because there are an increasing number of tools available to help researchers obtain their desired results.

Various epigenetic modifications are found in neurons

These modifications include classical epigenetic marks such as DNA methylation and histone modifications. Other epigenetic mechanisms in play include histone subunit exchange, cytosine methylation variants and non-coding RNAs. In recent years, there has been a rapid increase in the number of publications suggesting a role for epigenetics in regulating the Central Nervous System (CNS) and a growing body of evidence linking epigenetic mechanisms to neural plasticity and CNS disorders, such as schizophrenia and drug addiction (Table 1). More than a dozen neurological syndromes so far have been linked to mutations in single genes that encode DNA methyltransferase and histone modifying enzymes. This list includes neurological disorders that appear in early childhood (Rett syndrome) or later in life, such as Hereditary Sensory and Autonomic Neuropathy Type 1 (HSAN1).

The epigenetic tool kit

The development of methods such as chromatin immunoprecipitation (ChIP) and DNA methylation enrichment techniques, along with Next-Generation Sequencing tools, is allowing researchers to get a better picture of the genome-wide changes associated with neurological and other diseases. There are a number of techniques that are key to epigenetic analysis. These include:

- *Antibodies* to discriminate between the multiple post-translational modifications on the histone tails, and to detect methylated DNA
- *ChIP and ChIP-Seq* techniques to enable scientists to link specific states of chromatin to individual gene loci in a cell to understand how genes are regulated
- *Enrichment methods* for isolation of methylated DNA variants (5-methylcytosine, or 5-mC, and 5-hydroxymethylcytosine, or 5-hmC)
- *Bisulfite conversion* of methylated DNA prior to sequencing to allow detection of methylated cytosines

"What Roles do Epigenetic Mechanisms Play in Complex Human Diseases of the Nervous System?"

Using the right tools for ChIP

ChIP is a technically challenging method. Numerous factors can cause it to fail, so researchers who are not experts in the techniques 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 the researcher with their ChIP experiments. We provide all the critical components needed 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 over 1,000 papers in peer-reviewed journals. For the full list of kits and reagents for ChIP, please visit us at www.activemotif.com/chip.

"How is DNA Methylation Actively Regulated in Neurons?"

What is the role of DNA methylation in brain function?

DNA methylation of genes is found in all human tissues, including the brain. There are several methylation variants, and one of these, 5-hmC, is highly expressed in the brain relative to other parts of the body. This has led to speculation that 5-hmC may have a unique biological role in the CNS. Also, recent studies have shown a correlation between DNA methylation levels and mental health problems, such as anxiety and depression. In addition, mutations in genes involved in DNA methylation (*e.g.* MeCP2) are linked to neurological diseases. However, the mechanism by which DNA methylation exerts these effects is not fully understood.

Tools for DNA methylation analysis

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 (MeDIP) and/or immunofluorescence. For complete details on all of our DNA methylation products, please visit us at www.activemotif.com/dnamt.

Epigenetic techniques require validated antibodies

One of the greatest challenges for epigenetic-based research has been the lack of antibodies that show proper specificity and that have been validated for use in techniques such as ChIP and ChIP-Seq. Histones have multiple post-translational modifications along the so-called tail portion of the protein that appear to be involved in gene regulation and disease. Antibodies need to be able to clearly differentiate between these multiple small modifications. It is a major challenge to develop antibodies with sufficient specificity that will also perform in demanding techniques such as ChIP.

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 a test on the specificity of our histone antibodies with a unique Modified™ Histone Peptide Array (Catalog No. 13001). For the full list of our epigenetics antibodies please visit www.activemotif.com/abs.

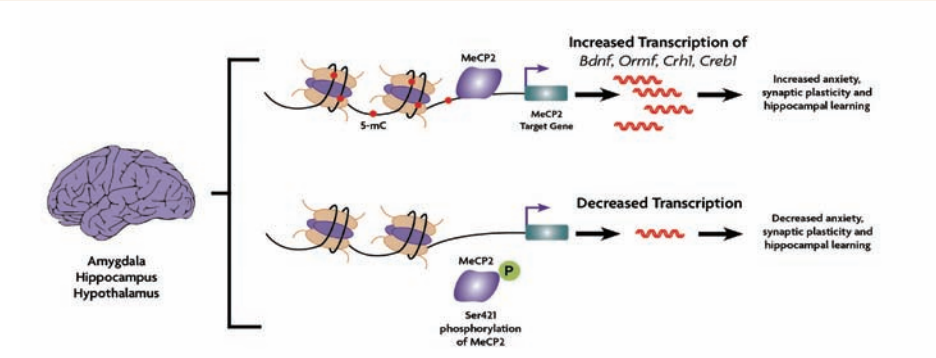
	Histone modification	DNA methylation	Noncoding RNA
Learning and memory	√	√	√
Addiction	√	√	√
Autism spectrum	?	?	?
Epilepsy	√	√	√
PTSD	√	√	
Alzheimer's disease	√	√	
Stress	√	√	√
Schizophrenia	√	√	
Bipolar disorder	√	√	√
Adult neurogenesis	√	√	

Table 1: Examples of neural processes and disorders associated with epigenetic modifications.

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's why our Epigenetic Services team provides a wide variety of ChIP services. This makes 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.



5-hmC and the Brain

Epigenetic regulation is an important part of human brain development. It links our environment and our genome and influences learning and memory processes by what many now refer to as behavioral epigenetics, or neuroepigenetics. Neurogenesis is said to continue in certain parts of the brain throughout adult life. However, it may be neuroepigenetic changes that hold the key to understanding disease-related risk factors associated with our neural development and the aging process. One of the most studied epigenetic modifications, DNA methylation, is at the center of research involving brain diseases, major psychosis and addictions.

DNA methylation

DNA methylation functions to regulate gene transcription in normal cells. Aberrant DNA methylation is responsible for repression of genes through promoter hypermethylation, which is implicated in tumorigenesis, and the ubiquitous hypomethylation common in early carcinogenesis. Abnormal DNA methylation is also linked to various neurological diseases.

