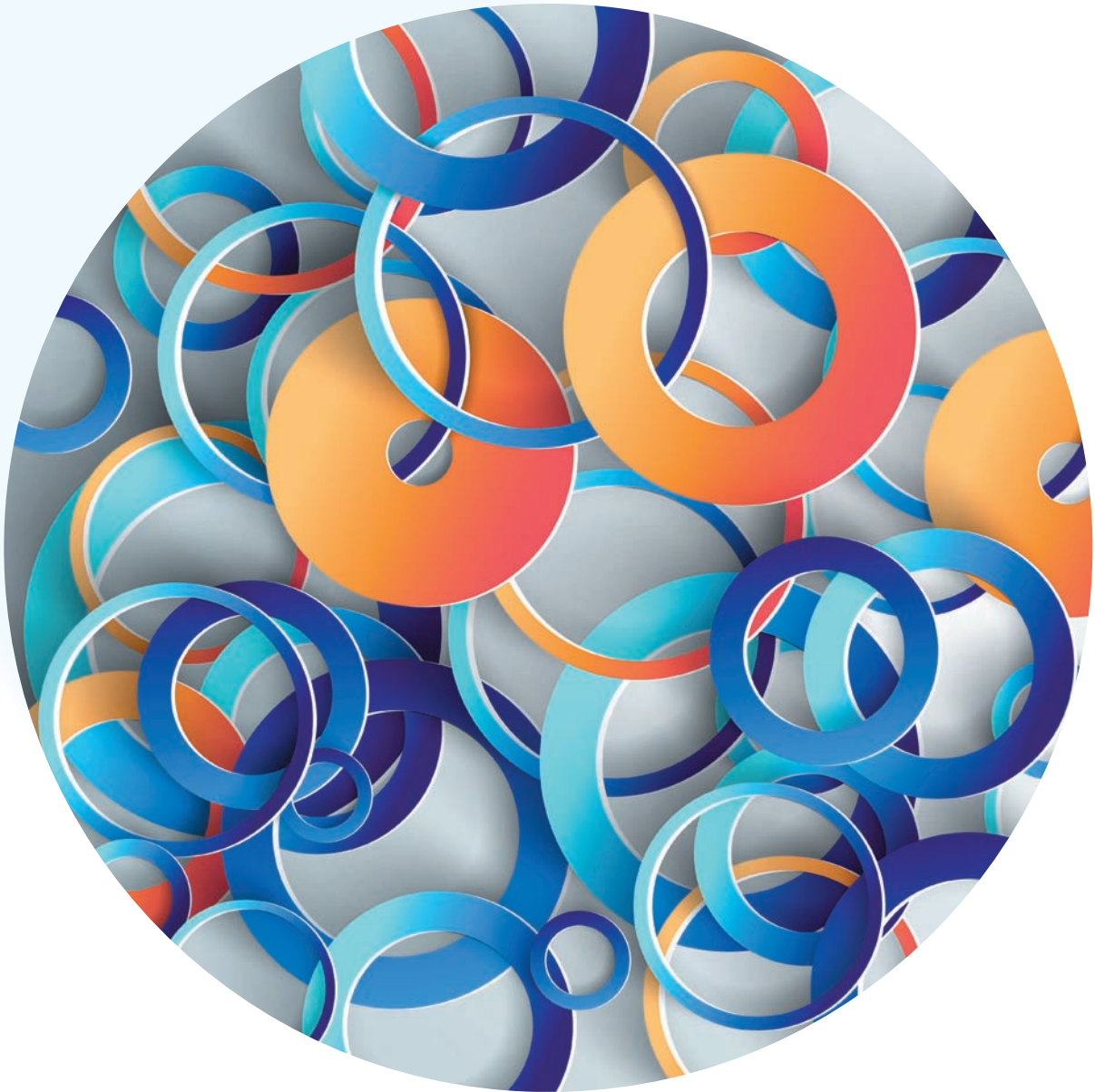
ACTIVE MOTIF®
Enabling Epigenetics Research

products & services

EPIGENETICS

ChIP / Histones / DNA Methylation /
Sample Prep / Antibodies / Services



Contents

Active Motif is the industry leader in providing innovative tools to enable epigenetics and gene regulation research. We provide superior products, service and support to serve our life science, clinical, pharmaceutical and drug discovery partners. We are recognized worldwide for our ground-breaking ChIP technologies, for our LightSwitch™ functional genomic products and genome-wide services. Whether you are an expert in the field of epigenetics or gene regulation or a researcher looking to integrate these areas of research into your studies, our comprehensive portfolio of products offers end-to-end solutions to meet the specific needs of your research.

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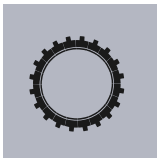
For distributors, visit
www.activemotif.com/distributors

WEB:
www.activemotif.com



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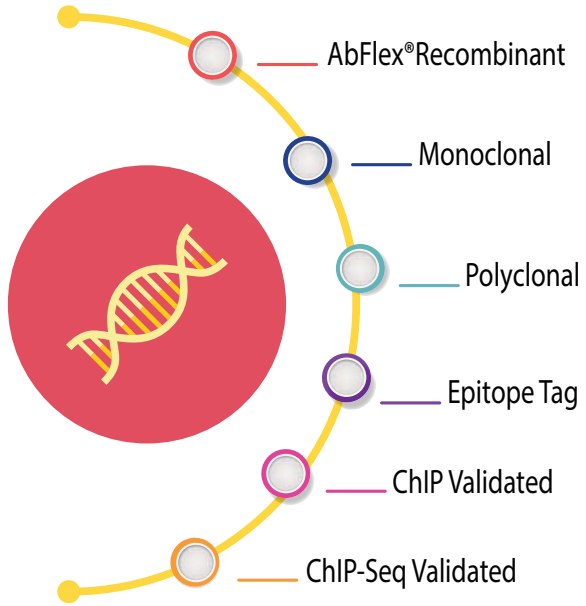
ANTIBODIES FOR EPIGENETICS & GENE REGULATION RESEARCH

ANTIBODY PORTFOLIO



Active Motif offers a wide range of antibodies specific for epigenetic modifications, chromatin modifying proteins, and transcription factors. From immunogen design to specificity screening and technique validation, our staff scientists have an established program to qualify our antibodies for use in various applications, including ChIP-Seq, EMSA, Western blot and immunofluorescence. Our years of experience in antibody development ensures that only the highest quality antibodies are offered for use in your research.

WE HAVE WHAT YOU WANT

The antibodies list below shows only a small sampling of the over 800 antibodies currently offered. For a complete, up-to-date list of all available antibodies, please visit www.activemotif.com/abs.

HISTONES & HISTONE MODIFICATIONS	
H1	H3K27me1
H2A	H3K27me2
H2A.X	H3K27me3
H2A.Z	H3S28ph
H2B	H3K36ac
H3	H3K36me2
H3K4me1	H3K36me3
H3K4me2	H3K56ac
H3K4me3	H3K79me1
H3K9ac	H3K79me2
H3K9me1	H4
H3K9me2	H4ac (pan-acetyl)
H3K9me3	H4R3me2a
H3S10ph	H4K5ac
H3T11ph	H4K12ac
H3K14ac	H4K16ac
H3K18ac	H4K20me1
H3K27ac	H4K20me2
H3R17me2a	H4K31me1

DNA METHYLATION	
3-mC	DNMT3A
5-caC	DNMT3B
5-fC	DNMT3L
5-hmC	TETs
5-mC	MBDs
DNMT1	MeCP2
DNMT2	Uhrf1

CHROMATIN MODIFIERS	
BRD3	Jhd2
BRD4	JMJD2A & 2D
CARM1	EHMT2
CGBP	LSD1 / KDM1A
DOT1L	MLL / HRX
EZH1& 2	MMSET / WHSC1
GCN5	PARPs
HDACs	PRMTs
HP1	Sirtuins
JARID1C / KDM5C	SUV39H1

STEM CELLS	
BMI-1	Notch1
c-Myc	Notch3
EED	YY1
EZH2	Oct4
KLF4	Ring1B
LIN28A	Sox2
Nanog	Sp1
NKX2.5	Suz12

GENE REGULATION	
Ago1/2/3	IKK
AML	MyoD
AP-1	NFκB
AR1	p53
Cas9	PARP
Dicer	PP2A
ER1	RNA pol II
FKHR	STAT
IκBα	Ubiquitin

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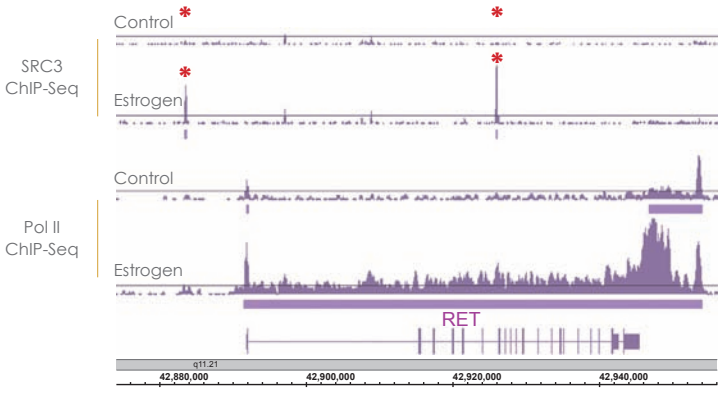
COMPETITIVE *AB*-VANTAGE

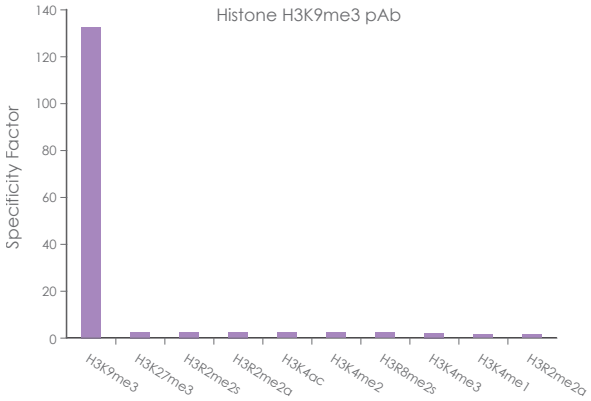
Not every antibody is suitable for ChIP

One of the challenges for epigenetics research is the lack of antibodies that have been validated for use in techniques such as ChIP and ChIP-Seq. The problem is compounded by antibody suppliers who do not manufacture or test the antibodies they sell, and who sell them to one another and then to researchers. At Active Motif, we manufacture and rigorously test our antibodies in-house to ensure their quality and performance. Our ChIP validated antibodies (Figure 1) are validated according to the guidelines of the **ENCODE Consortium*** and are run through the same internal validation program used by our ChIP-Seq Services group.

In addition, we are the only company to test the specificity of our histone antibodies using our ground-breaking **MODified™ Histone Peptide Array** (Figure 2, page 36) that enables assessment of cross-reactivity of our antibodies against a panel of known histone modifications in a single experiment. Our array offers the most extensive coverage available for commercial arrays of similar format and enables the study of not only individual sites, but also the effects of neighboring modifications on recognition and binding.



▲ FIGURE 1: Active Motif's ChIP validated antibodies are qualified using the same internal validation program used by our Custom Services group.
ChIP-Seq data from control and estrogen-treated MCF-7 chromatin using antibodies against RNA pol II CTD phospho Ser2 (Catalog No. 61083) and against the estrogen-inducible transcription factor SRC3 (Catalog No. 39797).



▲ FIGURE 2: Active Motif's MODified Histone Peptide Array confirms the specificity of modification-specific histone antibodies.
Data produced using the MODified Histone Peptide Array to confirm the specificity of Histone H3K9me3 antibody (Catalog No. 39161).

VISIT OUR WEBSITE

Active Motif is continually adding new antibodies to its portfolio. For a complete, up-to-date list of available antibodies, please visit us at www.activemotif.com/abs.

*For ENCODE Consortium guidelines for antibody validation, please refer to Landt S.G. *et al.* (2012) Genome Res. 22, 1813–1831.

CHROMATIN IMMUNOPRECIPITATION

Active Motif offers a complete selection of validated reagents, instruments and kits to help streamline each step of the ChIP assay workflow. Whether you are performing ChIP for the first time or are an expert in need of a specialized ChIP protocol and reagents, Active Motif has the right products to meet your needs. For more complete information on available ChIP products, please visit us at www.activemotif.com/chip.

EPISHEAR™ SONICATORS

Probe sonicators and accessories for shearing chromatin in preparation for ChIP analysis.

ChIP-IT® ChIP KITS

ChIP-IT® offers a comprehensive suite of basic and specialized ChIP kits for performing standard chromatin immunoprecipitation or more innovative techniques, such as sonication-free ChIP, ChIP without the need for protein-specific antibodies, sequential ChIP, transcription factor ChIP, low-cell ChIP, RNA-ChIP, high resolution ChIP and various other methods. For optimal results & the most innovative ChIP technologies, rely on the *ChIP Experts*™.

ChIP ACCESSORIES

A variety of ChIP accessories, including ChIP DNA purification kits, Protein G magnetic beads and agarose columns, qPCR primer sets and controls, to complement your ChIP experiments.

EPISHEAR™ SONICATION PRODUCTS

Active Motif's EpiShear™ sonication systems are designed for ease of use and ultimate control for the highest degree of sample-to-sample reproducibility. Keypad programming and digital displays clearly display all parameters and options, making it easy to control functions such as amplitude, pulse cycles and processing times with the simple touch of a button.

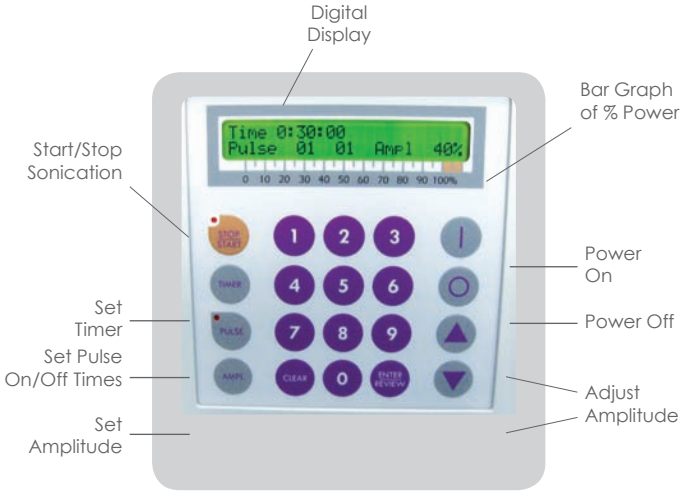
Experience the versatility of probe sonication

The EpiShear™ Probe Sonicator is ideal for shearing chromatin, DNA and RNA, or for cell disruption and homogenization. With a variety of microtip probe sizes available, you can process samples ranging from 200 µl to 50 ml. Each unit includes:

- an ultrasonic electric generator
- a piezoelectric converter
- a 1/8" microtip probe
- power & converter cables
- a wrench set for changing probes

▲ EpiShear Probe Sonicator

The unit is backed by a two-year warranty.
For more information, visit www.activemotif.com/probe.



TECHNICAL SPECS

Input Voltage:
Cat. No. 53051: 100V - 120V @ 50/60 Hz
Cat. No. 53052: 220V - 240V @ 50/60 Hz

Adjustable Amplitude: 20% - 100%

Programmable Timer: 1 sec - 10 hrs

Generator Dimensions:
8.0" W x 13.75" L x 5.75" H (203 mm x 349 mm x 146 mm) 7.0 lbs. (2.2 Kg)

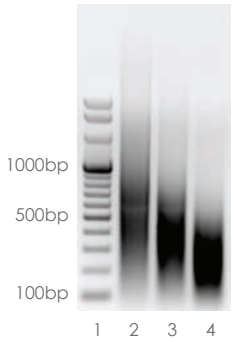
Sample size (probe dependent):
200 µl - 50 ml
(500 µl - 15 ml with included 1/8" probe)

Adjustable Pulse: 1 - 59 seconds

Frequency: 20 kHz; Power: 120 watts

ORDERING INFORMATION

Product	Format	Cat. No.
EpiShear™ Probe Sonicator	110V	53051
	230V	53052



▲ FIGURE: Gel analysis of sheared HeLa chromatin.
Three samples of chromatin were prepared from HeLa cells for ChIP analysis using the EpiShear Probe Sonicator (1/8" probe) with the ChIP-IT® Express Kit (Catalog No. 53008) components and protocol using 5, 10 and 20 pulses at 25% amplitude. Each pulse consisted of a 20-second sonication followed by a 30-second rest on ice to prevent heat build up. The samples were then run on a 1% agarose gel.

PROTEIN G BEADS & READY-TO-ChIP AGAROSE COLUMNS

For researchers who routinely perform ChIP with their own optimized protocols, Active Motif is making it faster & easier to obtain consistency in chromatin preparation and improve the quality of your ChIP reactions with the use of our low background Protein G Magnetic Beads or Agarose Columns and our ChIP-IT® Fixation Buffer.

Magnetic pull-down for faster, more efficient processing

Active Motif offers the ChIP-IT® Protein G Magnetic Beads, the same Protein G magnetic beads that are used in our best-selling ChIP-IT® Express Kits (page 8) to streamline the protocol and enable ChIP to be performed in just 1 day. Because the beads are pre-blocked and there is no resin loss, the use of magnetic beads allows for rapid processing and improved efficiency of your ChIP procedure, giving you the advantage of:

- ✓ Fewer steps
- ✓ Minimal sample loss
- ✓ High specificity
- ✓ Less background
- ✓ Validated use for various applications including ChIP & Co-IP

▲ Protein G Agarose Column

Improve the reproducibility of your optimized ChIP protocol

Active Motif's Protein G Agarose Columns contain the same high-affinity Protein G agarose beads used in our highly popular ChIP-IT High Sensitivity® Kit (page 12). The beads have been specifically engineered to bind IgG with high affinity and to eliminate non-specific binding. The beads offer many advantages for ChIP over traditional agarose beads including:

- ✓ High binding capacity: 10 µg IgG / µl bead
- ✓ Better reproducibility
- ✓ No sample loss
- ✓ Filtration method is faster & easier than centrifugation
- ✓ Adaptable to various applications

ORDERING INFORMATION

Product	Format	Cat. No.
Protein G Agarose Columns	30 rxns	53039
	5 rxns	53037
ChIP-IT® Protein G Magnetic Beads	40 rxns (1 ml)	53033
	200 rxns (5 ml)	53034
ChIP-IT® Fixation Buffer	3 ml	53038

LOW CELL CHIP-SEQ KIT

Complete ChIP-Seq workflow from limited cell numbers

Active Motif's Low Cell ChIP-Seq Kit provides a complete ChIP-Seq workflow for chromatin preparation, immunoprecipitation, and next generation sequencing library preparation from limited amounts of cells or small tissue biopsies. The Low Cell ChIP-Seq Kit not only reduces sample input requirements, but it also resolves issues often associated with low cell ChIP, including poor signal-to-noise ratios, inefficient library amplification, and high duplication rates.

- ✓ Reproducible ChIP-Seq data from as little as 1,000 to 50,000 cells, depending on protein abundance
- ✓ Includes reagents for chromatin preparation, immunoprecipitation and the Next Gen DNA Library Kit (page 16)
- ✓ Maximize unique data by using Molecular Identifiers (MIDs)
- ✓ Better peak calling from improved signal-to-noise ratios

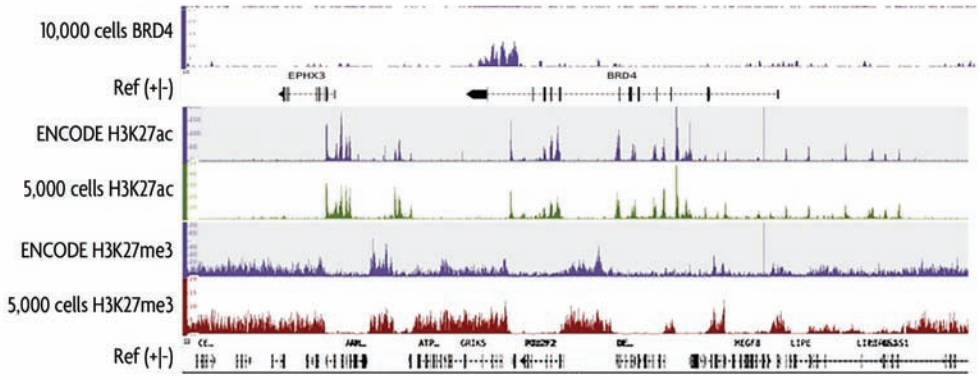


FIGURE 1: Low Cell ChIP-Seq peaks compared to ENCODE data sets.