5-hmC and the brain

Ten-eleven translocation (TET) enzymes catalyze 5-methylcytosine (5-mC) conversion to 5-hydroxymethylcytosine (5-hmC). 5-hmC is considered the sixth base in mammalian DNA because of its increasing functional significance apart from an intermediary in the demethylation process. While 5-hmC is enriched in many active genes, it appears more prominent in the brain. 5-hmC is approximately 10 times more abundant in brain cells than in stem cells, being highly enriched in the cerebellum and hippocampus. It has been shown that, in areas of enriched 5-hmC, there is loss of 5-mC and vice versa, suggesting unique functions in neural development and maintenance. Interrogation of 5-hmC genome patterns in the brain has revealed important interdependencies, one being the correlation between 5-hmC and methyl CpG binding protein 2 (MeCP2), a member of the MBD family that is important for neuronal maturation. While 5-hmC has limited binding affinity to most MBD proteins, in the case of MeCP2, both 5-mC and 5-hmC can bind it with high affinity (Figure 1) raising the question of how each affects neuronal processes. While MeCP2's repressive properties are important for normal brain function, it has recently been shown that mutations in MeCP2 proteins are associated with certain diseases. In the case of Rett syndrome, there is a strong correlation between 5-hmC and MeCP2 binding when these mutations are present.

As researchers continue to study 5-hmC's contribution to disease and to normal brain function, Active Motif offers highly characterized and validated 5-hmC antibodies for a number of important applications, including hMeDIP-Seq, IF and IHC. For more information please visit us at www.activemotif.com/dnamethabs.

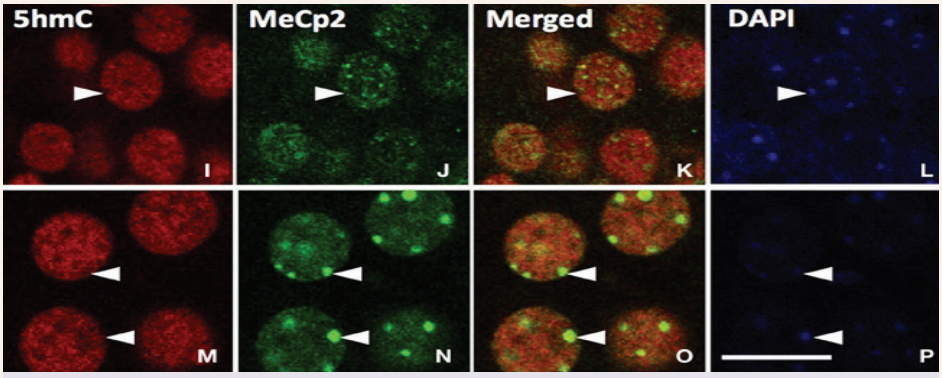
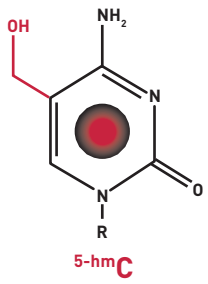


Figure 1: 5-hmC antibody (Catalog No. 39769) shown in confocal microscopy.
The homogeneously (euchromatic) distributed MeCP2 co-localized with 5-hmC (top, arrowhead), but the aggregated (heterochromatic) MeCP2 dissociated with 5-hmC in more mature neurons (bottom, arrowhead). It is shown that MeCP2 are mostly distributed at DAPI-dense regions with heterochromatin where 5-hmC is usually absent during neuronal maturation (legend edited for content).

Product	Format	Catalog No.
5-Hydroxymethylcytosine (5-hmC) antibody (pAb)	100 µl	39769

Tools for Neuroepigenetic Research



5-Hydroxymethylcytosine (5-hmC) is an epigenetic modification that is known to play a major role in the Central Nervous System (CNS) in the regulation of development, neuroplasticity and disease. Due to its importance in neuroepigenetic research, tools to accurately detect and quantify 5-hmC are highly warranted. Active Motif offers a comprehensive portfolio of products specific for 5-hmC, including antibodies, kits for 5-hmC enrichment, assay-ready enzymes and custom services to aid in this area of research.

5-hmC is highly abundant in the CNS where it regulates various processes, including development, aging and neuroplasticity. High 5-hmC levels in the developing brain are associated with maintenance of active transcription. In adult neurons, alternating DNA methylation and demethylation are observed in response to neuronal activity that suggests 5-hmC is involved in cognitive function and development of psychiatric disorders such as stress-induced PTSD. Also, aberrant 5-hmC distribution is linked to the etiopathology of various neurodegenerative disorders, including Alzheimer's and Huntington's disease.

Assays for 5-hmC DNA enrichment

To analyze 5-hmC at the resolution of individual genomic loci by PCR or sequencing, Active Motif offers two methods for hydroxymethylated DNA enrichment. Both methods utilize magnetic beads for faster processing.

Our hMeDIP Kit is an antibody-based technique that utilizes a highly specific 5-hmC antibody to immunoprecipitate DNA fragments containing 5-hmC from the rest of the genomic DNA. The assay is optimized to work with both single-stranded and double-stranded DNA.

Alternatively, the Hydroxymethyl Collector™ Kit utilizes a β-Glucosyltransferase enzyme to label 5-hmC residues within double-stranded DNA fragments with a modified glucose moiety to enable distinction from other cytosine modifications during enrichment. This sensitive technique can enrich DNA fragments containing as little as two 5-hmC residues.

Assay-ready enzymes to study 5-hmC

Little is known about how 5-hmC functions as a transcriptional regulator or as an intermediary of DNA demethylation. Functional studies often involve assays to study these processes *in vitro*. For this purpose, Active Motif provides several enzymes for 5-hmC analysis to readily incorporate into your assays.

To study the process of DNA demethylation, Active Motif offers Recombinant Tet1 protein that catalyzes the conversion of 5-mC to 5-hmC and other oxidative cytosine variants.

Active Motif also offers the β-Glucosyltransferase enzyme to chemically label 5-hmC with a glucosyl moiety and the PvuRts1I restriction endonuclease that specifically cleaves hydroxymethylated DNA. These enzymes distinguish 5-hmC from other cytosine variants and are valuable tools for analysis of hydroxymethylated DNA patterns in the genome. For more information, please visit www.activemotif.com/dnametenzymes.