ChIP-Seq data shows peaks from BRD4 (10,000 cells), H3K27ac (5,000 cells) and H3K27me3 (5,000 cells) are high quality and comparable to ENCODE data sets using 20 million cells. Libraries were constructed with the Next Gen DNA Library Kit (page 16) reagents included in teh Low Cell ChIP-Seq Kit.

FIGURE 2: MID-containing Low Cell ChIP-Seq Library Molecules.

ChIP-Seq libraries generated with the Low Cell ChIP-Seq Kit contain sample indices (indicated in dark blue) for sample multiplexing during sequencing, and MIDs (indicated in teal) for identification of unique library molecules. MIDs enable users to save more of their sequencing data by retaining molecules that would otherwise be recognized as duplicates and be discarded.

ORDERING INFORMATION

Product	Format	Cat. No.
Low Cell ChIP-Seq Kit	16 rxns	53084
ChIP Buffer	50 ml	37516
Blocking Reagent AM1	0.1 ml	37496
BSA (10 mg/ml)	0.1 ml	37497
Blocker	0.1 ml	37498
Protein G Agarose Beads	1.2 ml	37499
TE, pH 8.0	35 ml	37515

ChIP-IT HIGH SENSITIVITY®

Most sensitive transcription factor ChIP Kit available

The ChIP-IT High Sensitivity® Kit's specially formulated buffers enable highly efficient enrichment from low abundance targets, such as transcription factors, or from limited sample amounts or low affinity antibodies. During the immunoprecipitation reaction, low-background Protein G agarose beads and an antibody blocker reduce non-specific binding. Specialized ChIP buffers are designed to enhance enrichment and reduce the presence of non-specific DNA. In addition, ChIP filtration columns are used for faster, easier and more consistent capture and wash steps. The result is a kit that is optimized to deliver both higher signals and lower background when compared to other commercially available ChIP kits (Figure 1).

- ✓ Perform ChIP reactions on low abundance transcription factors (TFs), with limited sample material or using low-affinity antibodies (Figure 2)
- ✓ Enrich DNA from as little as 1,000 cells per IP reaction
- ✓ Validated across multiple sample types
- ✓ Proven performance in ChIP-Seq, ChIP-chip and qPCR

For more information, visit www.activemotif.com/chipiths.

TECHNICAL SPECS

Recommended for use with:
- low abundance targets (e.g. TFs)
- limited sample material
- low affinity antibodies

Cell requirements (# cells / ChIP rxn):
- high abundance target: ≥ 1K cells
- low abundance target: ≥ 50K cells

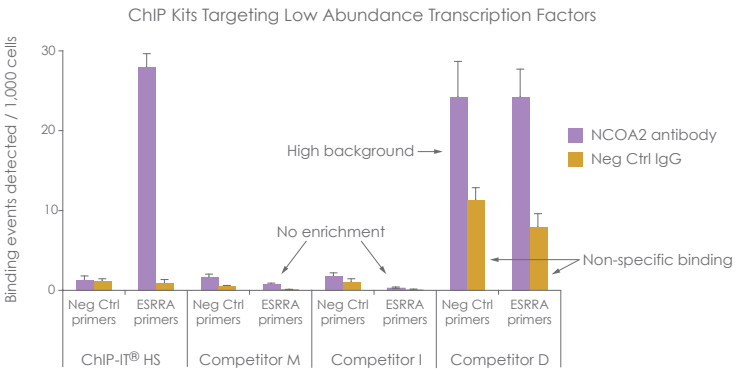
Recommended # cells for chromatin preparation: 100K cells

Capture method: Column filtration

IP method: Protein G agarose beads

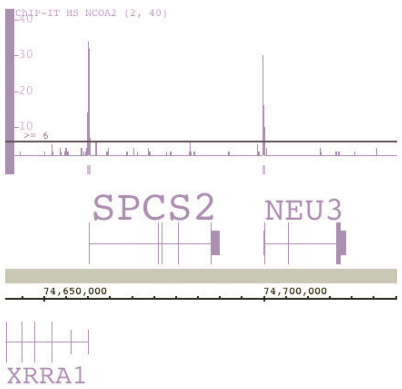
Procedure length: 2 - 3 days

Compatible downstream applications:
- qPCR
- ChIP-chip
- ChIP-Seq



▲ FIGURE 1: ChIP-IT High Sensitivity shows better enrichment than competitor ChIP Kits.

A comparison of ChIP kits targeting a low abundance transcription factor was performed using an antibody for the low abundance NCOA2 protein and a negative control IgG. Following enrichment, qPCR was performed using the ChIP-IT® qPCR Analysis Kit (Catalog No. 53029) in order to allow stringent normalization of the data for direct comparison of the results.



▲ FIGURE 2: ChIP-Seq data showing peaks from transcriptional co-factor NCOA2-enriched DNA generated using ChIP-IT High Sensitivity.

ChIP-Seq data showing peaks from transcriptional cofactor NCOA2 in the promoters of two genes. ChIP was performed using the ChIP-IT High Sensitivity Kit, confirmed with the ChIP-IT qPCR Analysis Kit and followed by sequencing on the Illumina Next-generation sequencing platform.

ORDERING INFORMATION

Product	Format	Cat. No.
ChIP-IT High Sensitivity® Kit	16 rxns	53040
High Sensitivity Chromatin Preparation Kit	16 rxns	53046
ChIP-IT® qPCR Analysis Kit	10 rxns	53029

ChIP-IT® EXPRESS

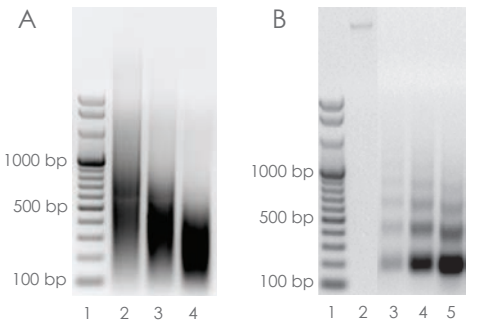
Single day optimized ChIP with ChIP-IT® Express

Active Motif's ChIP-IT Express Kits use ChIP-IT® Protein G Magnetic Beads (page 7) that reduce processing time with fewer steps and reagents, making it possible to perform ChIP in just 1 day (Figure 1, opposite page). Kits that use either sonication or enzymatic chromatin shearing protocols are available. Both kits offer the following advantages:

- ✓ **Complete Solution** – includes all necessary components for performing ChIP
- ✓ **Results in just 1 day** – 4-hour IP, easy-to-follow streamlined protocol
- ✓ **Convenient** – procedure is already optimized for superior results
- ✓ **Sensitive** – as few as 100,000 cells needed per ChIP reaction

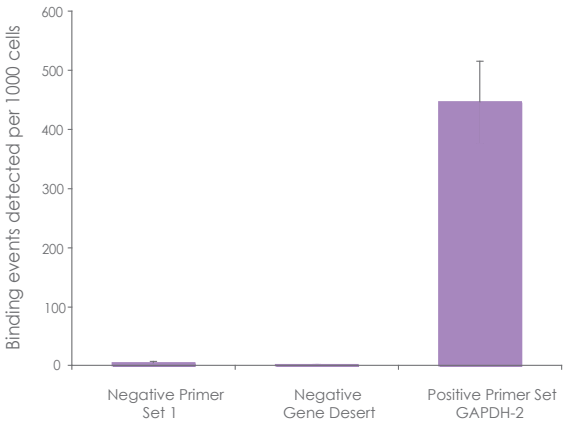
For complete details, please visit www.activemotif.com/chipitexpress.

▲ ChIP-IT Express ChIP Kit



◀ FIGURE 1: Comparison of DNA fragmentation results from the ChIP-IT Express Sonication and Enzymatic shearing procedures.

Sheared chromatin prepared using either (A) the ChIP-IT Express Shearing Kit and the EpiShear™ Probe Sonicator or (B) the ChIP-IT Express Enzymatic Shearing Kit.



◀ FIGURE 2: H3K4me3 enrichment at GAPDH promoters, but not gene deserts.

The ChIP-IT Express Kit was used to perform ChIP using MCF-7 chromatin and Histone H3K4me3 antibody qPCR analysis of enriched DNA using Negative and Positive Control Primers, and primers specific to a gene desert show enrichment of H3K4me3 at GAPDH promoters, but not at gene deserts, as expected.

TECHNICAL SPECS

Available versions*:
- Enzymatic (ChIP-IT® Express Enzymatic)
- Sonication (ChIP-IT® Express)

Recommended for use with:
- high abundance proteins
- ChIP validated antibodies

Sufficient reagents for:
- 10 chromatin preparations
- 2 shearing optimizations
- 25 ChIP reactions

Cell requirements: ≥100K cells / ChIP rxn

Capture method: Magnetic

IP method: Protein G Magnetic Beads

Procedure length: 4 hours

*Protein G Magnetic Beads are also sold separately (*see Ordering Information on page 6*).

ORDERING INFORMATION

Product	Format	Cat. No.
ChIP-IT® Express Kit	25 rxns	53008
ChIP-IT® Express Enzymatic Kit	25 rxns	53009

TAG-ChIP-IT®

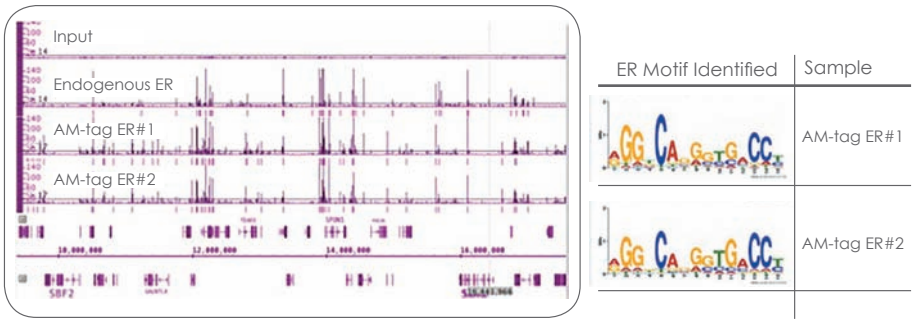
Perform ChIP without protein-specific antibodies

Active Motif's Tag-ChIP-IT® Kit addresses a commonly encountered issue when considering ChIP, the lack of available ChIP-grade antibodies to a target of interest. A common solution to this issue is to use an epitope tag, such as FLAG, HA, and GFP, to tag the protein of interest. However, since these tags are not specifically designed for ChIP, they have a high and unpredictable failure rate. Tag-ChIP-IT is the first epitope-tagging procedure that is specifically optimized for ChIP.

How does it work?

Tag-ChIP-IT utilizes a unique AM-tag (patent pending) designed specifically for ChIP consisting of a small, unstructured sequence that can be attached to any protein of interest. Furthermore, we have designed a high specificity antibody to the tag for maximum pull-down efficiency during immunoprecipitation (IP). Combined with optimized reagents and a streamlined protocol, this unique ChIP method enables researchers the ability to perform ChIP on any target without the need for target-specific antibodies. For more complete information, please visit us at www.activemotif.com/tagchip.

- ✓ Ideal for targets lacking ChIP-qualified antibodies
- ✓ Improved sensitivity & specificity over FLAG, GFP and HA tags
- ✓ Negligible cross-reactivity with mammalian systems
- ✓ Study isoforms, mutations & truncations with ease



▲ FIGURE: Tag-ChIP-IT identifies same estrogen receptor (ER) binding motifs as published ChIP-Seq data.
ER cDNA was cloned into pAM_1C Empty Vector and transiently transfected into cells. Cells were induced with estradiol and ChIP was performed on harvested chromatin using the Tag-ChIP-IT Kit. Enriched DNA was submitted for Next-generation sequencing. Data was compared to published ChIP-Seq results from ER antibody enrichment performed under the same 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. Results show the ER motif was identified in both Tag-ChIP-IT samples.

WHAT'S IN THE BOX?

The Tag-ChIP-IT Kit contains all the buffers and reagents necessary for ChIP analysis using the AM-tag. The pAM_1C Empty Vector **is available separately** and is used 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 is used to isolate chromatin and perform IPs using AM-Tag antibody specific for the AM-tag.

ORDERING INFORMATION

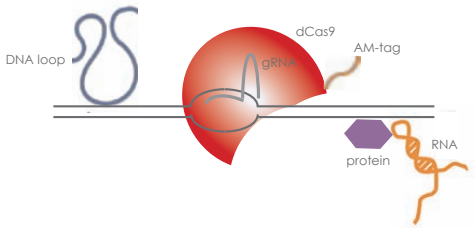
Product	Format	Cat. No.
Tag-ChIP-IT® Kit	16 rxns	53022
pAM_1C Empty Vector	20 µg	53023
FuGENE® HD Transfection Reagent	0.2 ml	32042
	0.5 ml	32043

enChIP

Determine CRISPR/Cas9 specificity with enChIP

To enable you to assess Cas9 specificity, Active Motif's enChIP Kit (Engineered DNA-binding molecule-mediated Chromatin Immunoprecipitation) offers a modified ChIP assay that utilizes the CRISPR/Cas9 system to target a specific DNA locus for immunoprecipitation (Figure 1). Using the enChIP Kit for your genome editing experiments enables you to biologically validate each target sequence for specificity. Sequences demonstrating off-target effects can be excluded, saving valuable time and resources.

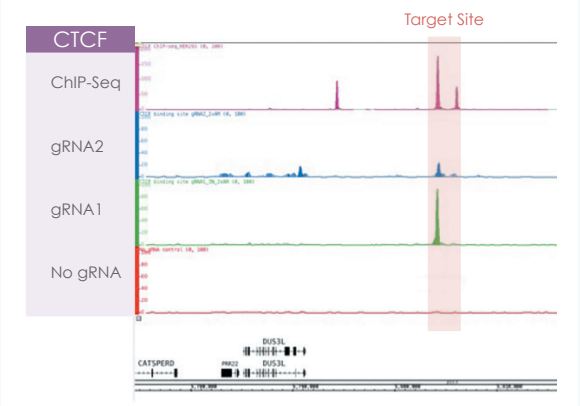
- ✓ Identify off-target binding events for each gRNA sequence
- ✓ Evaluate *cis*- and *trans*-interacting chromosomal looping events
- ✓ ChIP-optimized AM-tag design ensures highly efficient dCas9 enrichment
- ✓ Works well with open chromatin regions to detect promoter, enhancer and insulator elements



▲ FIGURE 1: Illustration of enChIP design.

How does it work?

A guide RNA (gRNA) containing 20 nucleotides complementary to the desired genomic region is expressed in combination with an enzymatically inactive Cas9 protein (dCas9) tagged with an AM-tag that is specifically designed for ChIP. The gRNA directs dCas9 to the target sequence and an RNA-DNA heteroduplex is formed. Chromatin immunoprecipitation is then performed using an antibody directed against the dCas9 AM-tag to enrich for genomic sequences bound by the gRNA/dCas9 complex. By using the enChIP Kit, off-target gRNA binding sites can be identified (Figure 2). This provides valuable information regarding the quality of gRNA design prior to use in genome editing experiments. Additionally, the enChIP Kit can be used to study *cis*- and *trans*-interacting chromosomal looping events. For more complete information, please visit us at www.activemotif.com/enchip.



▲ FIGURE 2: enChIP validates specificity of gRNA for a CTCF binding site.
Two gRNAs were designed targeting different 20 nt sequences within a 500 bp region of a CTCF binding site on chromosome 19. Each gRNA sequence was cloned into the pAM_dCas9 vector and transfected into HEK293T cells. A 'No gRNA' negative control was also performed. Chromatin was prepared and immunoprecipitated according to the enChIP Kit. Enriched DNA was analyzed by Next-generation sequencing and background was subtracted using the 'No gRNA' control. Data was compared to ChIP-Seq data for CTCF in the same cell line. Results show gRNA1 had a strong peak and specific binding at the target location. Data for gRNA2 revealed a large number of off-target binding events outside of the target location. This confirms that enChIP provides biological validation for CRISPR/Cas9 gRNA specificity.

ORDERING INFORMATION

Product	Format	Cat. No.
enChIP Kit	16 rxns	53125
pAM_gRNA Vector	10 µg	53121
pAM_dCas9 Vector	10 µg	53122
pAM_gRNA_CTCF Vector (Positive control)	10 µg	53123
pAM_dCas9_CTCF Vector (Positive control)	10 µg	53124

ChIP-IT® ChIP-SEQ

ChIP for Next-generation sequencing

The combination of ChIP with genome-wide analysis using Next-generation sequencing (ChIP-Seq) is a powerful approach that can provide insights into gene regulation, the impact of chromatin modifications on gene expression and signaling pathway mechanisms. Active Motif's ChIP-IT® ChIP-Seq Kit is designed to provide the highest quality ChIP-enriched DNA for use in sequencing on Illumina® Genome Analyzer II, HiSeq and MiSeq Next-generation sequencing systems.

ChIP-IT ChIP-Seq is robust enough for use with challenging antibodies that do not yield signal with other ChIP methods and sensitive enough to detect binding of low abundance transcription factors. The increased sensitivity is due to optimized ChIP buffers which reduce the presence of non-specific DNA, resulting in lower background and better enrichment. Better enrichment translates into larger ChIP-Seq peaks and more accurate peak calling, since a greater percentage of the sequence reads are mapped to binding sites rather than background DNA regions. To learn more, please visit us at www.activemotif.com/chipitseq.

TECHNICAL SPECS

Recommended for use with:
- low abundance targets (*e.g.* TFs)
- low affinity antibodies

Sufficient reagents for:
- 16 chromatin preparations
- 16 chromatin IPs
- 10 sequencing libraries

Cell requirements: ≥ 1.5M cells / ChIP rxn

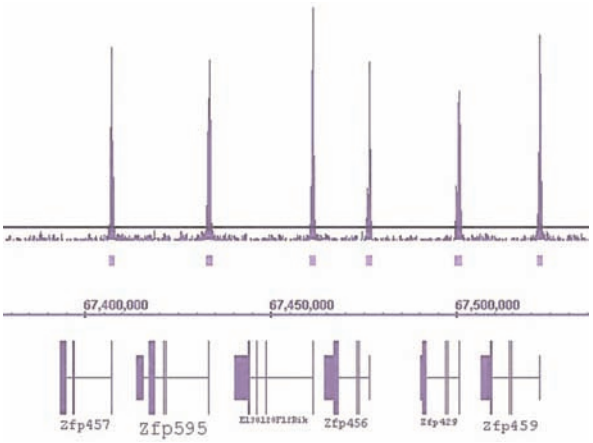
Recommended # cells for chromatin preparation: ≥ 4.5M cells

Capture method: Column filtration

IP method: Protein G agarose beads

Procedure length: 2 - 3 days

Compatible downstream applications:
- qPCR
- ChIP-chip
- ChIP-Seq



▲ FIGURE: ChIP-IT ChIP-Seq reveals that H3K4me3 peaks are present at the start site of all *Zfp* genes.

ChIP-IT ChIP-Seq was performed using a Histone H3K4me3 pAb (Catalog No. 39159) on mouse livers. The data shows the presence of H3K4me3 at the transcription start sites of several *Zfp* genes.

WHAT'S IN THE BOX?

The ChIP-IT ChIP-Seq Kit contains the same robust chromatin IP components of the ChIP-IT High Sensitivity® Kit (see opposite page) along with other components necessary for preparation of samples for ChIP-Seq analysis. Included in the ChIP-IT ChIP-Seq Kit are:

- Optimized protocol & buffers to perform ChIP from fresh or frozen cells or tissues
- DNA purification reagents for clean-up of ChIP-enriched DNA
- ChIP-IT® qPCR Analysis Kit for the verification of quality of the DNA prior to sequencing
- Library construction reagents and protocol for the preparation of 10 NGS libraries

ORDERING INFORMATION

Product	Format	Cat. No.
ChIP-IT® ChIP-Seq Kit	10 libraries	53041

ChIP KITS FOR DISEASE RESEARCH

For disease research, Active Motif provides the ChIP-IT® FFPE and ChIP-IT® PBMC assays to finally enable researchers to perform ChIP analysis of primary tissue samples. Performing ChIP on these sample types had been previously unsuccessful due to the difficulty in extracting high-quality chromatin from difficult-to-lyse, very limited, or degraded material.