Methylation variant profiling services

Active Motif's Custom Epigenetic Services include genome-wide hydroxymethylcytosine (hmC), formylcytosine (fC) and carboxylcytosine (caC) profiling. The services use antibodies that specifically recognize each modification for DNA immunoprecipitation to generate genome-wide enrichment profiles. For more complete information, please visit www.activemotif.com/meth-variant-svc.

Product	Format	Catalog No.
hMeDIP	10 rxns	55010
Hydroxymethyl Collector™	25 rxns	55013
Recombinant Tet1 protein, active	25 µg	31363
β-Glucosyltransferase enzyme	500 Units	55012
PvuRts1I restriction enzyme	50 Units	55011

NEW

Quantify Global Changes in DNA Methylation

Because global hypomethylation is a hallmark of human cancer and disease, simpler methods than HPLC or bisulfite sequencing are warranted for analyzing genome-wide DNA methylation to correlate variances with factors such as treatment conditions or clinical outcome. 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 to offer better specificity and reproducibility than other methods that utilize non-specific passive adsorption.

LINE-1 & global DNA methylation

The Global DNA Methylation – LINE-1 Kit is uniquely designed to quantitate methylation in Long Interspersed Nucleotide Element 1 (LINE-1) repeats in human genomic DNA. LINE-1 methylation serves as a surrogate readout for global DNA methylation levels. The ELISA-based assay provides a highly specific and sensitive high-throughput method for screening relative changes in 5-mC levels across samples to correlate with variables, such as treatment conditions, environmental and social factors and clinical prognosis (Figure 1).

DNA methylation occurs in the context of CpG dinucleotides that are mainly found within repetitive elements. It is largely understood that the global hypomethylation observed in cancers is primarily due to hypomethylation at these elements. Not surprisingly, aberrant LINE-1 methylation is also linked to the onset and poor prognosis of cancer. Assessing the methylation status of repetitive elements such as LINE-1 has emerged in the literature as a comparable method of ascertaining global methylation levels to supplant HPLC and other current methods used to measure total 5-mC as a percentage of total cytosine.

How does it work?

In the Global DNA Methylation – LINE-1 Assay, the genomic DNA of interest is enzymatically fragmented and hybridized to a biotinylated probe containing a human LINE-1 consensus sequence. Hybridized DNA is then immobilized onto a 96-well streptavidin-coated plate. Any unbound DNA fragments are then washed away and a 5-Methylcytosine antibody and a secondary antibody conjugated to horseradish peroxidase (HRP) are used for detection of methylated fragments. The colorimetric readout is then easily quantified by spectrophotometry. This LINE-1 targeted hybridization approach produces lower background and enables more specific and reproducible capture of methylated genomic DNA fragments than methods using passive adsorption. To learn more, please visit www.activemotif.com/gdm.

Global DNA Methylation – LINE-1

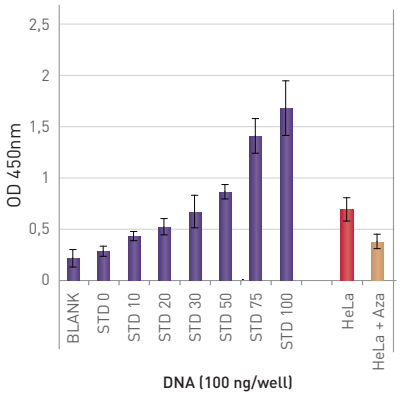


Figure 1: Global DNA Methylation Assay results showing a decrease in 5-methylcytosine levels resulting from treatment of HeLa cells with 5-azacytidine (Aza), a DNA methyltransferase inhibitor.

What's in the box?

The Global DNA Methylation – LINE-1 Kit contains an optimized protocol and all the necessary reagents to perform DNA fragmentation, hybridization, capture and colorimetric detection of 5-mC. For added convenience, methylated and non-methylated DNA standards containing known levels of LINE-1 methylation are included in the kit that can be utilized to generate a standard curve for determination of % 5-mC values of your samples.

Product	Format	Catalog No.
Global DNA Methylation - LINE-1 Kit	1 x 96 rxns	55017

NEW

ChIP-Bisulfite-Sequencing for Mapping Allele-Specific Methylation of Protein/DNA Binding Sequences

Active Motif's new ChIP-Bis-Seq Kit offers a method to directly interrogate both the chromatin and DNA methylation profile on the same sample. The kit combines our highly validated high sensitivity chromatin immunoprecipitation (ChIP) protocol to enrich for the sites of chromatin associations or modifications of interest, followed by a bisulfite conversion and sequencing (Bis-Seq) procedure to generate single base-pair resolution methylation profiles at these sites.

What is ChIP-Bis-Seq?

ChIP-Bisulfite-Sequencing (ChIP-Bis-Seq) enables direct interrogation of both the chromatin and DNA methylation profile on the same sample within a single experiment. ChIP-Bis-Seq combines a ChIP procedure for target-specific enrichment of protein-bound or modified chromatin with bisulfite conversion and sequencing (Bis-Seq) to provide a DNA methylation profile of ChIP-enriched chromatin sequences. This approach is ideally suited for genome-wide studies of the interplay between DNA methylation and chromatin features, such as in assessing allele-specific DNA methylation variances (*e.g.* imprinting, X-inactivation), or in evaluating global gene regulatory effects.

How does it work?

The ChIP-Bis-Seq Kit consists of three modules. The first is Active Motif's ChIP-IT® High Sensitivity Kit which is used to immunoprecipitate chromatin associated with a protein or modification of interest in order to reduce the representation to targeted sites of the genome. The second kit module contains methylated adapters and enzymes to enable preparation of a Next-Generation Sequencing (NGS) library from the ChIP DNA. By using methylated adapters, the cytosines within the adapter sequence will remain unchanged during bisulfite conversion and retain their original sequence. The third kit module includes reagents to perform bisulfite conversion on the adapter-ligated DNA. The converted library is PCR amplified, size selected and submitted for NGS.