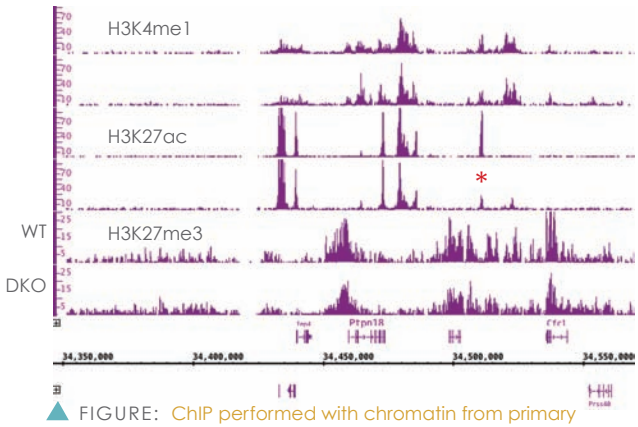
ChIP-IT® FFPE for ChIP analysis of FFPE archived tissue samples

Active Motif's ChIP-IT FFPE assay is the first-of-its-kind to enable extraction and ChIP analysis of chromatin from FFPE samples for Next-generation sequencing, enabling researchers to uncover epigenetic variances in archived tissue. The assay comes in *two* modules:

- ChIP-IT® FFPE Chromatin Preparation Kit – extract high-quality ChIP-grade chromatin from preserved human, mouse or rat FFPE material. The kit has been successfully used to extract chromatin from FFPE blocks stored over 10 years under suboptimal conditions.
- ChIP-IT® FFPE Kit – perform ChIP using the only ChIP kit available that can enrich for ChIP DNA using FFPE-extracted chromatin. The assay produces ChIP-enriched DNA in sufficient amounts and quality for analysis by qPCR or Next-generation sequencing.

ChIP-IT® PBMC for ChIP analysis of difficult-to-lyse T and B cells

Active Motif's ChIP-IT PBMC Kit is the only ChIP kit available to enable ChIP on difficult-to-lyse peripheral blood mononuclear cells, or PBMCs, including lymphocytes (T cells, B cells & NK cells) and monocytes. PBMCs are highly resistant to lysis under conditions normally suitable for other cell types. We have developed an optimized chromatin extraction method that yields high-quality chromatin from primary cells for ChIP analysis and Next-generation sequencing. ChIP reagents have also been optimized to enable enrichment of DNA from nanogram quantities of chromatin and still produce robust signal and minimal background.



▲ FIGURE: ChIP performed with chromatin from primary mouse T cells using the ChIP-IT PBMC Kit.

Epigenetic profiling was performed in a mouse model harboring a knockout (DKO) of a lymphoma related gene. Within the region of the genome shown, there is a clear loss of the enhancer associated H3K27ac modification at a location upstream of the *Cfc1* gene (red asterisk*).

ORDERING INFORMATION

Product	Format	Cat. No.
ChIP-IT® FFPE Chromatin Preparation Kit	5 rxns	53030
ChIP-IT® FFPE Kit	16 rxns	53045
ChIP-IT® PBMC Kit	16 rxns	53042

SPECIALIZED ChIP KITS & ACCESSORIES

Active Motif includes a variety of ChIP Kits for specialized applications along with ChIP accessory products in our vast portfolio of ChIP-related products and services. As the *ChIP Experts*™, we are continually striving to develop and provide new and innovative ChIP products and services to meet our customer's needs.

Specialized ChIP Kits

- Re-ChIP-IT® – perform sequential ChIP to simultaneously assay two unique binding events at the same genomic locus.
- RNA ChIP-IT® – recover immunoprecipitated RNA for RT-PCR analysis to study RNA-protein interactions in a chromatin context.
- ChIP-Bis-Seq (ChIP-Bisulfite-Sequencing) – provides single nucleotide resolution DNA methylation profiling by combining ChIP with bisulfite conversion and sequencing (Bis-Seq).

ChIP Accessory Products

- ChIP Control qPCR Primer Sets – large variety of qPCR primer sets for use as positive and negative controls for the more common ChIP targets. For a complete list of available products and ordering information, please visit www.activemotif.com/qpcr.
- ChIP-IT® Control Kits – positive and negative control antibodies, a Bridging Antibody and a positive control PCR primer set to assess your ChIP procedure by endpoint PCR.
- ChIP-IT® Control qPCR Kits – positive control RNA pol II antibody, a Bridging Antibody to enhance the binding affinity of mouse monoclonal antibodies, a negative control IgG antibody, and species-specific positive and negative control qPCR primers.
- ChIP-IT® qPCR Analysis Kit – includes a standard curve DNA and primer pair, human and mouse positive and negative control qPCR primer sets and an analysis spreadsheet to simplify qPCR data analysis and assess the quality of ChIP-enriched DNA.
- Ready-to-ChIP Chromatin – offered from a number of ENCODE cell lines that have been optimally sheared by sonication and validated in ChIP as a control or test sample.
- Bridging Antibody – to enhance the binding affinity of mouse monoclonal antibodies.
- Dounce Homogenizer – available in two sizes for preparation of cell lysates or chromatin.

ChIP DNA Purification Kit

Active Motif's ChIP DNA Purification Kit is specifically formulated to remove common contaminants, such as reverse cross-linking reagents, Proteinase K or other digestive enzymes, detergents and salts from eluted ChIP DNA samples. The kit enables efficient and rapid preparation of ChIP DNA for analysis without the need for messy, labor intensive and time-consuming phenol/chloroform extraction. Our buffers and columns are specifically optimized for purification of ChIP DNA to produce the highest quality DNA for use in downstream chromatin IP and epigenetics applications.

▲ FIGURE: The DNA Purification Binding Buffer has a pH indicator dye so the pH of the solution can be easily determined.

- ✓ Specifically formulated for ChIP DNA clean-up
- ✓ 85-100% recovery of purified DNA
- ✓ Start with as few as 10,000 cells
- ✓ Enables recovery of DNA fragments as small as 50 bp
- ✓ Proven compatibility with ChIP and methylated DNA enrichment kits & protocols

ORDERING INFORMATION

Product	Format	Cat. No.
Re-ChIP-IT® Kit	25 rxns	53016
RNA ChIP-IT® Kit	25 rxns	53024
RNA ChIP-IT® Control Kit – Human	5 rxns	53025
ChIP-Bis-Seq Kit	10 libraries	53048
ChIP-IT® Control Kit – Human	5 rxns	53010
ChIP-IT® Control Kit – Mouse	5 rxns	53011
ChIP-IT® Control Kit – Rat	5 rxns	53012
ChIP-IT® Control qPCR Kit – Human	5 rxns	53026
ChIP-IT® Control qPCR Kit – Mouse	5 rxns	53027
ChIP-IT® Control qPCR Kit – Rat	5 rxns	53028
ChIP-IT® qPCR Analysis Kit	10 rxns	53029
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
Ready-to-ChIP Hep G2 Chromatin	10 rxns	53019
Ready-to-ChIP K-562 Chromatin	10 rxns	53020
Ready-to-ChIP NIH/3T3 Chromatin	10 rxns	53021
Bridging Antibody for Mouse IgG	500 µg	53017
Dounce Homogenizer	1 ml	40401
	15 ml	40415
ChIP DNA Purification Kit	50 rxns	58002

NEXT GEN DNA LIBRARY KIT

High complexity libraries from low cell inputs

The Next Gen DNA Library Kit is designed to prepare Next Generation Sequencing (NGS) libraries from ultra low DNA inputs, for example from ChIP samples, FFPE samples or cfDNA samples. High efficiency library preparation, combined with molecular identifiers (MIDs), maximizes the amount of unique data obtained from limiting samples.

- ✓ Minimize adapter dimer formation, even with just 10 pg input
- ✓ Maximize unique data using MIDs
- ✓ Multiplex up to 16 samples for efficient sequencing
- ✓ Compatible with Illumina sequencing platforms

HOW IT WORKS

Repair steps maximize available DNA.

In tube wash steps reduce sample loss.

Individual adapter attachment reduces adapter dimers.

Optional PCR Step allows for library amplification, if necessary.

TECHNICAL SPECS

Recommended for use with:
- ChIP DNA
- FFPE DNA
- cfDNA
- limited gDNA samples

Input:
- 100 ng PCR-free
- 10 pg with PCR

Procedure length: 2 - 3 hours

Downstream Applications:
- ChIP-Seq
- WGS
- Targeted sequencing

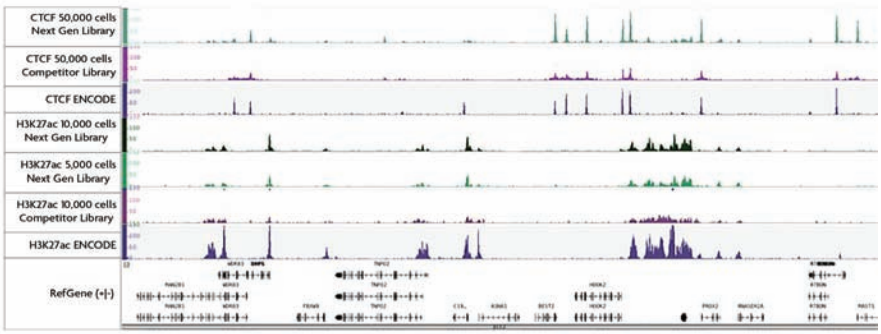


FIGURE 1: High quality ChIP-Seq libraries, even with lower cell numbers.

Active Motif's Low Cell ChIP-Seq protocol was used to prepare chromatin from GM12878 cells using the number of cell equivalents listed. ChIP-Seq libraries were prepared using either the Next Gen DNA Library Kit or a competitor kit. Results show strong peaks that match ENCODE data sets for the Next Gen Libraries, even if starting from low cell numbers.

ORDERING INFORMATION

Product	Format	Cat. No.
Next Gen DNA Library Kit	16 rxns	53216
Next Gen Indexing Kit	64 rxns	53264

ChIP NORMALIZATION

Correct for unwanted variation to reveal true 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 technical variation can lead to results that are difficult to interpret. To overcome this challenge, Active Motif has developed a ChIP Normalization spike-in strategy for normalization of ChIP qPCR and ChIP-Seq data to reduce the effects of technical variation and sample processing bias. This strategy can be applied across samples and antibodies to eliminate bias and reveal true biological variance induced by causal agents such as inhibitory compounds, mutations, or disease.

- ✓ Reduce technical variation and sample processing bias
- ✓ Detect latent or subtle biology not observed with standard ChIP analysis
- ✓ Can be applied across different antibodies and samples without bias
- ✓ Works with both qPCR and ChIP-Seq analysis
- ✓ Can be applied to any ChIP protocol

APPLICATION NOTES

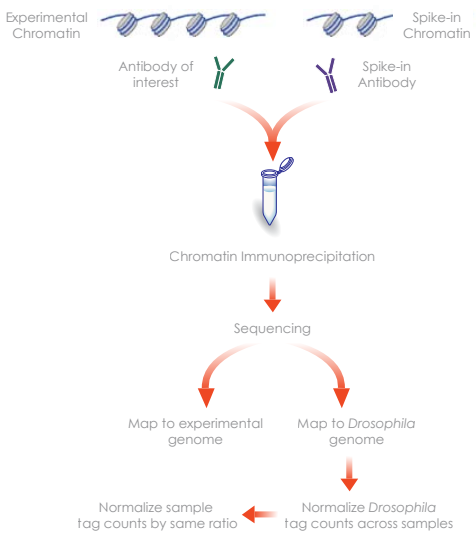
ChIP normalization can readily be implemented simply by integrating our spike-in reagents into your standard ChIP protocol as follows:

- 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.
- Spike-in Antibody that recognizes the *Drosophila*-specific histone variant, H2Av, is added to the reaction as a mechanism to reliably pull down the *Drosophila* chromatin fraction across samples.

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.

For more complete details, visit us at www.activemotif.com/spikein.

ChIP-Seq Normalization Workflow



ORDERING INFORMATION

Product	Format	Cat. No.
Spike-in Antibody	50 µg	61686
Spike-in Chromatin	15 rxns	53083
Drosophila Positive Control Primer Set Pbgs	96 rxns	71037
Drosophila Negative Control Primer Set 1	96 rxns	71028

CHROMATIN ASSEMBLY

Active Motif's Chromatin Assembly kits & reagents provide a complete and simple solution for chromatin reconstitution to enable the generation of substrates that closely mimic native chromatin for use in downstream assays. For more, visit www.activemotif.com/chromassembly.

NUCLEOSOME ASSEMBLY

Our Nucleosome Preparation Kit is designed to prepare mono- and oligonucleosomes from cells. Use to study histones and histone modifications, enzyme kinetics, or for inhibitor screening.

CHROMATIN ASSEMBLY KIT

Enables *in vitro* assembly of an extended array of ordered nucleosomes from supercoiled and linear DNA. The resulting chromatin closely resembles native *in vivo* chromatin to enable studies of histone modifications and associated proteins regulating the target DNA sequence. Assembled chromatin is ready to use in downstream assays such as *in vitro* transcription, ChIP and histone acetyltransferase (HAT) assays.

NUCLEOSOME ASSEMBLY CONTROL DNA

187 bp ds control DNA contains the well-characterized 601 sequence. Use as a control to validate the proper assembly of your *in vitro* chromatin assembly reactions or as a template to monitor assembly kinetics.

RECOMBINANT NUCLEOSOMES

Pre-assembled human nucleosomes comprised of octamers of core histone proteins (H2A, H2B, H3 & H4) bound by DNA for use in drug discovery screening (pg 44).

ORDERING INFORMATION

Product	Format	Cat. No.
Chromatin Assembly Kit	10 rxns	53500
Nucleosome Preparation Kit	20 rxns	53504
Nucleosome Assembly Control DNA	50 µg	53502

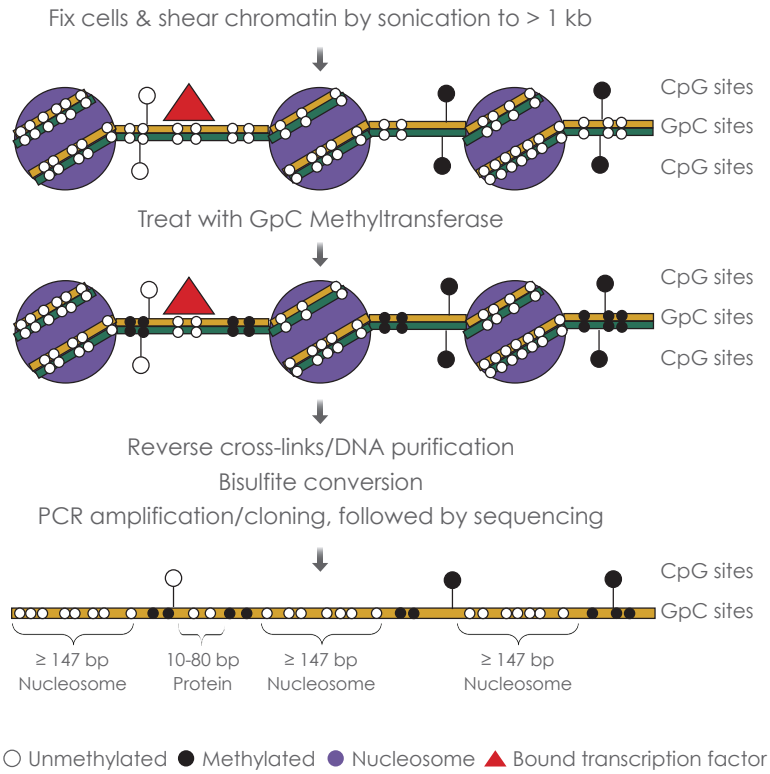
NOME-SEQ

Simultaneously analyze nucleosome occupancy & DNA methylation profiles

Active Motif's NOMe-Seq (Nucleosome Occupancy and Methylome Sequencing) Kit is the first commercially available assay to enable the high-resolution analysis of both nucleosome positioning and CpG methylation on the same DNA strand. Currently used methods can only determine either nucleosome occupancy or DNA methylation state at a specific locus. In contrast, NOMe-Seq is capable of providing genome-wide information about *both* epigenetic states simultaneously. Key features & advantages include:

- ✓ Single-molecule resolution analysis of nucleosome occupancy & CpG methylation
- ✓ Determine spatiotemporal correlation between nucleosome positioning and DNA methylation
- ✓ Highly sensitive assay detects even subtle changes in nucleosome position
- ✓ Lacks nucleosome occupancy bias observed with nuclease digestion techniques
- ✓ Chromatin fixation provides information regarding transcription factor binding sites

NOMe-Seq Workflow



NOMe-Seq is listed as one of the Top Ten Innovations 2013 by The Scientist.

TECHNICAL SPECS

Input: 750,000 cell equivalents
Conversion efficiency: ≥ 99%
DNA recovery: ≥ 80%
Format: Spin column
Elution volume: 50 µl
Procedure length: - GpC methyltransferase: 4 hours - Bisulfite conversion: 5 hours

ORDERING INFORMATION

Product	Format	Cat. No.
NOMe-Seq	10 rxns	54000

CELL FREE DNA PURIFICATION KIT

Isolate cell-free DNA from human plasma or serum.

Active Motif's Cell-Free DNA (cfDNA) Purification Kit is designed to isolate cell-free DNA from human plasma and serum samples. Cell-free DNA can serve as a valuable source of information for non-invasive detection and monitoring of disease states, however, it is often found in limited amounts in plasma and serum samples. Using Active Motif's fast, magnetic bead-based protocol, it is easy to isolate and concentrate cell-free DNA from fresh or frozen samples.

- ✓ Works with fresh and frozen samples
- ✓ Process input volumes from 100 µl to 10 ml
- ✓ Fast, magnetic bead-based purification is compatible with automation
- ✓ Low elution volumes to concentrate the cell-free DNA
- ✓ Compatible with Streck Cell-Free DNA BCT® Tubes

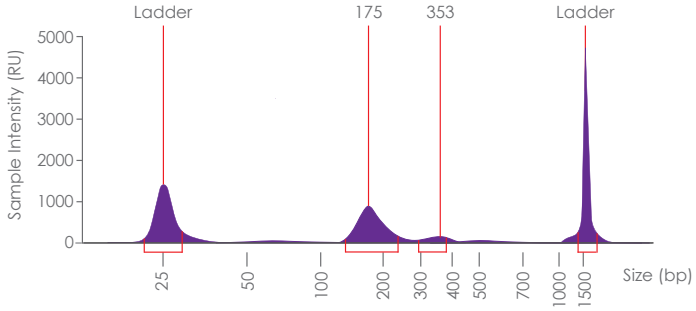


FIGURE 1: TapeStation assessment of the size of isolated cell-free DNA

Cell-free DNA was isolated from 5 ml of healthy human plasma using the Cell-Free DNA (cfDNA) Purification Kit. Isolated DNA was run on a TapeStation to assess the size of the purified DNA. Results show the majority of the purified sample is cell-free DNA at the expected size of 150-200 bp.

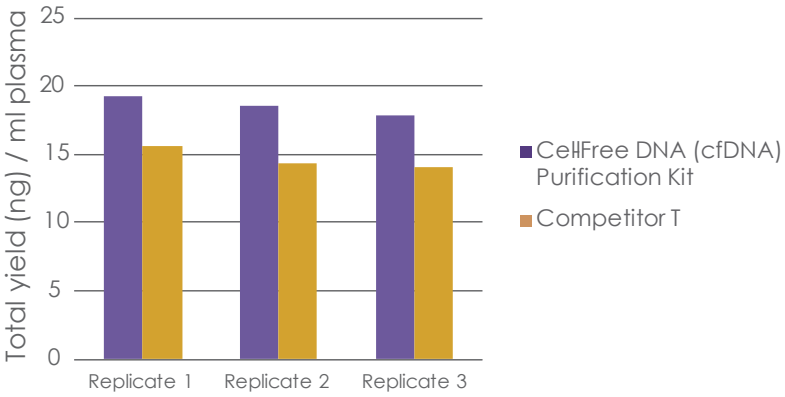


FIGURE 2: Automation compatibility with KingFisher™ Flex Purification System

The Cell-Free DNA (cfDNA) Purification Kit and a competitor magnetic bead-based cfDNA purification Kit (Competitor T) were used to isolate DNA from 600 µl of plasma from a normal human donor in triplicate using the KingFisher Flex Purification System. Results show the total DNA yield (ng) per ml plasma. The Cell-Free DNA (cfDNA) Purification Kit recovered more DNA than the alternative purification method.

ORDERING INFORMATION

Product	Format	Cat. No.
Cell-Free DNA (cfDNA) Purification Kit	100 ml	25503

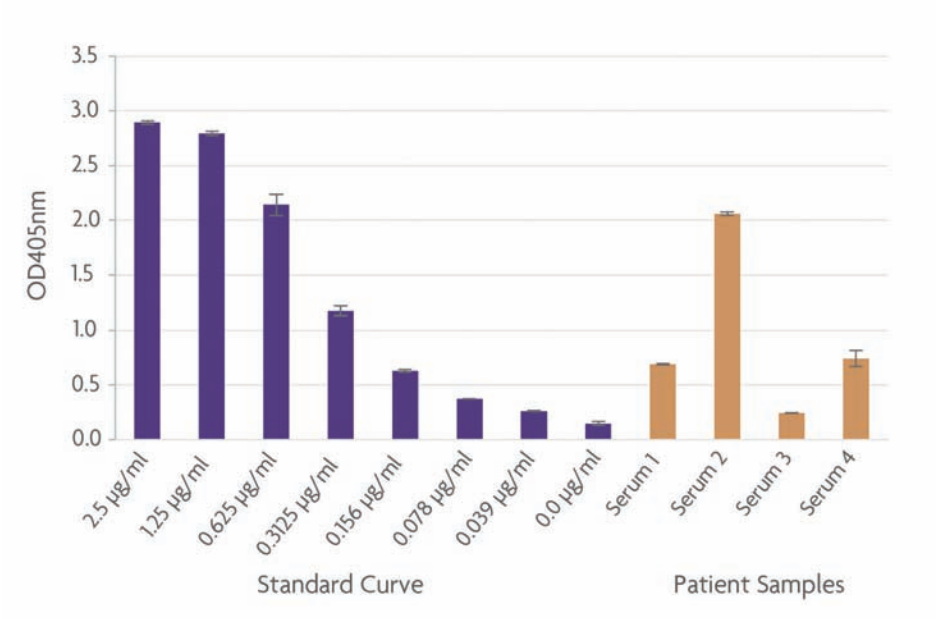
NU.Q™ TOTAL

Determin total levels of circulating cell-free nucleosomes.

Active Motif's Nu.Q ™Total Assay Kit is designed for the detection of global levels of cell-free circulating nucleosomes (cf-nucleosomes) in human serum in a high-throughput format. For added convenience and a more quantitative interpretation of results, the Nu.Q™ Total Assay Kit also include a recombinant nucleosome protein for use as a reference standard curve.

- ✓ **Sensitivity:** Detect circulating nucleosomes in as little as 10 µl of serum
- ✓ **Specificity:** Nucleosome epitope specific antibody enables detection of only intact nucleosomes
- ✓ **Quantification:** Recombinant nucleosomes enable relative quantification of total levels of circulating nucleosomes
- ✓ **Convenience:** Colorimetric assay in a simple 96-stripwell format for high and low throughput
- ✓ **Fast:** Results can be obtained in 5 hours

Active Motif's Nu.Q™ Total Assay Kit is powered by VolitionRx.



◀ FIGURE 1: Total circulating cell-free nucleosome detection.

The Nu.Q™ Total Assay Kit was used to assay human serum samples. The provided Positive Control was assayed from 0.039 - 2.5 µg/ml as a reference standard curve. Data shown are the results from wells assayed in duplicate with a 20 minute developing time. These results are provided for demonstration only.

ORDERING INFORMATION

Product	Format	Cat. No.
Nu.Q™ Total Assay Kit	1 x 96 rxns	53510

DNA METHYLATION

Methods for studying and quantifying DNA methylation, such as methylated DNA enrichment, bisulfite conversion, along with Next-generation sequencing allows researchers to get a better picture of the genome-wide and gene-targeted changes in DNA methylation and their association with disease.
Active Motif offers a number of products and services to simplify and streamline DNA methylation analysis. For more information, visit www.activemotif.com/dna-methylation.

ANTIBODIES & PROTEINS

Comprehensive portfolio of antibodies & proteins for the analysis of 5-mC and other DNA methylation variants (5-hmC, 5-fC, 5-caC & 3-mC).

BISULFITE CONVERSION

Bisulfite conversion assays for the analysis of DNA methylation patterns, genome wide or individual loci at single base-pair resolution

METHYLATED & UN-METHYLATED DNA ENRICHMENT

A wide selection of assays for enrichment of methylated & hypomethylated DNA for both 5-mC 5-hmC.

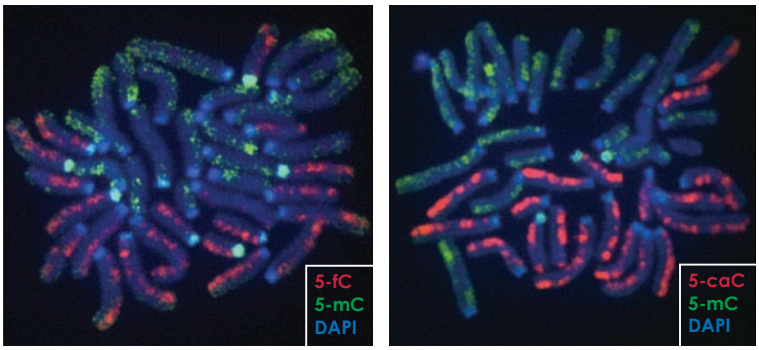
QUANTITATIVE ASSAYS

Simple, high-throughput ELISA-based assays for measuring changes in global DNA methylation levels, hydroxymethylation levels and DNMT activity.

DNA METHYLATION ANTIBODIES

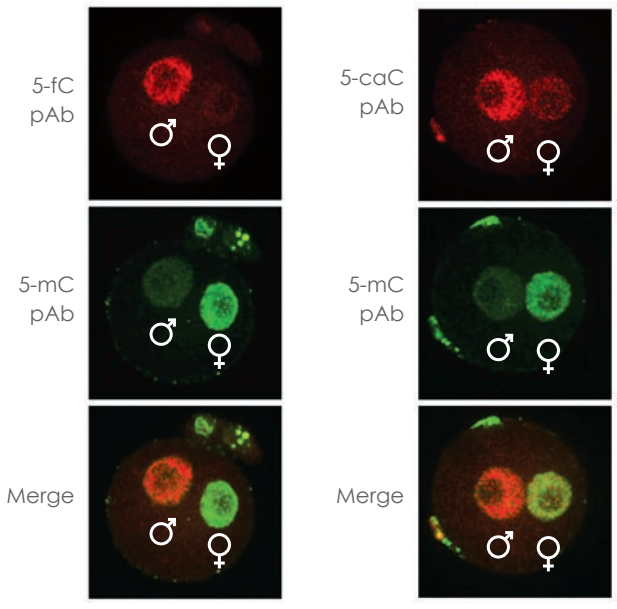
DNA methylation antibodies

Active Motif offers a broad selection of antibodies specific to 5-methylcytosine (5-mC), 5-hydroxymethylcytosine (5-hmC) and other DNA methyl variants for characterization of DNA methylation. These antibodies have been validated for numerous applications and show no cross-reactivity to non-specific methyl variants or non-methylated cytosines.



◀ FIGURE 1: Immunofluorescent images of mitotic chromosome spreads co-stained with Active Motif's DNA methylation antibodies.

5-Formylcytosine (5-fC, left) or 5-Carboxylcytosine (5-caC, right) antibodies (red, Catalog Nos. 61223 and 61225, respectively), a 5-methylcytosine (5-mC) antibody (green) and DAPI (blue) at the two-cell stage of mouse preimplantation development. The images reveal that, at the two-cell stage, only one of the two sister chromatids is enriched for 5-fC and 5-caC, consistent with findings that 5-fC and 5-caC levels are diminished by half in blastomeres with each round of DNA replication (Inoue *et al.**).



▲ FIGURE 2: Representative confocal images of fertilized oocytes co-stained with Active Motif's DNA methylation antibodies.

5-Formylcytosine (5-fC) and 5-Carboxylcytosine (5-caC) antibodies (red, Catalog Nos. 61223 and 61225, respectively), and a 5-methylcytosine (5-mC) antibody (green) (Inoue *et al.**).

* The images above were kindly provided by the laboratory of Yi Zhang, HHMI Investigator at the University of North Carolina at Chapel Hill. The data is described in detail in Inoue *et al.* (2011) *Cell Research* 21(12): 1670-1676.

ANTIBODIES AVAILABLE FOR:

- ✓ 3-mC
- ✓ 5-fC
- ✓ 5-caC
- ✓ 5-hmC
- ✓ 5-mC

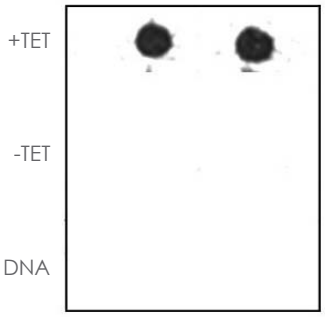
For a complete list of available antibodies and detailed information regarding isotypes, reactivity and validated applications, please visit www.activemotif.com/dnamethabs.

Active Motif also has antibodies specific for proteins that regulate DNA methylation, including DNA methyltransferase proteins (DNMTs), methyl-CpG binding domain (MBD) protein, and Ten-Eleven Translocation-1 (TET) dioxygenases. For a complete list of available DNA methylation related antibodies and detailed information, visit www.activemotif.com/dnamethabs.

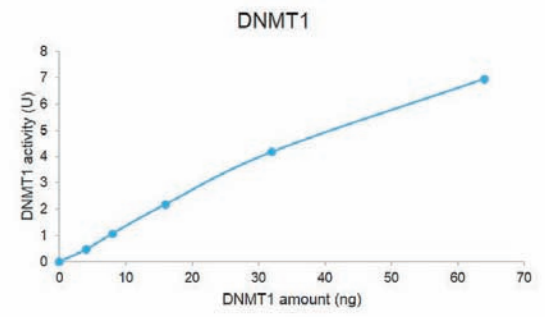
Proteins to study DNA methylation

Active Motif offers DNA modifying and restriction enzymes for DNA methylation analysis, including:

- Recombinant TET proteins – convert 5-mC DNA into 5-hmC DNA for studies of hydroxymethylation (Figure 3). For a list of available proteins, go to www.activemotif.com/tet.
- β-Glucosyltransferase – specifically modifies 5-hmC with the addition of a glucose moiety to distinguish 5-hmC from 5-mC by the use of glucosyl-sensitive restriction enzymes. Alternatively, can be used in combination with a radiolabeled UDP-glucose donor for direct labeling of hydroxymethylated residues.
- PvuRts1I restriction enzyme – distinguishes 5-hmC by directly cleaving 5-hmC DNA in its non-glucosylated form, but will not digest 5-mC or unmethylated cytosine residues. Also cleaves glucosylated 5-hmC DNA, but at a lower efficiency.
- DNA methyltransferase enzymes (DNMTs) – for use in studies of enzyme kinetics or for screening enzyme activity in response to compound treatment (Figure 4).



▲ FIGURE 3: Dot blot of DNA containing 5-mC shows the conversion of 5-mC to 5-hmC by Tet1.
DNA containing 5-methylcytosine that was incubated with 5 µg of recombinant Tet1 enzyme (+TET) or without Tet1 (-TET) .



▲ FIGURE 4: Recombinant DNMT1 protein activity assay.
Recombinant DNMT1 protein activity was measured using the DNMT Activity / Inhibition Assay (pg. 34) for 1 hour at 37°C.

ORDERING INFORMATION

Product	Format	Cat. No.
β-Glucosyltransferase enzyme	50 µl	55012
PvuRts1I restriction enzyme	50 Units	55011
Recombinant DNMT1 protein	20 µg	31404
Recombinant DNMT3A / DNMT3L Complex	20 µg	31415
Recombinant DNMT3B protein	20 µg	31413

ASIA: +81 (0)3 5225 3638 EUROPE: +32 (0)2 653 0001 NORTH AMERICA: 877 222 9543

GLOBAL DNA METHYLATION ASSAYS

Active Motif provides sensitive ELISA-based assays to enable a cost-effective, high-throughput and accurate method for measuring relative changes in global 5-methylcytosine (5-mC) and 5-hydroxymethylcytosine (5-hmC) levels compared to HPLC or other chromatographic methods. For more complete information, please visit us at www.activemotif.com/globalmethylation.

Global DNA Methylation – LINE-1 Kit

The Global DNA Methylation – LINE-1 Kit uses a unique hybridization approach to quantify global 5-mC levels that offers better specificity & reproducibility than methods utilizing non-specific passive adsorption. The assay is uniquely designed to quantitate methylation in Long Interspersed Nucleotide Element 1 (LINE-1) repeats in human genomic DNA, known as a surrogate marker for global DNA methylation. Kit advantages include:

- ✓ Simple ELISA-based assay to quantify global 5-mC levels in only 5 hours
- ✓ Detects 5-mC from as little as 10 ng DNA and as low as 0.5% methylation
- ✓ Unique LINE-1 probe hybridization method offers better specificity than passive adsorption
- ✓ Human Jurkat genomic DNA standards provide more biologically relevant standards for quantification of 5-mC
- ✓ Engineered to provide high specificity, consistency & low background

TECHNICAL SPECS

Modification: 5-mC
Input: 10 ng – 200 ng DNA
Readout: Colorimetric
Adsorption method: LINE-1 hybridization
Cross-reactivity: Human
Format: 96 stripwell microplate
Range of detection: 0.5% – 13%
Procedure length: 5 hours

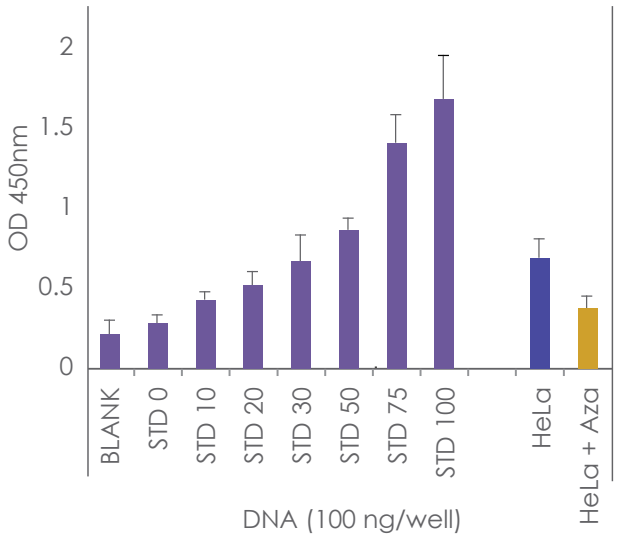


FIGURE 1: Global DNA Methylation Assay.
HeLa cells treated with 5-azacytidine (Aza) DNA methyltransferase inhibitor show decreased 5-mC levels, with standard curve estimating total 5-mC levels at approximatelt 30% in the untreated and close to 0 in treated cells.

ORDERING INFORMATION

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

Global 5-hmC Quantification Kit

Active Motif has developed the Global 5-hmC Quantification Kit to provide researchers with a robust and quantifiable method to analyze global changes in 5-hmC levels to better understand the influence of hydroxymethylation on development and disease. The Global 5-hmC Quantification Kit enables you to obtain the same quantitative results using less sample material and in a higher throughput and easier-to-use format than mass spectrometry.

- ✓ Higher throughput, simpler & more cost-effective than LC-MS/MS
- ✓ Highly sensitive – can detect a little as 0.02% 5-hmC
- ✓ Can detect 5-hmC from as little as 10 ng of input DNA
- ✓ Includes DNA standards for quantification of global 5-hmC levels
- ✓ Highly specific – no cross-reactivity with 5-mC or other methyl variants

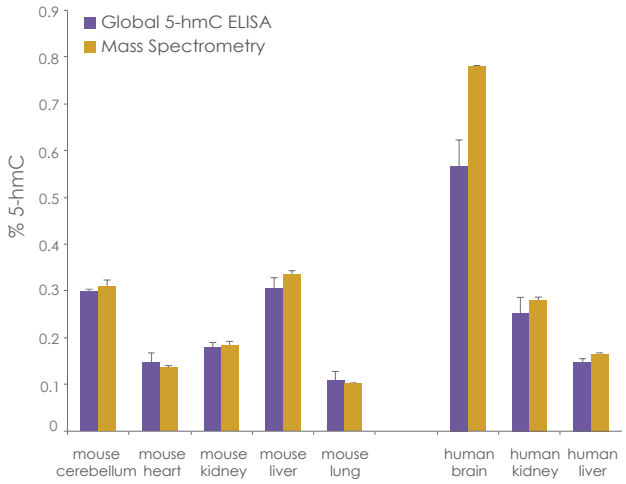


FIGURE 2: The Global 5-hmC ELISA generates 5-hmC quantification data comparable to mass spectrometry.

Percent 5-hmC in mouse and human tissues determined by the Global 5-hmC ELISA Kit shows equal quantification as mass spectrometry data using 10-fold less of starting material.

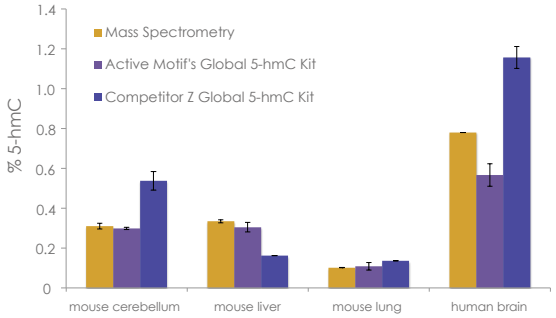


FIGURE 3: Comparison of various 5-hmC quantification methods.

Percent 5-hmC in mouse and human tissues determined with Active Motif's Global 5-hmC Quantification Kit show similar levels as determined by mass spectrometry while Competitor Z's Global 5-hmC Kit quantification differed significantly.

TECHNICAL SPECS

Modification: 5-hmC
Input: 10 ng – 50 ng DNA
Readout: Colorimetric
Adsorption method: Direct detection
Cross-reactivity: Wide Range
Format: 96 stripwell microplate
Range of detection: ≥ 0.02%
Procedure length: 5 hours

ORDERING INFORMATION

Product	Format	Cat. No.
Global 5-hmC Quantification Kit	1 x 96 rxns	55018

5-METHYLCYTOSINE (5-mC) DNA ENRICHMENT

Choose the DNA methylation kit that best suits your specific research needs

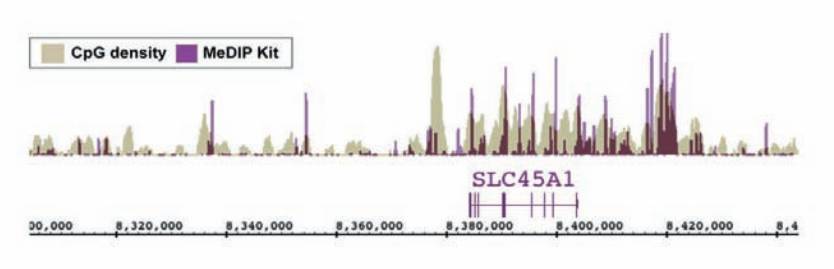
Active Motif's extensive portfolio of DNA methylation kits enables you to select from a variety of enrichment options. This allows you to customize features to achieve the results you desire from your DNA methylation research. For more information on these assays, visit www.activemotif.com/dnamt.

	Methylation at: CpG	Methylation at: non-CpG	DNA Enriched: ssDNA	DNA Enriched: dsDNA	Magnetic Beads	Capture Method
MeDIP	✓	✓	✓	–	✓	Antibody
MethylCollector™ Ultra	✓	–	–	✓	✓	Protein
hMeDIP	✓	✓	✓	✓	✓	Antibody
Hydroxymethyl Collector™	✓	✓	–	✓	✓	Chemical
HypoMethylCollector™	✓	–	–	✓	✓	Protein

MeDIP

Active Motif's MeDIP Kit uses a highly specific 5-methylcytosine (5-mC) capture antibody for methylated DNA immunoprecipitation (MeDIP). The monoclonal antibody is able to distinguish between 5-mC and 5-hmC, making this approach more selective than conventional bisulfite conversion or enzymatic methods.

- ✓ Highly specific antibody enriches only 5-mC methylated DNA
- ✓ Detects methylated cytosines in both CpG and non-CpG context
- ✓ Magnetic bead protocol for faster, more efficient enrichment
- ✓ One-step IP reaction



▲ FIGURE 1: Next-generation sequencing data generated using Active Motif's MeDIP Kit correlates well with CpG density.