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

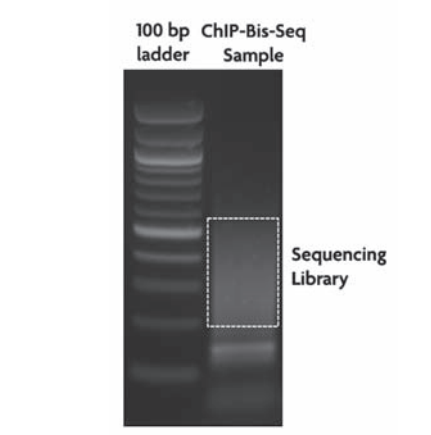


Figure 1: DNA gel for size selection of the PCR amplified ChIP-Bisulfite-Sequencing library.

Save time and money

Active Motif saves you time and effort by providing an optimized, validated start-to-finish solution at a lower price than purchasing components separately. Check out the list of included items in the box below.

What's in the ChIP-Bis-Seq Kit?

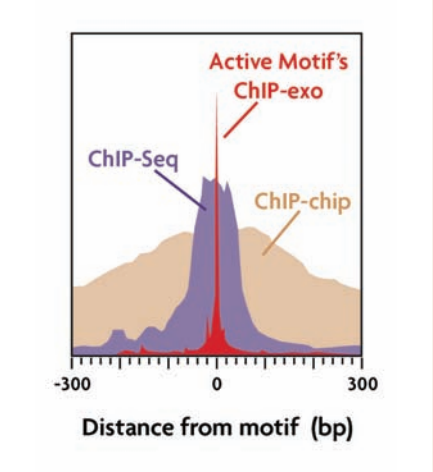
- ChIP-IT® High Sensitivity Kit – includes lysis buffers, protease inhibitors, protein G agarose beads, IP buffers and DNA purification reagents to prepare chromatin from cells or tissues for 16 ChIP reactions
- Library Preparation Reagents – includes end polishing enzymes and methylated adapters compatible with Illumina sequencing platforms to prepare 10 NGS libraries from your ChIP DNA
- Bisulfite Conversion – includes sodium bisulfite conversion reagents with specialized DNA purification columns suitable for use with converted, fragmented ChIP-enriched DNA

Product	Format	Catalog No.
ChIP-Bis-Seq Kit	10 libraries	53048

NEW

High-Resolution ChIP Analysis of Transcription Factors

Active Motif's new ChIP-exo Kit* provides high-resolution genome-wide mapping of transcription factor binding sites. The ChIP-exo method utilizes exonuclease-mediated digestion of DNA during the immunoprecipitation procedure to eliminate non-targeted DNA and increase binding resolution to within 20-95 base pair sequencing reads. ChIP-exo significantly improves the accuracy of identification of protein/DNA binding motifs and is ideal for discovery-based studies or evaluation of mutation and SNP effects.



What is ChIP-exo?

ChIP-exonuclease (ChIP-exo), a technology that was developed in the laboratory of B. Franklin Pugh, is a modified ChIP-Seq approach that includes an exonuclease digestion step during chromatin immunoprecipitation to excise non-cross-linked DNA and reduce fragments to the site of transcription factor binding prior to deep sequencing. By removing the excess unbound DNA, background signal is reduced so that fewer sequencing reads map to non-specific genomic regions. This also enables identification of weak peaks allowing detection of transcription factors that are inefficiently bound to the genome that would be indistinguishable from noise with traditional ChIP-Seq.

A high-resolution method for genome-wide mapping of protein binding sites offers many advantages. It improves identification of transcription factor consensus sequences that will aid in the discovery of new binding motifs (Figure 1). Additionally, studies of binding specificities at high resolution assist in deducing the impact of mutations and SNPs on transcriptional regulation and disease.

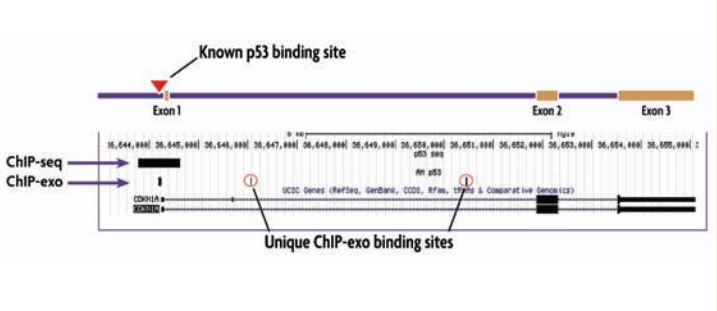


Figure 1: Identification of unique p53 binding sites. In addition to reducing the size of the identified p53 binding site (red triangle) as compared to ChIP-Seq results, Active Motif's ChIP-exo method identifies several other unique binding sites not revealed by ChIP-Seq (red circles).

How does the assay work?

Cells are fixed with formaldehyde to cross-link protein/DNA binding interactions. Cells are then lysed and chromatin is fragmented by sonication. An antibody directed against the protein of interest is conjugated to protein G magnetic beads for immunoprecipitation of the DNA of interest. With the chromatin still bound by the beads, the DNA is end-polished and P7-exo adapters are ligated onto the blunt ends. The nicked DNA is repaired and then digested by lambda and RecJF exonucleases to excise DNA in a 5´ to 3´ direction, trimming DNA to the site of the cross-linking and selectively eliminating the P7 adapter at the 5´ end. Following cross-link reversal and elution from the beads, the DNA is made double-stranded by P7 primer extension, and a P5-exo adapter is added to the exonuclease-treated ends.

The DNA library is PCR amplified and size selected before it is subjected to high-throughput sequencing. The sequence of the DNA is mapped back to the reference genome to determine the binding locations of the protein of interest. The 5´ ends of the DNA fragments on the forward strand indicate the left border of a protein/DNA interaction, while the 5´ ends of the DNA

ChIP-exo advantages

- 20-95 bp resolution of binding sites as compared to 300 bp for ChIP-Seq
- Provides high-resolution genome-wide mapping of transcription factors
- Exonuclease digestion of non-specific DNA reduces non-specific background
- Improves detection of inefficiently-bound transcription factors
- On-bead enzymatic reactions streamline sample processing

fragments on the reverse strand indicate the right border of a protein/DNA interaction. These borders demarcate the precise site of protein/DNA cross-linking, providing high-resolution (20-95 base pairs) identification of genomic binding sites (Figure 2).