DNA was enriched from adaptor ligated human PBMC DNA using Active Motif's MeDIP Kit. Next-generation sequencing was performed using the Illumina platform and sequence tags were mapped to generate a whole-genome DNA methylation profile. The image shows that the enriched regions (purple peaks) correlate well with CpG density.

TECHNICAL SPECS

- Modification: 5-mC
- Methylation: CpG and non-CpG
- Input: 100 ng - 1 µg fragmented gDNA
- DNA enriched: ssDNA
- Capture method: 5-mC antibody
- IP method: Magnetic beads
- Procedure length: 2 hours hands-on time with 1 overnight incubation

ORDERING INFORMATION

Product	Format	Cat. No.
MeDIP	10 rxns	55009

MethylCollector™ Ultra

Active Motif's MethylCollector Ultra Kit improves the enrichment of CpG-methylated DNA compared to alternative methyl-binding domain protein (MBD) or antibody IP methods by incorporating the **Methylated CpG Island Recovery Assay (MIRA)**. MIRA uses a combination of methyl-binding proteins (MBD2b and MBD3L1) to increase the affinity for methylated DNA. This unique protein complex provides greater specificity for methylated CpG dinucleotides than alternative MBD or antibody immunoprecipitation (MeDIP) techniques in less than half the time (Figures 2 & 3).

- ✓ Better enrichment than other MBD capture or antibody-based methods
- ✓ Magnetic bead-based protocol is completed in < 3 hours
- ✓ Low input – requires as few as 170 cells (~1 ng DNA) / reaction for qPCR
- ✓ Magnetic bead protocol for more rapid, efficient & reliable results

TECHNICAL SPECS

Modification: 5-mC
Methylation: CpG
Input: 1 ng - 1 µg fragmented gDNA
DNA enriched: dsDNA
Capture method: Protein (MIRA)
IP method: Magnetic beads
Procedure length: < 3 hours

MethylCollector comes in *two* versions:

MethylCollector™ Ultra Kit – roptimized for identification of methylated CpG DNA
HypoMethylCollector™ Kit– optimized for identification of hypomethylated CpG DNA

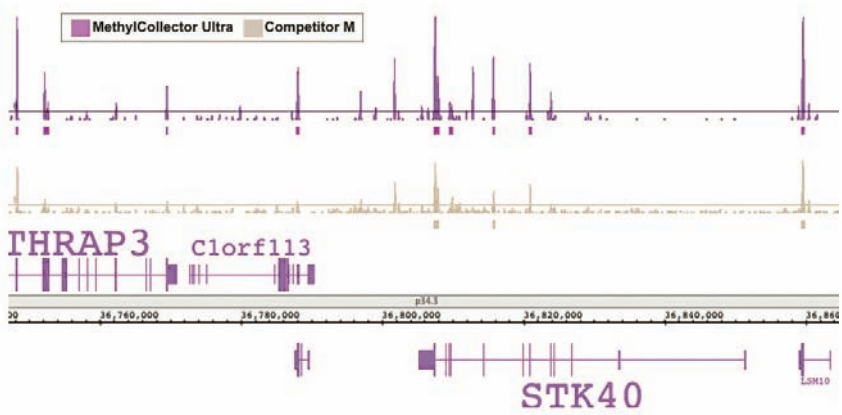


FIGURE 2: MethylCollector Ultra is more sensitive and robust than other MBD enrichment kits.
Comparison of the Active Motif's MethylCollector Ultra Kit and a competitor's MBD-based enrichment kit on human PBMC DNA. MethylCollector Ultra (top purple panel) identified more methylated regions and the peaks had greater intensity than peaks identified from the competitor kit (lower copper panel).

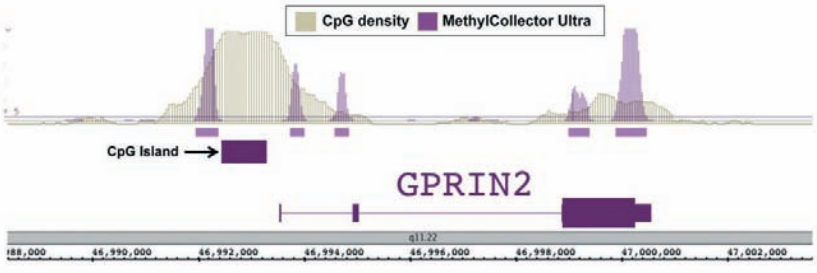


FIGURE 3: Next-generation sequencing data generated using MethylCollector Ultra detects methylation at CpG shores.
DNA was enriched from human PBMC DNA using Active Motif's MethylCollector Ultra Kit. Next-generation sequencing was performed and tags were mapped to generate a whole-genome DNA methylation profile. The data show one of many examples of DNA methylation detected at CpG shores rather than in the CpG island itself and agrees with recent findings showing that methylated sites occur in these CpG shore regions that are adjacent to CpG islands.

ORDERING INFORMATION

Product	Format	Cat. No.
MethylCollector™ Ultra	30 rxns	55005
HypoMethylCollector™	30 rxns	55004

5-HYDROXYMETHYLCYTOSINE (5-hmC) DNA ENRICHMENT

Active Motif's hMeDIP and Hydroxymethyl Collector™ Assays are specific for the enrichment of DNA containing 5-hydroxymethylcytosine (5-hmC) residues. While most conventional methods to analyze DNA methylation, such as bisulfite conversion, do not distinguish between 5-mC and 5-hmC residues. Active Motif's hMeDIP and Hydroxymethyl Collector™ enable researchers to isolate 5-hmC DNA separate from 5-mC DNA.

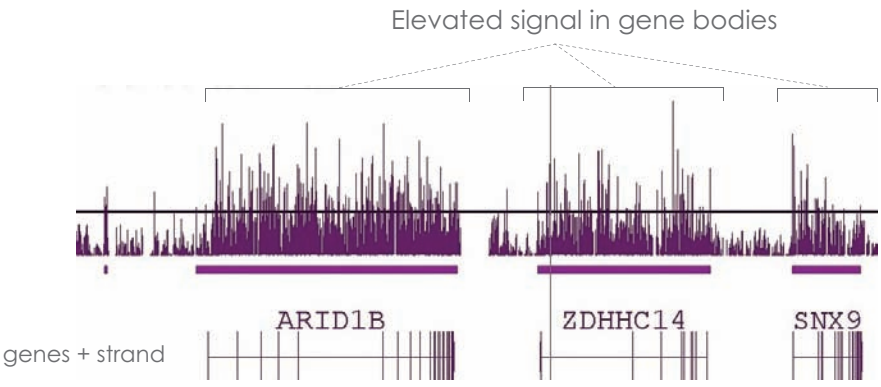
hMeDIP

The hMeDIP Kit contains a highly specific purified 5-hydroxymethylcytosine (5-hmC) antibody and necessary reagents to perform selective immunoprecipitation of either double-stranded or single-stranded genomic 5-hmC containing DNA fragments (hMeDIP). Enriched DNA is ready for downstream applications including PCR, microarray, or Next-generation sequencing analysis (Figure 1).

- ✓ Highly specific antibody capture of 5-hmC DNA
- ✓ Works with either dsDNA or ssDNA
- ✓ Low input – requires as little as 100 ng DNA/reaction
- ✓ Includes unmethylated, 5-mC and 5-hmC DNAs & PCR primer controls

TECHNICAL SPECS

Modification: 5-hmC
Methylation: CpG and non-CpG
Input: 100 ng - 1 µg fragmented gDNA
DNA enriched: dsDNA and ssDNA
Capture method: 5-hmC antibody
IP method: Magnetic beads
Procedure length: 2 hours hands-on time with 1 overnight incubation



◀ FIGURE 1: hMeDIP-Seq performed on human brain DNA.

Data shows that 5-hmC is enriched primarily in the coding region of genes, rather than the promoter or regulatory regions.

ORDERING INFORMATION

Product	Format	Cat. No.
hMeDIP	10 rxns	55010

Hydroxymethyl Collector™

Hydroxymethyl Collector utilizes chemical labeling of 5-hmC residues to ensure no cross-reactivity with 5-mC, and the method is not limited by the specific properties or consensus sequences that constrain traditional methods, such as glucosyl-sensitive restriction enzyme digestion. The result is reduced background and increased sensitivity to enable enrichment of DNA fragments containing as few as two 5-hmC residues. Enriched DNA can be used in the analysis of individual genes by PCR and qPCR, or with microarrays and sequencing for genome-wide analysis (Figure 2).

- ✓ Chemical labeling of 5-hmC DNA with no cross-reactivity to 5-mC
- ✓ Enables detection of non-CpG methylation (CpA or CpT)
- ✓ Uses dsDNA for easier library preparation for NGS
- ✓ Can enrich DNA fragments containing as few as two 5-hmC residues

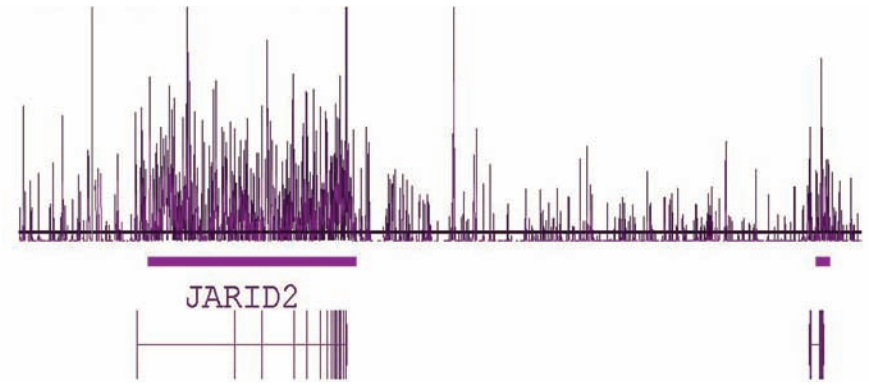
TECHNICAL SPECS

Modification: 5-hmC
Methylation: CpG and non-CpG
Input: 5 ng - 2.5 µg (or 1 - 10 µg for HMC-Seq) fragmented gDNA
DNA enriched: dsDNA
Capture method: Chemical labeling
IP method: Magnetic beads
Procedure length: < 5 hours

Hydroxymethyl Collector comes in *two* versions:

Hydroxymethyl Collector™ Kit – recommended for analysis of individual genes by PCR

Hydroxymethyl Collector™-Seq Kit – optimized for use in combination with NGS for 5-hmC genome-wide analysis



▲ FIGURE 2: Hydroxymethyl Collector data of enrichment of 5-hmC enrichment across the *JARID2* gene.

Human tiling array data showing enrichment of 5-hmC across the entire length of the *JARID2* gene in chromosome 6 using human brain DNA that was enriched with Hydroxymethyl Collector.

ORDERING INFORMATION

Product	Format	Cat. No.
Hydroxymethyl Collector™	25 rxns	55013
Hydroxymethyl Collector™–Seq Kit	25 rxns	55019
UDP-Azide-Glucose	1 vial	55020

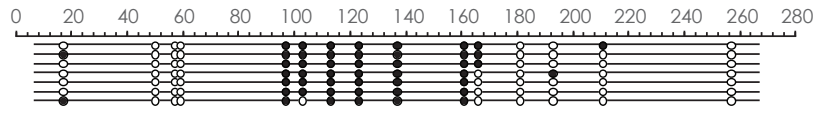
BISULFITE CONVERSION

Bisulfite Conversion Kit

Active Motif's Bisulfite Conversion Kit enables bisulfiteconversion of DNA in as little as 1.5 hours, while minimizing DNA fragmentation, degradation and sample loss. The included spin columns combine desulfonation and DNA purification into a single step to quickly generate purified, converted DNA suitable for PCR amplification and sequencing, endonuclease digestion or other downstream applications.

- ✓ Rapid conversion of DNA in as little as 1.5 hours
- ✓ Minimal DNA fragmentation, degradation & sample loss
- ✓ Thermocycling strategy enables more effective conversion reactions
- ✓ ≥ 99% conversion efficiency of unmethylated cytosines
- ✓ Highly sensitive – requires as little as 0.2 ng (50 cells) of input DNA

TECHNICAL SPECS
Input: 0.2 ng – 2 µg purified DNA
Conversion efficiency: ≥ 99%
DNA recovery: ≥ 80%
Format: Spin column
Elution volume: 30 µl
Procedure length: 1.5 hours



▲ FIGURE 1: Bisulfite sequencing results for the *CCND2* locus in PC-9 human lung adenocarcinoma cells.
Red circles - methylated CpGs; white circles - unmethylated CpGs.

FFPE Bisulfite Conversion Kit

Active Motif's FFPE Bisulfite Conversion Kit is designed for DNA methylation analysis of formalin- or paraformalin-fixed, paraffin-embedded (FFPE) tissues at single-base-pair resolution. This kit provides optimized reagents for tthe recovery of high quality DNA with minimal degradation and greater than 99% conversion efficiency.

- ✓ Minimal DNA fragmentation, degradation & sample loss
- ✓ Works with up to 4 x 20 µm FFPE tissue sections or 35 mg unsectioned core samples per reaction
- ✓ ≥ 99% conversion efficiency of unmethylated cytosines
- ✓ Successful bisulfite conversion using 5 pg - 2 µg input DNA

TECHNICAL SPECS
Input: 5 pg - 2 µg purified DNA
Conversion efficiency: ≥ 99%
DNA recovery: ≥ 80%
Format: Spin column
Elution volume: 20 µl
Procedure length: 5 hours

ORDERING INFORMATION

Product	Format	Cat. No.
Bisulfite Conversion Kit	50 rxns	55016
FFPE Bisulfite Conversion Kit	40 rxns	55021

ChIP-Bis-Seq

Active Motif's ChIP-Bisulfite-Sequencing (ChIP-Bis-Seq) Kit combines the target-specific selection of chromatin immunoprecipitation (ChIP) with bisulfite conversion and sequencing (Bis-Seq) to provide single nucleotide resolution of immunoprecipitated DNA. By combining the two techniques, information about both the chromatin context and the methylation profiles of each DNA strand can be evaluated, enabling better understanding of the biological context and significance regarding gene regulation, gene expression, mechanisms of chromatin modification and pathway analysis. Key features & advantages include:

- ✓ Interrogate both chromatin and DNA methylation profiles in the same sample
- ✓ Evaluate allele-specific differences in DNA (imprinting, X-inactivation)
- ✓ Compatible with histone and transcription factor antibodies
- ✓ Optimized ChIP protocol provides reduced background caused by non-specific binding and higher quality DNA for analysis
- ✓ Reduce sequencing costs by focusing on specific genomic regions of interest
- ✓ Save time and money by using our already validated ChIP-Bis-Seq workflow

TECHNICAL SPECS

Input: 1.5- 4.5 x 10^6 cell equivalents

Conversion efficiency: ≥ 99%

DNA recovery: ≥ 80%

Format: Spin column

Elution volume:
- ChIP: 36 µl
- Library Preparation: 11 µl
- Bisulfite conversion: 24 µl

Procedure length:
- ChIP: 2 days
- Library preparation: 2 hours
- Bisulfite conversion: 5.5 hours

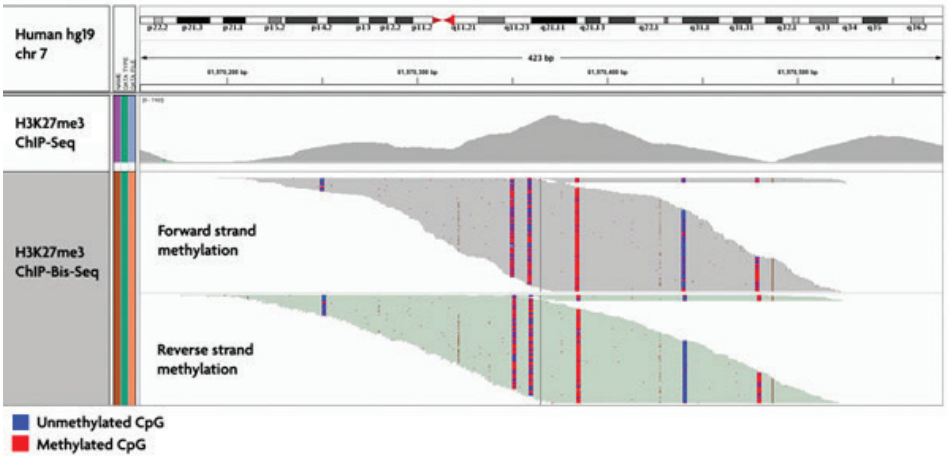


FIGURE 2: DNA methylation profile using the ChIP-Bis-Seq kit on H3K27me3 enriched DNA from PC9 cells

Example sequencing results using the ChIP-Bis-Seq Kit to analyze the DNA methylation profile of DNA that was enriched for Histone H3K27me3 in PC9 cells. The top track shows a 423 bp region of chromosome 7 from Human Hg19 genome. The next track reveals the ChIP-seq peak profile for Histone H3K27me3. The final track shows the DNA methylation profile of forward and reverse reads as analyzed by bisulfite conversion of the ChIP-enriched DNA. The blue bars represent unmethylated CpGs, while the red bars represent CpGs containing methylation.

ORDERING INFORMATION

Product	Format	Cat. No.
ChIP-Bis-Seq	10 rxns	53048

ASIA: +81 (0)3 5225 3638 EUROPE: +32 (0)2 653 0001 NORTH AMERICA: 877 222 9543

DNMT ACTIVITY / INHIBITION ASSAY

Non-radioactive assay to screen for DNA methyltransferase activity

Active Motif's DNMT Activity / Inhibition Assay is a highly sensitive and rapid microplate-based assay to simplify the measurement of DNA methyltransferase activity or to screen for DNMT inhibitors without the need for radioisotopes or expensive equipment. The assay employs a unique and highly sensitive method that detects methyltransferase activity using recombinant methyl-CpG binding domain (MBD) protein that is capable of binding methylated DNA with a higher affinity than antibodies, increasing the sensitivity of the assay relative to antibody-based methods (Figure 1).

- ✓ **Non-radioactive** – colorimetric assay is easily quantified by spectrophotometry on a microplate reader at 450 nm
- ✓ **Sensitive** – activity can be detected from as little as 0.5 ng of purified enzyme or 0.5 µg of nuclear extract (Figure 2)
- ✓ **Fast** – assay can be completed in < 3 hours
- ✓ **Flexible** – 96 stripwell microplate for low or high throughput

For more information and complete details, please visit us at www.activemotif.com/dnmt.

TECHNICAL SPECS

Recommended for assaying:
- Purified enzyme activity
- Nuclear extract activity
- DNMT inhibitors

Recommended range per well:
- Purified enzyme: 0.5 ng - 100 ng
- Nuclear extract: 0.5 µg - 10 µg

Readout: Colorimetric

Detection method: MBD2b protein

Adsorption method: Universal CpG-enriched DNA substrate

Format: 96 stripwell microplate

Range of detection: 0.5 - 100 ng/well

Procedure length: 3 hours

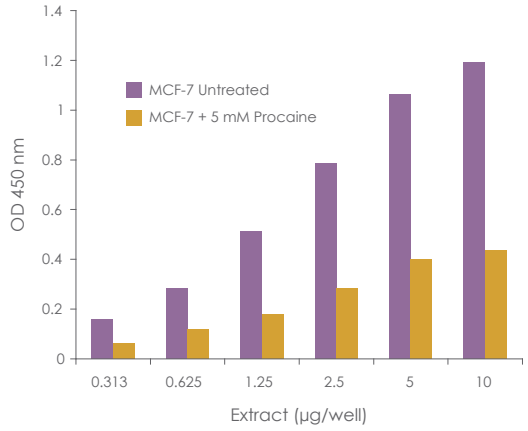


FIGURE 1: DNMT Activity / Inhibition Assay shows DNMT inhibition in MCF-7 cells treated with procaine, a DNA methyltransferase inhibitor, vs untreated cells.

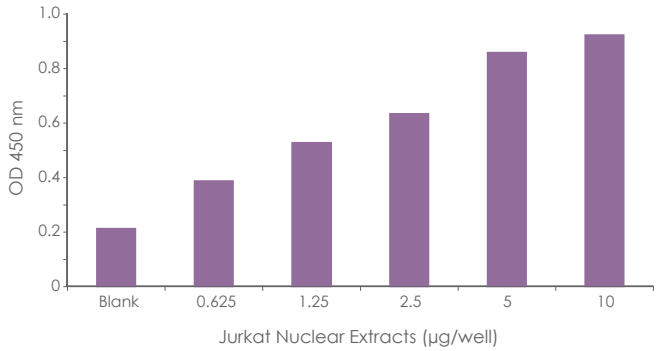


FIGURE 2: The DNMT Activity / Inhibition Assay range of detection.
Range of detection is demonstrated with increasing concentrations of Jurkat nuclear extract prepared using Active Motif's Nuclear Extract Kit (Catalog No. 40010).

ORDERING INFORMATION

Product	Format	Cat. No.
DNMT Activity / Inhibition Assay	1 x 96 rxns	55006

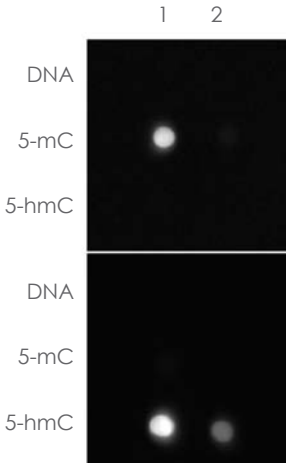
DNA METHYLATION STANDARDS AND CONTROLS

Methylated DNA standard kits

Active Motif offers DNA standards that can be used as controls in methylation analysis experiments. These include our:

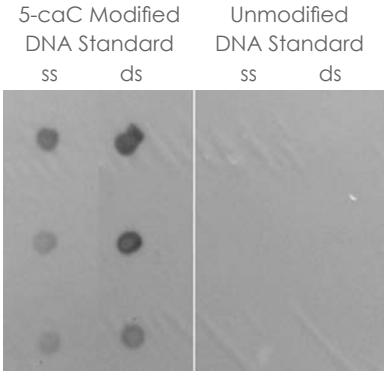
- Methylated DNA Standard Kit – includes 100% unmethylated, 5-mC & 5-hmC recombinant DNA standards derived from the *APC* gene promoter. Each standard is 338 bp and contains multiple cytosine residues in both CpG and non-CpG contexts. In addition to the DNA standards, PCR primers specific to the *APC* promoter are also provided (Figure 1).
- 5-Carboxylcytosine DNA Standard Kit – includes two double-stranded DNA oligonucleotides, one containing a total of 12 5-caC modifications and the other containing only unmodified cytosine residues (Figure 2).

In addition, Active Motif also provides Jurkat genomic DNA and Fully Methylated Jurkat DNA for use as controls in DNA methylation analysis experiments.



◀ FIGURE 1: Dot blot analysis of Methylated DNA Standard Kit samples.

Fifty nanograms of the DNA standards included in the Methylated DNA Standard Kit were spotted onto a positively charged nylon membrane:
DNA (unmethylated DNA), 5-mC (5-methylcytosine methylated DNA), 5-hmC (5-hydroxymethylcytosine methylated DNA).
Top panel: Dot blot probed with 5-Methylcytosine antibody (Catalog No. 39649).
Bottom panel: Dot blot probed with 5-Hydroxy-methylcytosine antibody (Catalog No. 39769).
Lane 1: single-stranded DNA
Lane 2: double-stranded DNA



▲ FIGURE 2: Dot blot analysis shows specificity of Active Motif's 5-Carboxylcytosine antibody for 5-caC.

DNA from the 5-Carboxylcytosine DNA Standard Kit were spotted onto a nylon membrane to show specificity of 5-Carboxylcytosine antibody (Catalog No. 61225) for 5-caC.

ORDERING INFORMATION

Product	Format	Cat. No.
Methylated DNA Standard Kit	3 x 2.5 µg	55008
5-Carboxylcytosine DNA Standard Kit	0.5 µg	55014
Fully Methylated Jurkat DNA	10 µg	55003
Jurkat genomic DNA	10 µg	55007

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HISTONE ANALYSIS

Active Motif offers a wide range of products to analyze histones and histone modifications, as well as the "writers", "readers" and "erasers" that regulate histone activity. These tools have been developed for use in basic research as well as clinical and drug discovery applications. For more, please visit www.activemotif.com/histone.

HISTONE QUANTIFICATION ELISAS

Simple, high-throughput quantitative assays for detecting global changes in histones and histone modifications in response to stimulus, drug treatment or disease related alterations in cell signaling and gene regulation.

HISTONE PEPTIDE ARRAYS

Specificity is a major problem with histone modification antibodies. The MODified™ Histone Peptide Array enables researchers to screen antibodies, enzymes and proteins for cross-reactivity and specificity. In addition, the array can be used to study substrate recognition by histone-modifying enzymes and reader domains.

HISTONE PTM MULTIPLEX ASSAY

Active Motif partnered with Luminex®, the industry leader in multiplexing, to develop the first epigenetic assay with multiplexing capability for high-throughput analysis of histone modifications.

HISTONE PURIFICATION KITS

Proprietary technology for isolating high quality purified core histones to enable studies of changes in histones and histone modifications due to disease, enzymes or drug treatment using mass spectrometry or similar sensitive quantitative analysis methods.

ENZYME ACTIVITY ASSAYS

High throughput simple-to-use assays to enable you to accurately and efficiently measure global changes in HAT, HDAC and HDM activity.

MODIFIED™ HISTONE PEPTIDE ARRAY & PRODUCTS

Screen for binding specificity with the broadest coverage of modifications

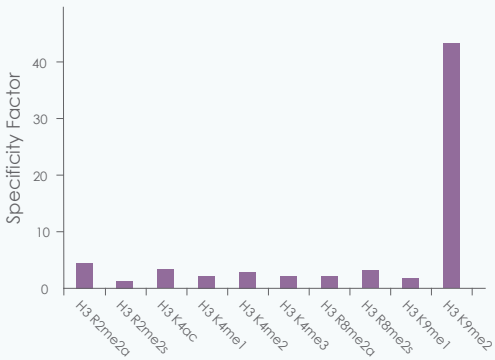
Active Motif's MODified™ Histone Peptide Array* offers the most extensive coverage of histone modifications available from a commercial array for the most accurate screening of binding specificity and cross-reactivity of antibodies and proteins using a simple Western blot-like detection method. Each array contains 384 different histone modification combinations in duplicate. Modifications include acetylation, methylation, phosphorylation and citrullination on the N-terminal tails of histones H2A, H2B, H3 and H4. Up to four modifications are represented per 19mer peptide to study not only individual modifications, but also to examine the effects of neighboring modifications on site recognition and binding. The array is compatible with either ECL-based or colorimetric detection systems. For more information, visit www.activemotif.com/modified.

- ✓ **Broadest coverage available** – 59 histone modifications displayed in 384 unique combinations
- ✓ **Study neighboring effects** – combines up to four modifications per peptide
- ✓ **Simple Western blot-like detection** – works with either ECL-based or colorimetric detection

APPLICATION NOTES

Also available as part of our MODified™ products are the:

- MODified™ Array Labeling Kit – contains blocking and wash buffers, rabbit and mouse HRP-conjugated antibodies and ECL reagents for chemiluminescent detection of the MODified Histone Peptide Array. For added convenience, positive control c-Myc antibody is included for recognition of the array's control c-Myc tag.
- Free Array Analyze Software – free PC-compatible software designed for use with the MODified Histone Peptide Array to analyze spot intensities from the MODified array and generate a graphical plot of the binding interactions (Figure 1). Information about spot intensity, averages and errors can be saved in Excel-compatible files. Up to three individual modifications can be displayed in superposition to the experimental data for visualization of neighboring effects.



▲ FIGURE 1: The MODified Histone Peptide Array confirms the specificity of modification-specific histone antibodies.
The MODified Histone Peptide Array and MODified Array Labeling Kit were used to screen Active Motif's Histone H3K9me2 antibody (Catalog No. 39683) for cross-reactivity.

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

ORDERING INFORMATION

Product	Format	Cat. No.
MODified™ Histone Peptide Array	1 array	13001
	5 arrays	13005
MODified™ Array Labeling Kit	5 rxns	13006

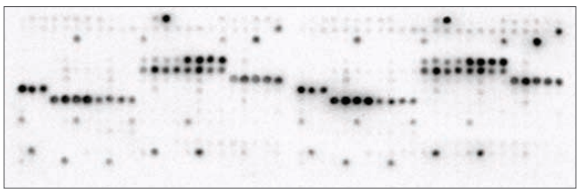
MODified™ Protein Domain Binding Kit – screen protein domains for reactivity with histone modifications

There are several classes of reader domains (e.g. bromodomains, chromodomains) that influence gene regulation and chromatin remodeling by interacting with specific histone modifications. The MODified Protein Domain Binding Kit is designed for researchers interested in screening these domains for reactivity with specific histone modifications. The assay is engineered to be used in conjunction with Active Motif's MODified Histone Peptide Array (opposite page). After blocking the MODified Histone Peptide Array with blocking buffer and adding your His-tagged reader domain of interest, the kit provides all the buffers and reagents needed to screen binding specificity of the His-tagged reader domain and for chemiluminescent detection of the arrays (Figure 2A). The array image can then be analyzed using the Array Analyze Software (Figure 2B & opposite page).

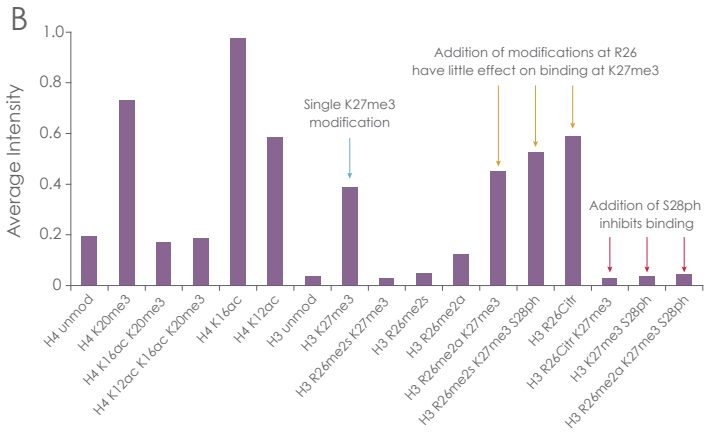
Domain	Binding Site	Function	Examples
Bromo	Acetylated lysine residues	Regulates chromatin structure and gene expression as part of HATs or chromatin remodeling factors	$TAF_{II}250$, PCAF, GCN5
Chromo	Methylated lysine residues	Associated with the assembly of protein complexes on chromatin	HP1β, MPP8,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

▲ Examples of common protein domains and their binding specificity

A



B



▲ FIGURE 2: The MODified Protein Domain Binding Kit shows the binding specificity of JMJD2A.

Shown are (A) a representative image of a developed MODified Histone Peptide Array and (B) data produced by the Array Analyze Software to analyze the binding specificities of the JMJD2A reader domain to various histone modifications using the MODified Protein Domain Binding Kit and Histone Peptide Array.

WHAT'S IN THE BOX?

The MODified Protein Domain Binding Kit contains interaction buffer, wash buffer, anti-His6-tag antibody, HRP-conjugated secondary antibody and ECL reagents for chemiluminescent detection. For added convenience, His-tagged recombinant G9a protein, a known histone methyltransferase that targets Histone H3K9, is included for use as a positive control.

MODified Histone Peptide Arrays *are not included* in the kit and must be purchased separately.

ORDERING INFORMATION

Product	Format	Cat. No.
MODified™ Protein Domain Binding Kit	5 rxns	13007

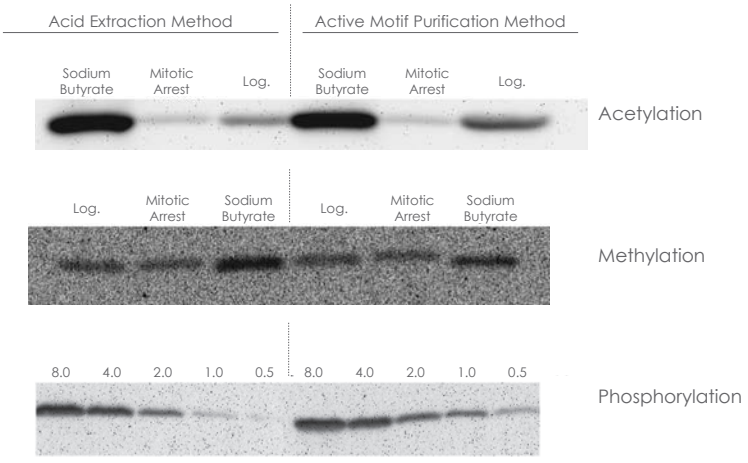
HISTONE PURIFICATION KITS

The only commercial assays available for isolating purified core histones

When using mass spectrometry or similar sensitive quantitative analysis methods to study changes in histones and histone modifications associated with disease, enzymes or drug treatments, the highest quality samples are required. Active Motif's Histone Purification Kits enable you to obtain the purest, highest quality histones from cell or tissue samples for use in downstream analysis. Active Motif is the only company that offers commercial assays for purification of core histones. Our proprietary method yields the highest quality samples by improving purity and preservation of native modifications above crude acid extraction methods (Figure 1).

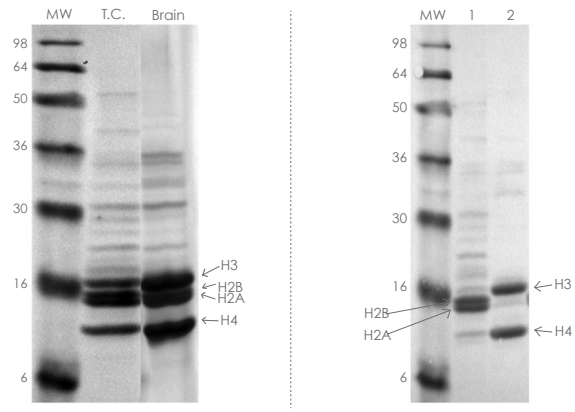
Active Motif Histone Purification Kits use a unique purification resin and a series of proprietary elution buffers to eliminate contaminating acid-insoluble cellular proteins that are left behind with standard acid extraction procedures. Removal of these impurities prevents additional enzymatic alterations to histone modifications, ensuring you have the highest quality histone samples for use in your downstream analysis. The results are improved yield, purity, and preservation of modifications above what is achievable with crude acid extraction (Figure 2).

- ✓ **Unique** – only commercial kits available for histone purification
- ✓ **Quality** – enhanced histone purity and preservation of histone modifications
- ✓ **Scalability** – 3 throughput formats available to suit your needs
- ✓ **Flexibility** – works with cell or tissue samples



▲ FIGURE 1: Histone purification preserves post-translational modifications better than standard acid extraction methods.

Active Motif's Histone Purification method preserves acetyl, methyl and phosphoryl modifications as well or better than crude acid extraction methods.



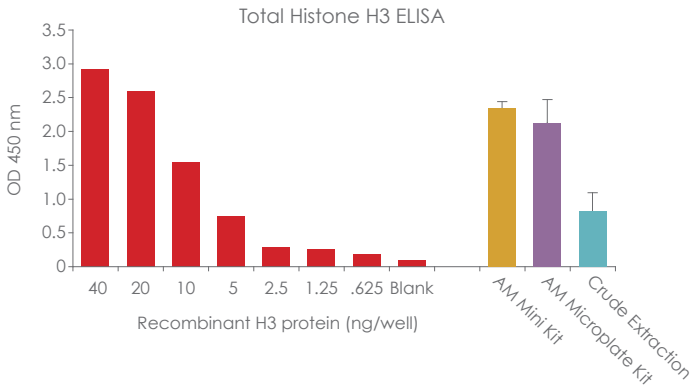
▲ FIGURE 2: Histone purification isolates fractions of core histones.

SDS-PAGE of histone fractions purified from both tissue and cell samples using the Histone Purification Kit.

ASIA: +81 (0)3 5225 3638 EUROPE: +32 (0)2 653 0001 NORTH AMERICA: 877 222 9543

Histone purification kits for quality sample preparation

Achieving the best results is highly reliant on the quality of your input samples. With histone purification, you can significantly improve the consistency and reliability of your analysis of histones and their modifications. Purified histones are ready for quantitative mass spectrometry or other types of analysis, such as in Western blot with Active Motif's collection of **histone modification antibodies** (pages 2-3), for **chromatin assembly** (page 19) or as substrates in functional assays, including Active Motif's **Histone Modification ELISAs** (Figure 3, page 42).



▲ FIGURE 3: Significantly improve sample preparation for downstream applications with histone purification.

Active Motif's Histone Purification Mini Kit and Histone Purification Microplate Kit produce significantly higher histone yields than traditional crude acid extraction methods as measured using the Total Histone H3 ELISA Kit, page 42.

Which kit is right for you?

Active Motif's Histone Purification proprietary histone purification method is available in three different format options to suit the throughput needs of your downstream analysis. Use Table 1 below to determine which kit is right for you. To learn more, please visit www.activemotif.com/histonepur.

Kit	Application	Format	Elution	Capacity	# Purifications
Histone Purification Kit	 • Low throughput • Large sample quantities	Gravity Flow	Separate H2A/H2B & H3/H4 fractions	0.5 - 2.5 mg	10
		Spin Column	H2A, H2B, H3 & H4 in a single fraction	0.5 - 2.5 mg	10
Histone Purification Mini Kit	 • Medium throughput • Mid-range sample quantities	Mini Spin Column	H2A, H2B, H3 & H4 in a single fraction	0.1 - 0.5 mg	20
Histone Purification Microplate Kit	 • High throughput • Low sample quantities	96 Stripwell Plate Spin Column	H2A, H2B, H3 & H4 in a single fraction	< 0.48 mg	96

▲ Specifications for Active Motif's Histone Purification Kits

ORDERING INFORMATION

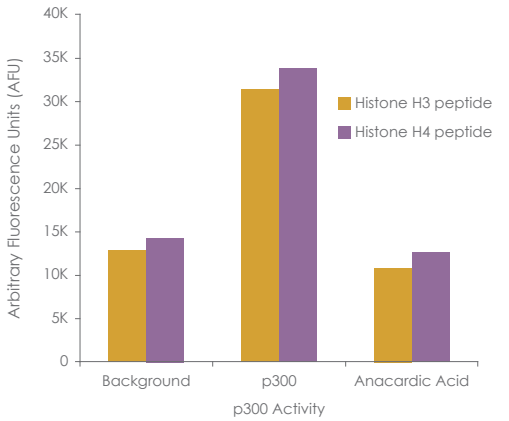
Product	Format	Cat. No.
Histone Purification Kit	10 rxns	40025
Histone Purification Mini Kit	20 rxns	40026
Histone Purification Microplate Kit	96 rxns	40027

HAT & HDAC ASSAYS

Active Motif's highly sensitive microplate-based Histone Acetyltransferase (HAT) & Histone Deacetylase (HDAC) Assay Kits offer a safer and faster alternative to radioisotopic methods to screen activity or inhibitors of histone acetyltransferases and histone deacetylases from cell or nuclear extracts, immunoprecipitates and purified enzymes.

Histone Acetyltransferase (HAT) Assay Kit

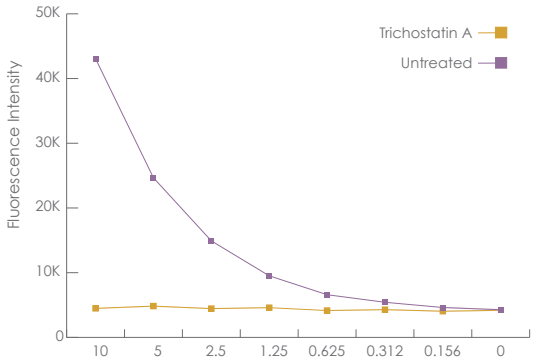
The HAT Assay Kit offers a simple fluorescent method to measure the histone acetyltransferase activity of your samples or to screen HAT inhibitors. The quick and easy procedure gives you results in 30 minutes (Figure 1). In addition to optimized buffers and reagents, the assay includes N-terminal histone H3 and H4 peptide substrates for screening HAT enzymes and a positive control p300 catalytic domain protein to screen for inhibitors. Anacardic acid is also provided for use as a HAT inhibitor control. A standard curve can be generated with either β-mercaptoethanol or Coenzyme A in order to quantitate the fluorescence of your HAT enzyme to determine specific activity.



▲ FIGURE 1: The HAT Assay Kit was used to analyze the effect of anacardic acid, a HAT inhibitor, on p300 activity.