What's in the box?

ChIP-exo Kits contain lysis buffers, protease inhibitors, protein G magnetic beads, exonuclease and polymerase enzymes and reaction buffers, dNTPs, sequencing adapters, indexing primers and DNA purification and size selection beads sufficient to prepare chromatin and perform 12 immunoprecipitation and sequencing library reactions. The kit includes a streamlined protocol that has been adapted for use on Illumina sequencing platforms.

For more complete information on the ChIP-exo Kit, please visit us at www.activemotif.com/chipexo.

*Technology is covered under U.S. Patent No. 8367334 b2.

ChIP-exo Flow Chart

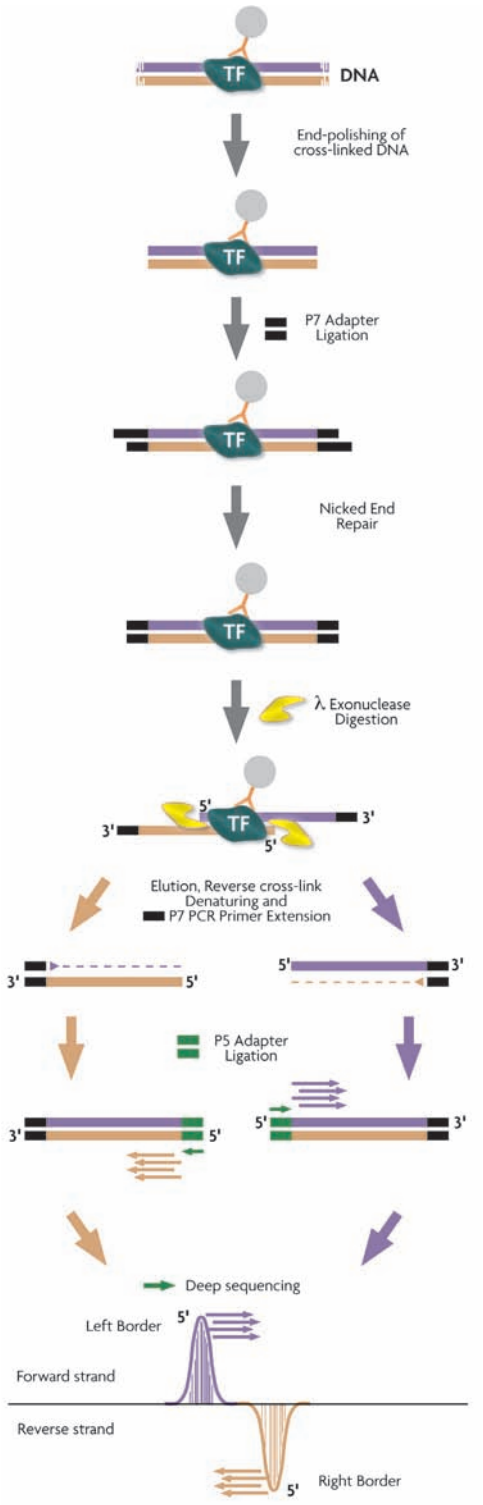


Figure 2: Schematic of the ChIP-exo Kit workflow following chromatin preparation and capture with antibody-conjugated beads.

Product	Format	Catalog No.
ChIP-exo Kit	12 rxns	53043

NEW

Nucleosome Substrates for More Biologically Relevant Screening Assays

Whether you are performing drug discovery screening, analyzing enzyme kinetics or monitoring changes in histone modifications, substrate selection is critical. Nucleosomes offer the advantage of providing a more biologically relevant substrate when compared to histone proteins alone or synthetic peptides. To best mimic cellular biology with your *in vitro* assay, use Active Motif's Nucleosome Preparation Kit to prepare nucleosomes from your samples, or utilize one of our pre-assembled Recombinant Nucleosomes.

Prepare your own nucleosomes

Active Motif's new Nucleosome Preparation Kit contains lysis buffers, protease inhibitors, digestion buffer, enzymatic shearing cocktail and an optimized protocol to isolate intact nucleosomes from your samples (Figure 1). Simply adjust your enzymatic digestion time to modify the amount of mononucleosomes or oligonucleosomes you extract. Use your isolated nucleosomes as substrates in the analysis of histone post-translational modifications, enzyme kinetics or inhibitor screening studies.

Looking for ready-to-use nucleosome substrates?

Active Motif's Recombinant Nucleosomes save you time and effort by providing pre-assembled human nucleosomes. Each recombinant nucleosome is comprised of octamers of unmodified core histone proteins (H2A, H2B, H3 and H4) bound by DNA. These intact nucleosomes are ideal substrates for *in vitro* studies. Nucleosomes are available to study both Histone H3.1 and Histone H3.3 variants. Additionally, choose from unlabeled or biotin-labeled nucleosomes to give you flexibility in your experimental design.

To illustrate the usefulness of nucleosome substrates, recombinant nucleosomes were compared with histone octamers in an activity assay using SET domain-containing histone methyltransferase enzymes NSD1-SET and NSD2-SET (Figure 2). The results show there is greater methyltransferase activity when using recombinant nucleosomes as a substrate as compared to octamers alone.

Nucleosome Preparation Kit

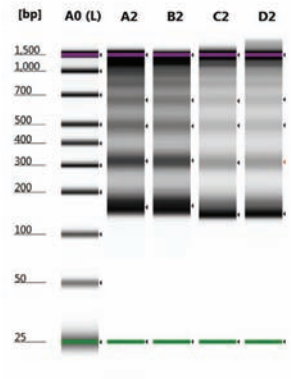


Figure 1: DNA analysis of HeLa (A2, B2) and MCF-7 (C2, D2) cells enzymatically digested into mono- and oligonucleosomes using Active Motif's Nucleosome Preparation Kit.

1. No enzyme
2. No substrate
3. Histone Octamers
4. Recombinant Nucleosomes A
5. Recombinant Nucleosomes B

Figure 2: Histone methyltransferase activity assay comparing recombinant nucleosomes and histone octamers as substrates.