Histone Deacetylase (HDAC) Assay Kit

The HDAC Assay Kit utilizes a peptide substrate that contains an acetylated lysine residue that can be deacetylated by Class I, IIB and IV HDAC enzymes (Class III HDAC enzymes, or the Sirtuins, require the addition of the NAD+ cofactor in the assay). Once the substrate is deacetylated, the lysine reacts with the Developing Solution to produce either a colorimetric or fluorescent product to enable measurement of HDAC activity (Figure 2). A deacetylated assay standard is provided in each kit to enable calculation of HDAC activity.



▲ FIGURE 2: Untreated and Trichostatin A-treated HeLa nuclear extracts were assayed for HDAC activity using the fluorescent version of the HDAC Assay Kit.

ORDERING INFORMATION

Product	Format	Cat. No.
HAT Assay Kit (Fluorescent)	1 x 96 rxns	56100
HDAC Assay Kit (Fluorescent)	1 x 96 rxns	56200
HDAC Assay Kit (Colorimetric)	1 x 96 rxns	56210

HISTONE DEMETHYLASE ASSAY

Screen LSD1 for histone demethylase activity

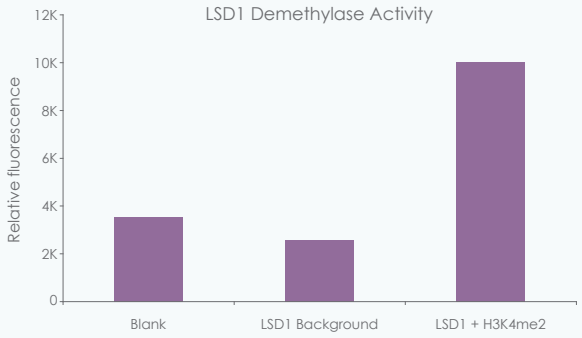
Lysine specific histone demethylase 1 (LSD1, also known as KDM1) specifically demethylates H3K4 and H3K9 and regulates gene silencing during various biological processes, including transcriptional regulation and chromatin remodeling. Active Motif's Histone Demethylase Assay provides a simple, high-throughput and accurate method to screen LSD1 demethylase activity or inhibitor compounds. Assay features include:

- ✓ Fluorescent assay complete with optimized buffers for measuring LSD1 activity
- ✓ Use of full-length H3K4me2 instead of peptide substrate more closely resembles *in vivo* conditions
- ✓ Includes both positive control LSD1 and a formaldehyde standard for quantitation
- ✓ Simple, fast microplate-based procedure can be completed in < 2.5 hours

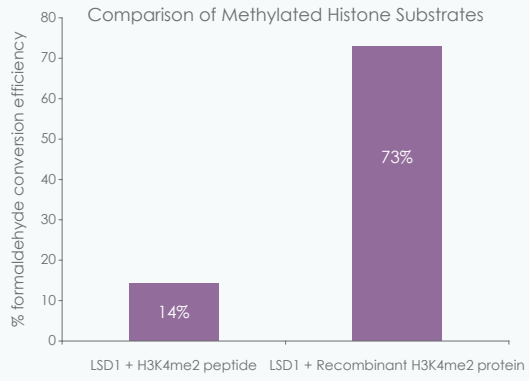
APPLICATION NOTES

The Histone Demethylase Assay is designed to detect formaldehyde released when LSD1 demethylates the recombinant histone H3K4me2 substrate. The formaldehyde by-product then reacts with the Detection Reagent to generate a fluorescent signal used for quantitation of enzyme activity (Figure 1).

Because the provided recombinant Histone H3K4me2 substrate more closely resembles native histone, the Histone Demethylase Assay enables more accurate measurement of LSD1 demethylase activity. As shown in Figure 2, the LSD1 enzyme is able to more efficiently demethylate the provided recombinant histone H3K4me2 protein than a histone H3K4me2 peptide substrate. For more complete details, visit www.activemotif.com/lsd1.



▲ FIGURE 1: Fluorescent detection of LSD1 demethylase activity using the Histone Demethylase Assay.



▲ FIGURE 2: Comparison of demethylase efficiency using the Histone Demethylase Assay shows LSD1 demethylates H3K4me2 protein substrate more efficiently than H3K4me2 peptide.

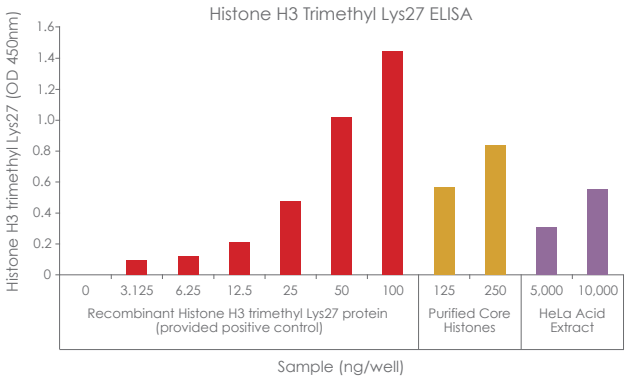
ORDERING INFORMATION

Product	Format	Cat. No.
Histone Demethylase Assay (Fluorescent)	48 rxns	53200

HISTONE MODIFICATION ELISA KITS

Assay cellular response by quantifying changes in histone modification levels

Active Motif's Histone Modification ELISA Kits provide a sensitive, high-throughput method for quantifying global changes in the level of specific histone modifications, including lysine methylation and acetylation, serine phosphorylation, as well as total H3 from purified core histones (page 38-39), or histones isolated by acid extraction. Using our expertise in histone antibody development, we identified optimal antibody pairs for the highly specific and sensitive detection of histone modifications and provided them in a simple sandwich ELISA format for your convenience and ease of use. Each kit includes modification-specific recombinant protein controls to generate standard curves for quantitation. In addition, the Total Histone H3 ELISA can be used to normalize the amount of histone modification in your samples when run in parallel with the methylated or acetylated histone ELISAs.



▲ FIGURE: The Histone H3 trimethyl Lys27 ELISA was used to assay H3K27me3 levels in purified HeLa core histones and HeLa acid extract. Control Recombinant Histone H3K27me3 protein provided in the kit was assayed as a reference standard curve.

- ✓ **Convenient** – colorimetric assays in a simple sandwich ELISA format
- ✓ **Fast** – can be completed in < 3 hours
- ✓ **Scalable** – stripwell microplate format for manual or high throughput analysis
- ✓ **Quantitative** – includes recombinant modified histones for use as standards for quantitation

For an up-to-date list of available Histone Modification ELISAs, please visit www.activemotif.com/hiselisa.

ORDERING INFORMATION

Product	Format	Cat. No.
Histone H3 monomethyl Lys4 (H3K4me1) ELISA	96 rxns	53101
Histone H3 dimethyl Lys4 (H3K4me2) ELISA	96 rxns	53112
Histone H3 trimethyl Lys4 (H3K4me3) ELISA	96 rxns	53113
Histone H3 acetyl Lys9 (H3K9ac) ELISA	96 rxns	53114
Histone H3 dimethyl Lys9 (H3K9me2) ELISA	96 rxns	53108
Histone H3 trimethyl Lys9 (H3K9me3) ELISA	96 rxns	53109
Histone H3 phospho Ser10 (H3S10ph) ELISA	96 rxns	53111
Histone H3 acetyl Lys14 (H3K14ac) ELISA	96 rxns	53115
Histone H3 monomethyl Lys27 (H3K27me1) ELISA	96 rxns	53104
Histone H3 trimethyl Lys27 (H3K27me3) ELISA	96 rxns	53106
Histone H3 acetyl Lys27 (H3K27ac) ELISA	96 rxns	53116
Histone H3 phospho Ser28 (H3S28ph) ELISA	96 rxns	53100
Total Histone H3 ELISA	96 rxns	53110

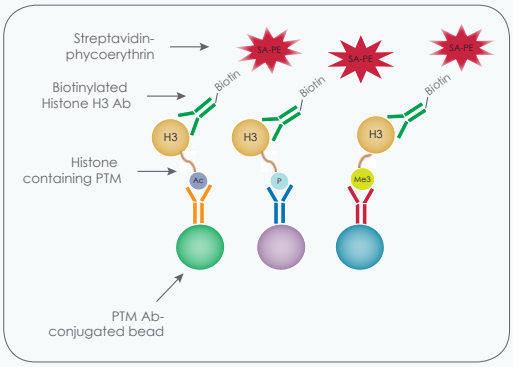
HISTONE H3 PTM MULTIPLEX ASSAY

Simultaneously screen specific and off-target effects on histone modifications

Active Motif partnered with Luminex®, the industry leader in multiplexing, to develop the Histone H3 PTM Multiplex Assay, the first multiplex epigenetic assay for high throughput profiling of histone post-translational modifications (PTMs) for use with MAGPIX®, Luminex® 200™ or FLEXMAP 3D® instruments. This unique assay enables you to rapidly and efficiently screen clinical and compound-treated samples and gather more information with smaller input, in less time and at a lower cost than traditional Western blot, immunofluorescence or chromatographic methods.

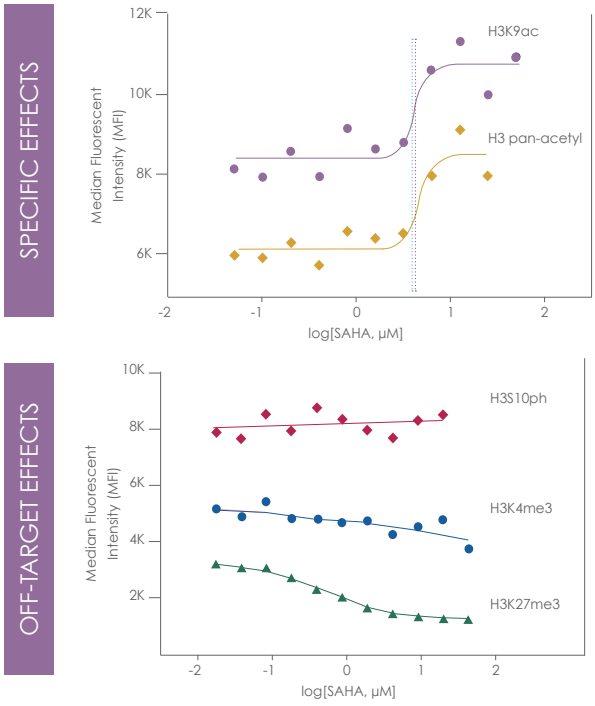
APPLICATION NOTES

The microplate-based assay works as a solution-based sandwich ELISA. Histone modifications in the N-terminus are captured using antibodies conjugated to fluorescent-labeled magnetic beads. Each bead emits a unique fluorescent signal to enable detection of multiple targets within the same well. A biotinylated H3 C-terminal antibody is used to capture histones and streptavidin-phycoerythrin (SA-PE) is then added to bind biotin and produce a signal. Fluorescent and SA-PE signals are read using a Luminex instrument and used to decipher both the bead identity and the number of binding events pertaining to each histone modification. For info on **Custom Services**, please go to page 59.



What do I need for the assay?

- ✓ The Histone H3 PTM Multiplex Kit (Catalog No. 33115) contains buffers and reagents for performing the assay.
- ✓ Ab-conjugated Beads for the individual PTMs *are sold separately* to enable customization of analyte selection. For an up-to-date list of available PTM targets, visit www.activemotif.com/luminex.
- ✓ Inclusion of Histone H3 Total Ab-conjugated Beads (Catalog No. 33116) in your multiplex enables you to normalize values for comparison of relative amounts of PTMs across samples.



▲ FIGURE: The Histone H3 PTM Multiplex Assay shows increased histone acetylation in response to SAHA-mediated HDAC inhibition. Results also show off-target effects. The dashed lines represent IC50 values for pan-acetyl and H3K9ac.

ORDERING INFORMATION

Product	Format	Cat. No.
Histone H3 PTM Multiplex Kit	96 rxns	33115

REAGENTS FOR DRUG DISCOVERY

One of the current challenges of epigenetic drug discovery is designing robust enzymatic assays for high-throughput inhibitor screens. The key to a robust enzymatic assay is obtaining epigenetic recombinant proteins that are affordable, highly active, and most closely mimic native enzymatic conditions. Active Motif is a leading provider of reagents, assays, and services for epigenetic drug discovery. We offer a large collection of full-length high quality HMTs, HDMs, HATs, HDACs, reader domains and enzyme complexes for many relevant drug targets, including DNMT1, EZH2, KDM5A, KDM5B, BRD4, PRC2 and MLLs. In addition, we also provide a wide selection of histone-related substrates (including full-length unmodified and modified histones and nucleosomes), antibodies and small molecules to provide a complete solution for designing assays for epigenetic drug discovery.

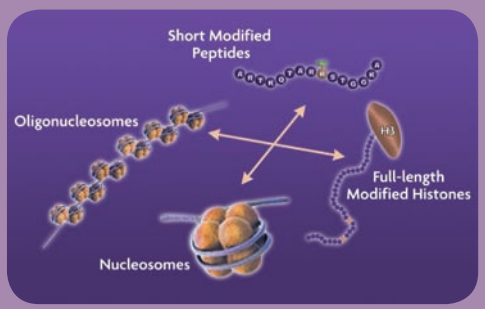
EPIGENETIC PROTEINS & ANTIBODIES

There is no need to waste valuable time and resources generating proteins and antibodies for assay development. Active Motif offers a comprehensive portfolio of over 500 ready-to-use purified recombinant epigenetic proteins, reader domains (visit www.activemotif.com/proteins, custom and bulk orders available) and over 800 antibodies for developing more efficient epigenetic assays.

HISTONE SUBSTRATES

Results are vastly improved when your assay mimics actual cellular biology. Active Motif offers a large collection of recombinant unmodified & modified histones, histone octamers and pre-assembled nucleosomes to provide you the best choice of substrate for use in your assay. For complete details visit us at www.activemotif.com/recombhis.

WHICH SUBSTRATE IS RIGHT FOR YOU?



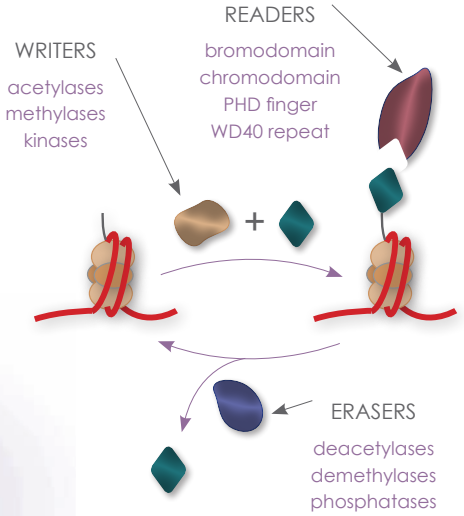
Choosing the correct histone substrate for your assay is key to achieving the best results. Opinion leaders in epigenetics recognize the power of reconstituting recombinant chromatin for creating biologically relevant substrates.

SMALL MOLECULES

Active Motif also offers an expanding collection of small molecule inhibitors and activators that modulate the activity epigenetic targets, including methyltransferases, demethylases, acetyltransferases, deacetylases and bromodomains. For a complete list of available products, go to our website at www.activemotif.com/smallmol.

THE ACTIVE MOTIF ANTIBODY PORTFOLIO

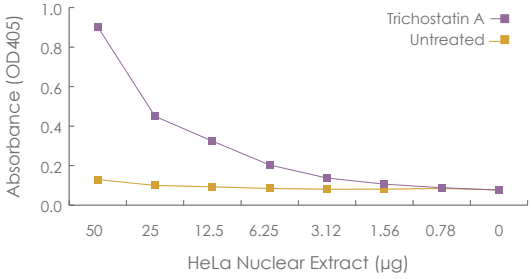
- ✓ Manufacturing in-house, and quality-tested for a range of applications
- ✓ Available and stocked in 1 mg sizes, ready to ship!
- ✓ BULK quantities up to 100 mg available upon request
- ✓ Recombinant mononucleosomes, polynucleosomes, and histones - containing all relevent modifications



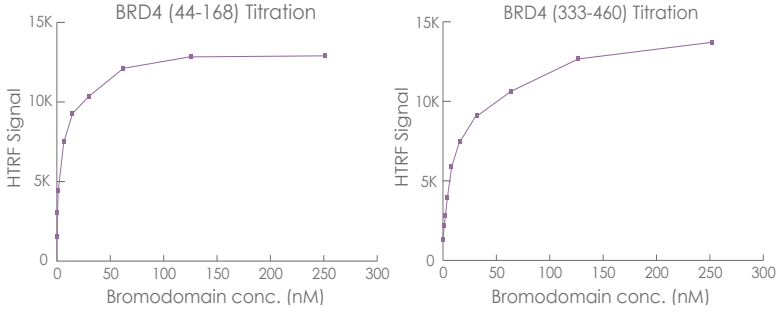
Featured Protiens

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 (page 40), the HDAC Assay Kits to analyze histone deacetylase activity (page 40, Figure 1), and our Histone Demethylase (HDM) Assay to analyze the lysine demethylase activity of LSD1 (page 41).

Active Motif recombinant proteins are manufactured to meet the highest standards of purity and activity required for conventional drug discovery assays, such as HTRF (Figure 2), mass spectrometry, AlphaLISA and Alphascreen. These assays are routinely incorporated in our quality control process to demonstrate the nM range activities needed for use in identification of potent inhibitors. We have optimized our protein production and testing to facilitate seamless integration of our recombinant proteins into drug discovery pipelines. We instill confidence in our proteins by providing comprehensive application data that is relevant to drug discovery platforms and unparalleled customer support for our drug discovery partners.



▲ FIGURE 1: Untreated and Trichostatin A-treated HeLa nuclear extracts were assayed for HDAC activity using the colorimetric version of the HDAC Assay Kit.



▲ FIGURE 2: BRD4 (44-168) and BRD4 (333-460) bromodomains (right) tested by HTRF. 3.3 µM histone peptide H4K5/8/12/16(tetra-acetyl) was incubated with the protein indicated in HTRF assay reaction buffer. Anti-FLAG antibody was used to detect the reaction products.

LIGHTSWITCH™ LUCIFERASE ASSAY SYSTEM

The LightSwitch™ Luciferase Reporter Assay System provides a comprehensive genome-wide approach for studying gene element function and identifying gene regulatory networks. In addition to over 30,000 ready-to-use promoter & 3´UTR reporter constructs, Active Motif also offers services for custom cloning and mutagenesis, pathway screening, sequence variant analysis and miRNA target validation. For more detailed information on LightSwitch products & services, please visit us at www.activemotif.com/lightswitch.