Product	Format	Catalog No.
Nucleosome Preparation Kit	20 rxns	53504
Recombinant Nucleosomes (H3.1)	20 µg	31466
Recombinant Nucleosomes (H3.1) - biotinylated	20 µg	31467
Recombinant Nucleosomes (H3.3)	20 µg	31468
Recombinant Nucleosomes (H3.3) - biotinylated	20 µg	31469

NEW

Epigenetic Services: A Novel ChIP-Seq Spike-in Strategy

A persistent problem with chromatin immunoprecipitation is that traditional ChIP-Seq protocols are not always able to detect global changes in histone modifications caused by treatment of samples with epigenetic inhibitors. Active Motif's Epigenetic Services team has a solution. We have created and validated a ChIP-Seq spike-in normalization strategy that is able to reveal these differences. This method is now available as part of our end-to-end ChIP-Seq Service.

Problem:
- ChIP-Seq does not always detect inhibitor-induced global changes in histone modifications

Solution:
- ChIP-Seq Normalization to a spiked-in control

How does it work?

ChIP-Seq reactions: A standard ChIP-Seq reaction is set up using experimental chromatin (*e.g.* human) and an antibody of interest (*e.g.* H3K27me3 antibody). However, in addition, *Drosophila melanogaster* 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 H2Av antibody provides a mechanism to reliably pull down a small fraction of *Drosophila* chromatin. Following ChIP, immunoprecipitated DNA sequences are analyzed by Next-Generation Sequencing (NGS).

Normalization: Following NGS, sequence tags are aligned to the experimental reference genome (*e.g.* human) and the *Drosophila* genome. Differences in *Drosophila* tag counts are equalized across samples. Human tag counts are then normalized using the same ratio used to equalize *Drosophila* tag counts.

Results: Biases that are introduced during Next-Generation library amplification and sequencing also occur in the *Drosophila* spike-in chromatin. Normalization using our spike-in strategy eliminates these biases to enable ChIP-Seq analysis to reveal any significant biological changes in your samples (Figure 1).

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.

For more information, or to request a quote, please go to www.activemotif.com/services-normalize.

NEW

Validate the Specific mRNA Targets of miRNAs

MicroRNAs (miRNAs) regulate many important cellular processes, including development, differentiation, proliferation and apoptosis. Because changes in miRNA expression levels have been found in a variety of cancers, as well as in cardiac disease and neurological disorders, miRNA-mediated regulation is an important topic in biomedical research. Active Motif's new miRNA Target IP Kit was designed to identify the physical interactions of miRNAs with endogenous mRNA transcripts in order to validate the binding targets of specific miRNAs.

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). In mammalian cells, the interaction of a miRNA with a 3´UTR usually results in repression of translation.

The targeting of a 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 (Diagram 1).

Validate miRNA/mRNA interactions using Ago IP

Active Motif's new 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 (Figure 1). 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.

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

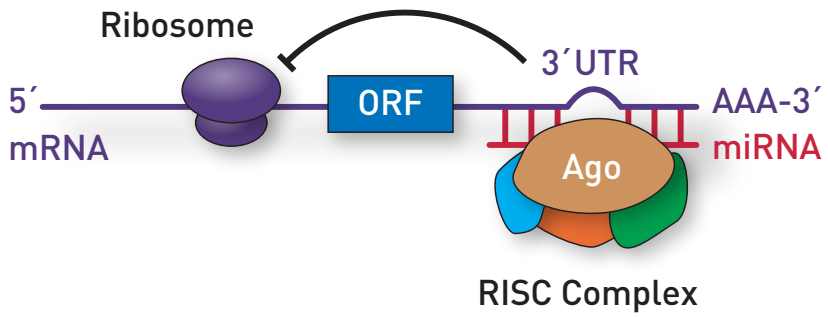


Diagram 1: The miRNA contained in a RISC complex enables precise silencing of specific mRNA transcripts.
The key components in a RISC complex are an Argonaute protein (Ago) and a miRNA. The Ago protein binds the miRNA, positioning it in a conformation that enables the RISC to base-pair in a Watson-Crick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

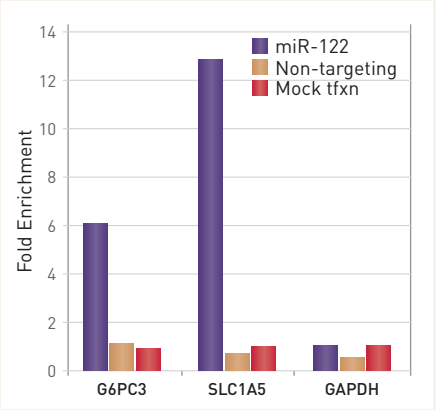


Figure 1: miR-122 targets G6PC3 and SLC1A5.
The miRNA Target IP Kit was used on samples of HT1080 cells that were transfected with a miR-122 mimic or a non-targeting miRNA control for 8 hours, or that were mock transfected. Following IP using the Ago1/2/3 antibody or Negative Control IgG included in the kit, qRT-PCR was performed on the samples using primers for G6PC3 and SLC1A5, which are known targets of miR-122, and for GAPDH, a common housekeeping gene that is not known to be targeted by miR-122.

miRNAs in Neural Development & Neurological Diseases

MicroRNAs (miRNAs) are a class of small, non-coding RNA molecules that act as post-transcriptional regulators in many different biological processes. Observed changes in expression levels of specific miRNAs, both upregulated and down, demonstrate that they are involved not just in the development and function of the brain, but also in the occurrence and progression of many neurological diseases. Consequently, miRNAs are proving to be useful as novel biomarkers in studies of ischemia & reperfusion, traumatic brain injury, as well as in disorders, such as epilepsy and Alzheimer's, Parkinson's & Huntington's diseases.

Post-transcriptional regulators

miRNAs regulate gene expression by binding to the 3´UTRs of mRNA transcripts. This interaction results in either inhibition of translation or transcript degradation. Most miRNAs target multiple mRNAs, and it has been shown that a single miRNA can regulate multiple signaling pathways simultaneously. In addition, individual mRNAs frequently contain binding sites for more than one miRNA. This creates a complex feedback network that regulates and fine-tunes the expression levels of individual genes, as well as entire pathways, in a tissue and cell-state dependent manner.

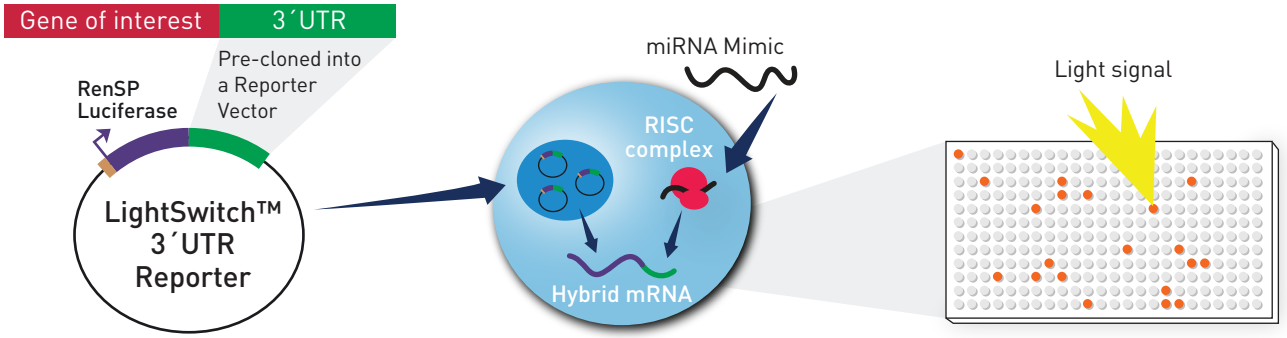
miRNAs in neurological disorders

A review article by Wang *et al. (Biomed. Rep.* (2014) 2(5): 611-619.) provides an overview of research articles that study the role miRNAs play in a number of neurological disorders. It highlights papers that show how the altered expression of specific miRNAs can play a neuroprotective role by modulating the expression of genes involved in diseases of the central nervous system. For example, Harraz *et al. (PNAS USA* (2012) 109: 18962-18967.) shows miR-223 overexpression can decrease levels of both GluR2 and NR2B, which protects the brain from neuronal cell death during stroke.

Study the impact of miRNAs

Active Motif's LightSwitch™ System is ideal for assessing the functional impact of miRNA-3´UTR interactions. It includes a collection of over 12,000 human 3´UTRs that can be purchased as ready-to-transfect LightSwitch luciferase reporter vectors. Combined with our large collections of miRNA Mimics & Inhibitors, you have everything needed to study miRNA-3´UTR interactions, validate miRNA targets, and to measure RNA stability and the functional impact of miRNAs on a gene-by-gene basis (see below). For more information, please visit us at www.activemotif.com/ls-3utr.

Studying miRNA-3´UTR Interactions with the LightSwitch™ Luciferase Assay System



1. Choose a 3´UTR of interest from our collection of over 12,000 ready-to-transfect constructs.
2. Co-transfect reporter construct with miRNA mimics or inhibitors.
3. Measure luciferase activity to determine the effect on translation of miRNA binding to the hybrid luciferase-3´UTR mRNA.

Tools to Streamline Epigenetic Drug Research

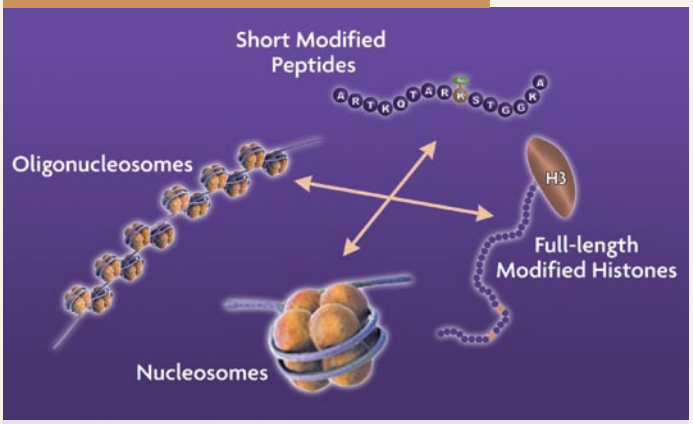
Drug discovery and development programs focused on epigenetics have attracted much attention and investment in recent years. This is driven by the potential to develop novel therapeutic strategies that can reverse epigenetic and transcriptional abnormalities. Active Motif is a leading provider of innovative technologies for epigenetics and gene regulation research. Our assays, reagents and services can be easily integrated into your research to streamline the drug discovery workflow.

Biologically relevant assay substrates

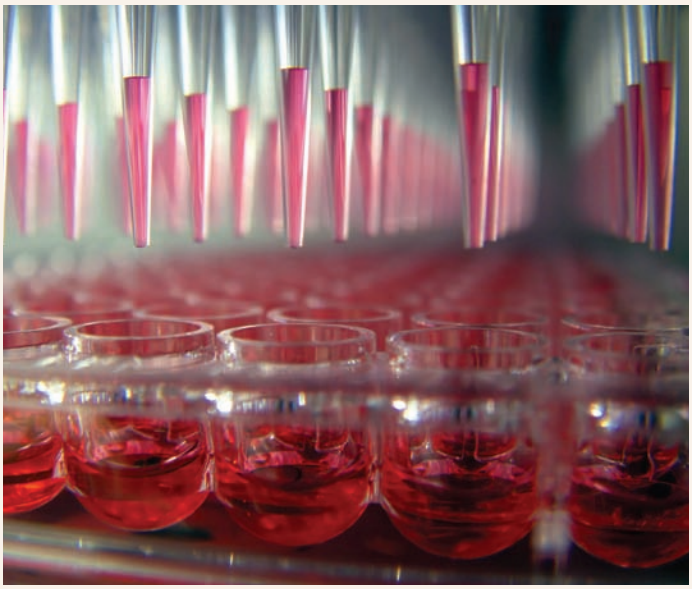
The outcome of your *in vitro* biochemical assays is vastly improved when the system mimics actual cellular biology. To provide you the best choice of substrate for use in your assay, Active Motif offers over 60 different recombinant full-length modified H3 and H4 histones that are engineered using patented technology to have structural and functional similarity to their native counterparts. These can be used as stand-alone substrates or assembled along with recombinant H2A and H2B to generate nucleosomes and oligonucleosomes. By choosing the most biologically relevant substrate, you can ensure the most accurate results.

To analyze epigenetic modifications or protein interactions in a chromatin context, Active Motif's Chromatin Assembly Kit provides an end-to-end solution for designing your own chromatin. The technique involves an ATP-dependent process to produce a chromatin substrate that mimics native *in vivo* chromatin structure. The assembled chromatin is ready to use in downstream assays, such as *in vitro* transcription assays, chromatin immunoprecipitation and enzyme activity assays.

Which Histone Substrate is Right for Your Assay?



Choosing the correct histone substrate for assay development is key to achieving the best results. Opinion leaders in epigenetics recognize the power of reconstituting recombinant chromatin for creating biologically relevant substrates.



Assay-ready proteins, enzymes and domains

There is no need to waste valuable time and resources generating proteins for assay development. With Active Motif's comprehensive portfolio of over 300 purified assay-ready recombinant proteins – including transcription factors, histones, DNA- and histone-modifying enzymes, and bromodomains – you are sure to find what you need to develop more efficient assays for your drug discovery and development program.

Assays for enzymatic activity

Active Motif offers a variety of robust, sensitive, high-throughput assays for quick and easy screening and profiling of cellular changes in the activity of histone modifying enzymes. These include our HAT Assay Kit to measure the activity of histone acetylases, the HDAC Assay Kits to analyze histone deacetylase activity and our Histone Demethylase Assay to analyze the lysine demethylase activity of LSD1.

For more details, go to www.activemotif.com/info to download a copy of our Tools for Drug Discovery product brochure.

NEW

Small Molecules to Modulate Epigenetic Processes

Active Motif has an expanding collection of small molecule compounds (activators and inhibitors) that target the activity of proteins that regulate epigenetic changes in DNA methylation, chromatin remodeling and histones. These include targets to DNA Methyltransferases (DNMTs), Histone Acetyltransferases (HATs), Histone Deacetylases (HDACs), Histone Methyltransferases (HMTs), Histone Demethylases (HDMs), and the BET family bromodomains. As epigenetic aberrations are implicated in many diseases, these modulators are important tools for use in lead generation and assay development.

Description	Target	Format	Cat. No.
HISTONE DEACETYLASE			
Apicidin	HDAC inhibitor	1 mg 5 mg	14041 14040
BML-210	HDAC inhibitor	5 mg 25 mg	14049 14048
CUDC-101	HDAC inhibitor	5 mg 25 mg	14061 14060
HPA (Hexyl-4-pentynoic acid)	HDAC inhibitor	10 mg 50 mg	14035 14034
MS-275	HDAC inhibitor	5 mg 25 mg	14043 14042
Panobinostat	HDAC inhibitor	5 mg 25 mg	14045 14044
Phenylbutyrate Na	HDAC inhibitor	1 g	14033
Romidepsin	HDAC inhibitor	1 mg	14083
Trichostatin A	HDAC inhibitor	1 mg 5 mg	14039 14038
Tubastatin A	HDAC inhibitor	1 mg 5 mg	14085 14084
Valproic acid	HDAC inhibitor	5 g	14021
Vorinostat (SAHA)	HDAC inhibitor	10 mg 50 mg	14027 14026

Description	Target	Format	Cat. No.
LYSINE METHYLTRANSFERASE & LYSINE DEMETHYLASE			
BIX-01294	HMT inhibitor	5 mg 25 mg	14073 14072
Chaetocin	HMT inhibitor	200 µg	14051
Daminozide	HDM inhibitor	50 mg 250 mg	14059 14058
DMOG	HDM inhibitor	10 mg 50 mg	14063 14062
GSK-J1 (cell impermeable)	HDM inhibitor	5 mg 25 mg	14069 14068
GSK-J4 (cell permeable)	HDM inhibitor	5 mg 25 mg	14071 14070
IOX1	HDM inhibitor	5 mg 25 mg	14057 14056
ML-324	HDM inhibitor	5 mg 25 mg	14079 14078
Tranylcypromine hemisulfate	HDM inhibitor	50 mg 250 mg	14047 14046

Description	Target	Format	Cat. No.
METHYLATION			
5-Azacytidine	DNA methyltransferase inhibitor	50 mg 250 mg	14103 14102
Decitabine (5-Aza-2´-deoxycytidine)	DNA methyltransferase inhibitor	10 mg 50 mg	14101 14100
RG108	DNA methyltransferase inhibitor	5 mg 25 mg	14105 14104

Description	Target	Format	Cat. No.
HISTONE ACETYLTRANSFERASE			
C646	HAT inhibitor	5 mg 25 mg	14053 14052
CTPB	HAT activator	5 mg 25 mg	14065 14064
Garcinol	HAT inhibitor	10 mg 50 mg	14077 14076

Description	Target	Format	Cat. No.
SIRTUIN			
AK-7	SIRT2 inhibitor	5 mg 25 mg	14055 14054
BML-278	SIRT1 activator	5 mg 25 mg	14025 14024
EX-527	SIRT1 inhibitor	5 mg 25 mg	14029 14028
Piceatannol	SIRT1 activator	5 mg 25 mg	14037 14036
Resveratrol	SIRT1 activator	100 mg 500 mg	14023 14022
SirtAct	SIRT1 activator	5 mg 25 mg	14081 14080
Sirtinol	SIRT2 inhibitor	5 mg 25 mg	14075 14074
Splitomycin	Sir2p inhibitor	5 mg 25 mg	14087 14086

Description	Target	Format	Cat. No.
BROMODOMAIN			
JQ1 (racemic)	Bromodomain inhibitor	5 mg 25 mg	14067 14066

Description	Target	Format	Cat. No.
OTHER			
FK-866 HCl	NAD biosynthesis inhibitor	5 mg 25 mg	14031 14030

For an up-to-date list of available activators & inhibitors, please visit www.activemotif.com/smallmol.

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