THE LIGHTSWITCH™ PRODUCT PORTFOLIO:

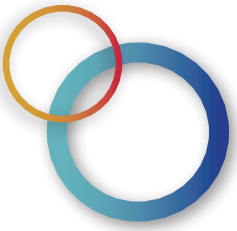
- Over 30,000 pre-cloned human promoter, lncRNA promoter & 3´UTR reporters
- miRNA mimics and inhibitors
- Validated promoter & 3´UTR controls
- LightSwitch™ Luciferase Assay reagents
- Synthetic miRNA target reporter constructs
- Synthetic promoter response elements
- Custom Cloning & Mutagenesis Services

APPLICATIONS: TRANSCRIPTIONAL REGULATION

- Study the mechanisms by which the expression of a lncRNA gene is induced or repressed
- Determine the effect of sequence variations on lncRNA promoter function
- Verify computational predictions and supplement microarray or Next-generation sequencing data

APPLICATIONS: miRNA / POST-TRANSCRIPTIONAL REGULATION

- Validate the 3´UTR targets of any miRNA
- Measure the effect of miRNA or siRNA on transcript stability or translation efficiency
- Quantify the impact of a 3´UTR in post-transcriptional gene regulation
- Assess miRNA function in response to various stimuli



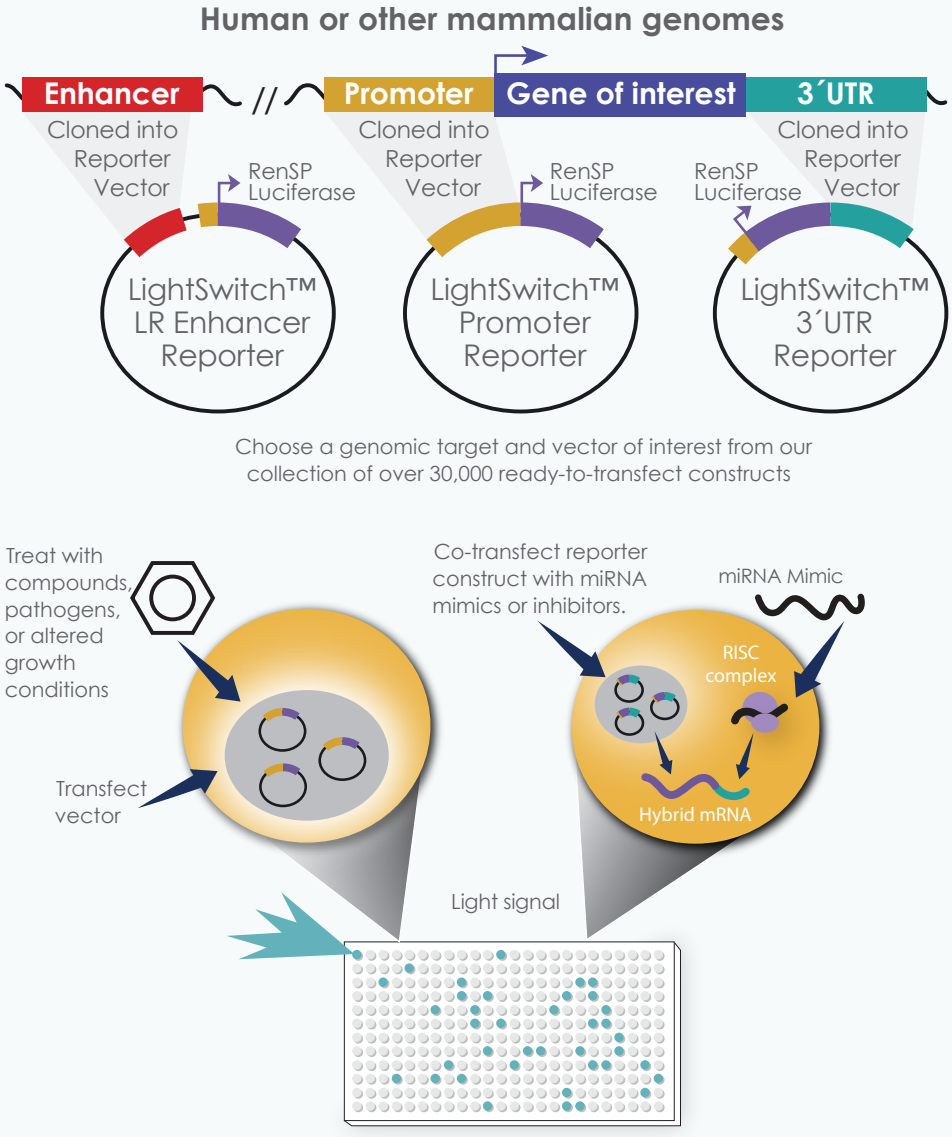
LIGHTSWITCH™ LUCIFERASE ASSAY SYSTEM

Genome in - Function Out

With a genome-wide library of over 30,000 cloned regulatory elements, custom cloning and mutagenesis services for those regulatory elements not in the collection, a highly optimized Renilla luciferase (RenSP) vector system and assay reagents, the LightSwitch™ Luciferase Assay System is the optimal solution for your gene regulation studies.

LIGHTSWITCH™ REPORTER ASSAY SYSTEM

The LightSwitch™ Reporter Assay System is a complete solution for performing gene regulation studies and/or high-throughput screening. All LightSwitch vectors utilize our RenSP luciferase engineered for gene regulation experiments to produce higher levels of luminescence with a shorter half-life than other Renilla luciferases. LightSwitch™ reporter vectors provide industry leading sensitivity and dynamic range which not only enables you to assess the activity of regulatory elements, but also map functional motifs or characterize the effects of sequence variation on gene expression.



miRNA TARGET IP KIT

Identify specific mRNA targets of miRNAs

To better understand miRNA-UTR interactions, Active Motif's miRNA Target IP Kit was designed to capture and identify the physical interactions of miRNAs with endogenous mRNA transcripts for validation of the binding targets of specific miRNAs. The targeting of a microRNA (miRNA) to a specific mRNA is mediated through the formation of an RNA Induced Silencing Complex (RISC), containing a combination of various RNA-binding proteins along with the Argonaute (Ago) protein and miRNA (Diagram 1). The Active Motif miRNA Target IP Kit utilizes a pan-Ago antibody that recognizes Ago1, Ago2 and Ago3 proteins for immunoprecipitation (IP) of miRNA/mRNA interactions as part of the RISC complex (Figure 1).

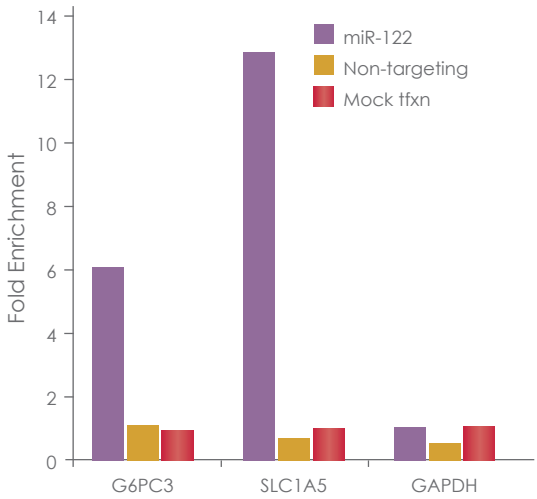


FIGURE 1: miR-122 targets G6PC3 and SLC1A5.
The miRNA Target IP Kit was used on HT1080 cells transfected with either a miR-122 mimic, a non-targeting miRNA control, or a mock plasmid control. Following IP using the Ago1/2/3 antibody or Negative Control IgG included in the kit, qRT-PCR was performed using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

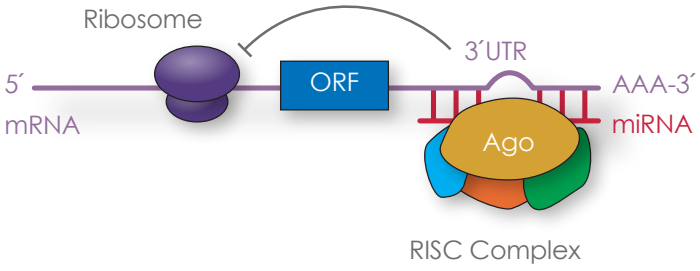


DIAGRAM 1: The miRNA within a RISC complex enables precise silencing of specific mRNA transcripts.
The key components in a RISC complex are an Ago protein and a miRNA. The Ago protein binds the miRNA, positioning it in a conformation that enables the RISC to base-pair in a Watson-Crick manner with a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

ORDERING INFORMATION

Product	Format	Cat. No.
miRNA Target IP Kit	10 rxns	25500

ASIA: +81 (0)3 5225 3638 EUROPE: +32 (0)2 653 0001 NORTH AMERICA: 877 222 9543

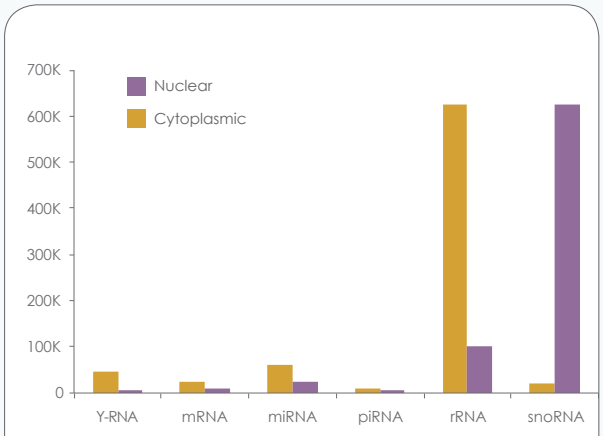
RNA SUBCELLULAR ISOLATION KIT

RNA Subcelular Isolation Kit

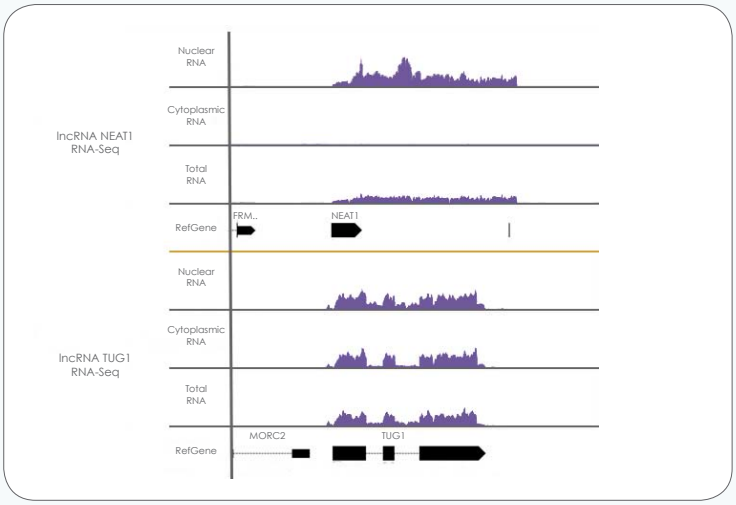
Active Motif's RNA Subcellular Isolation Kit is designed to efficiently isolate separate nuclear and cytoplasmic RNA fractions for downstream analysis. This method can be used to isolate long and short RNAs, including long non-coding RNAs (lncRNAs), microRNAs (miRNAs), short interfering RNAs (siRNAs), PIWI-interacting RNAs (piRNAs), and small nucleolar RNAs (snoRNAs) from cells or tissue without cross-contamination or the use of phenolic compounds.

ADVANTAGES

- ✓ Works with cells or tissue
- ✓ Method avoids the use of phenolic compounds
- ✓ Isolates RNA between 15 - 7K nucleotides in size
- ✓ Isolates lncRNAs, miRNAs, siRNAs, piRNAs, snoRNAs, and other short and long RNAs
- ✓ Enhanced detection of low abundance transcripts
- ✓ Reduced background from other intronic or mature RNAs
- ✓ Purified RNA is validated for use in RT-qPCR and RNA-Seq



▲ FIGURE1: The RNA Subcellular Isolation Kit shows small RNA distribution frequency between cytoplasmic and nuclear fractions. Cytoplasmic and nuclear RNA were isolated from HeLa cells using the RNA Subcellular Isolation Kit. RNA was processed and size selected to remove large RNA. 75 bp single read sequencing was performed and the data was mapped to miRBase. A frequency of the small RNA (sRNA) distribution for each fraction shows the specificity of subcellular isolation.



◀ FIGURE 2: RNA-Seq data confirms lncRNA localization
Nuclear, cytoplasmic and total RNA were isolated from HeLa cells. RNA was subjected to ribosomal RNA depletion during NGS library preparation. Samples were then sequenced using the Illumina HiSeq and 100 bp paired end reads with 50 M reads per sample. NEAT1 is a lncRNA that is primarily located in the nucleus, while TUG1 is a lncRNA known to have both nuclear and cytoplasmic localizations

ORDERING INFORMATION

Product	Format	Cat. No.
RNA Subcellular Isolation Kit	30 rxns	25501

EPIGENETIC & GENE REGULATION SERVICES

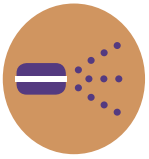
The Active Motif Custom Services team makes cutting-edge research accessible to the wider life science community. We provide services for state-of-the-art epigenetics and gene regulation analysis techniques to accelerate your research. For more information on Active Motif Custom Services, please visit us at www.activemotif.com/services.

ATAC-Seq

ChIP-Seq

RNA-Seq

DNA Methylation Analysis



Histone PTM Quantitation

RIME

ChIP SERVICES

Choose the global leader in end-to-end ChIP services

Given the importance of ChIP-Seq data sets for development and disease research, obtaining the highest quality data is crucial. Active Motif offers the most diversified portfolio for ChIP Services. As the *ChIP Experts*™, we bring over a decade of experience providing services, with over 15,000 samples processed, and the highest level of expertise of any service provider.

ChIP-Seq Services

Active Motif offers a comprehensive suite of standard and specialized ChIP-Seq Services. Our standard ChIP-Seq Services include:

- FactorPath™ ChIP-Seq – map protein-DNA interactions & histone modifications
- HistonePath™ ChIP-Seq – genome-wide profiling of histone modifications & interactions
- TranscriptionPath™ ChIP-Seq – measure global transcription rates across the genome
- ChIP-qPCR – custom analysis of any gene

In addition, Active Motif offers specialized ChIP-Seq Services that include:

- Super-enhancer Profiling – choose from our validated super-enhancer targets
- ChIP-Seq Spike-in – a novel ChIP normalization strategy to reveal latent biology
- ChIP Antibody Validation – verify if your antibody works for ChIP applications

For more detailed information about Active Motif specialized ChIP-Seq Services, visit pages 52-57 of the brochure or go to our website at www.activemotif.com/services.

SUPER-ENHANCER PROFILING SERVICE

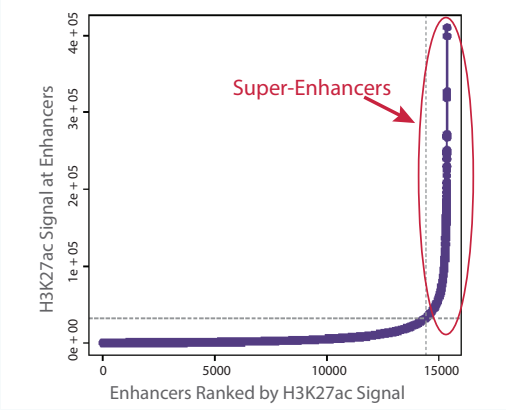
Specialized ChIP-Seq service for Super-Enhancer identification & analysis

Active Motif provides comprehensive specialized ChIP-Seq data generation and analysis services for genome-wide Super-Enhancer profiling. Our end-to-end Super-Enhancer Service* includes sample preparation, ChIP reactions using ChIP validated antibodies, library construction, sequencing on an Illumina instrument and comprehensive data analysis. To learn more, visit www.activemotif.com/services-superenhancer.

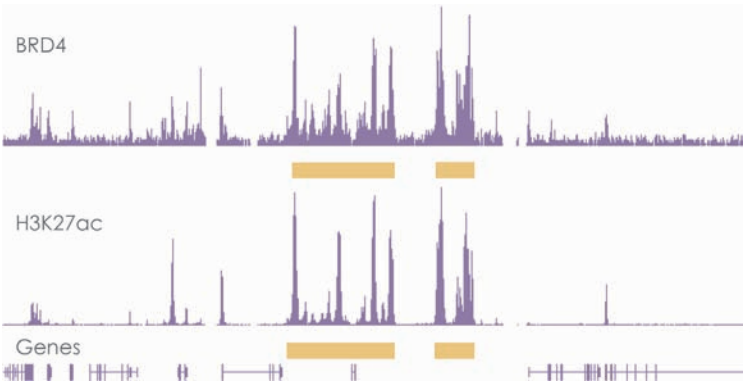
- ✓ Identify master regulators of cellular identity
- ✓ Regulatory regions associated with disease
- ✓ Reveal mechanisms of BRD4 inhibitors

TECHNICAL NOTES

Super-Enhancers are regulatory regions that control the expression of master transcription factors that function as master regulators of cellular identity. Misregulation of these transcriptional elements have been implicated in diseases such as cancer. Due to the association with disease, Super-Enhancers are important drug development targets. Active Motif utilizes validated antibodies for Super-Enhancer molecular signatures to perform chromatin immunoprecipitation followed by Next-generation sequencing to enable the most comprehensive analysis of these master regulatory domains.



▲ FIGURE 1: Identification of Super-Enhancers.
Enhancers are plotted in increasing order based on ChIP-Seq peak intensity. Super-Enhancers are the population above the inflection point of the curve.



▲ FIGURE 2: BRD4 and H3K27ac ChIP-Seq data identify Super-Enhancers.
The Super-Enhancer is defined by the clustering of high intensity peaks (copper hashes). This Super-Enhancer is marked by high intensity BRD4 and H3K27ac ChIP-Seq signal.

VALIDATED ANTIBODIES FOR SUPER-ENHANCER PROFILING

- ✓ BRD4
- ✓ H3K4me1
- ✓ SOX2
- ✓ NANOG
- ✓ H3K27ac
- ✓ OCT4
- ✓ MED1

* Patent No. 9,181,580, Syros Pharmaceuticals.

ChIP-SEQ SPIKE-IN NORMALIZATION

Novel ChIP-Seq normalization strategy to reveal latent & subtle biological effects

As a leader in ChIP innovation, Active Motif has developed ChIP-Seq Spike-in, an instrumental technical advancement in ChIP that enables more accurate sample comparisons and reveals latent biological effects.

Normalization of ChIP data reduces the effects of technical variation and sample processing bias and is universally applicable to any ChIP experiment. As part of our custom ChIP-Seq services, we can apply our ChIP-Seq Spike-in normalization strategy (page 18) across your ChIP samples and antibodies to eliminate bias and reveal real biological differences.

- ✓ Uncover latent or subtle biology
- ✓ Monitor consistency between samples
- ✓ Reduce effects of technical variation
- ✓ Eliminate sample bias

TECHNICAL NOTES

How does it work?

ChIP-SEQ REACTIONS:

- A standard ChIP-Seq reaction is set up using your experimental chromatin and antibody of interest.
- *Drosophila melanogaster* spike-in chromatin is added, or "spiked-in", to each reaction as a minor fraction of total chromatin.
- An antibody recognizing the *Drosophila*-specific histone variant, H2Av, is added to the reaction to reliably pull down a small fraction of *Drosophila* chromatin.
- Following ChIP, immunoprecipitated DNA sequences are analyzed by Next-generation sequencing (NGS).

NORMALIZATION:

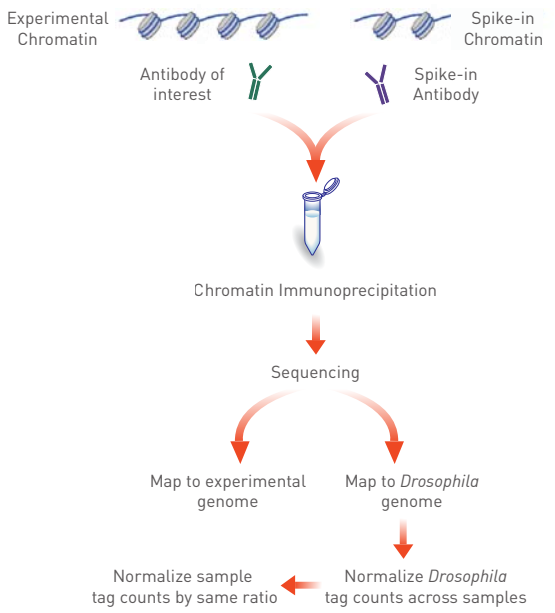
- Following NGS, sequence tags are aligned to the reference (*e.g.* human) and the *Drosophila* genome.
- Variances in *Drosophila* tag counts are equalized across samples.
- The same ratio used to equalize *Drosophila* tag counts is applied to human tag counts for normalization.

RESULTS:

Biases 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 the observation of any significant biological changes in your ChIP-Seq samples (reference the ChIP-Seq Normalization Workflow, opposite page).

For more on ChIP-Seq Spike-in Services or for ordering information, visit www.activemotif.com/services-normalize.

CHIP-SEQ NORMALIZATION WORKFLOW



A standard ChIP reaction is set up using experimental chromatin and an antibody of interest. *Drosophila* Spike-in chromatin and a Spike-in antibody that recognizes the *Drosophila* chromatin are also added to the reaction. Since variation introduced during the ChIP procedure will also occur with the immunoprecipitated Spike-in chromatin, the Spike-in signal can serve as a reference to normalize the test sample signals.

SPIKE-IN NORMALIZATION UNVEILS BIOLOGICAL EFFECTS OF COMPOUND TREATMENTS

Active Motif's ChIP-Seq Spike-in Normalization strategy reveals EZH2 inhibitor-induced changes in H3K27me3 levels that were previously undetected using a standard ChIP-Seq protocol.*

▲ **FIGURE:** 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.

WHY?

Without Spike-in normalizaion (-), uneven amplification of the ChIP DNA during preparation of Next-Gen sequencing libraries led to loss of differences between samples. With Spike-in normalization (+) the bias in PCR amplification was corrected and the difference between samples is clearly visible.

*Egan, B. *et al.* An alternative approach to ChIP-Seq normalization enables detection of genome-wide changes in histone H3 lysine 27 trimethylation upon EZH2 inhibition. *PLoS One.* 11:e0166438

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ChIP ANTIBODY VALIDATION

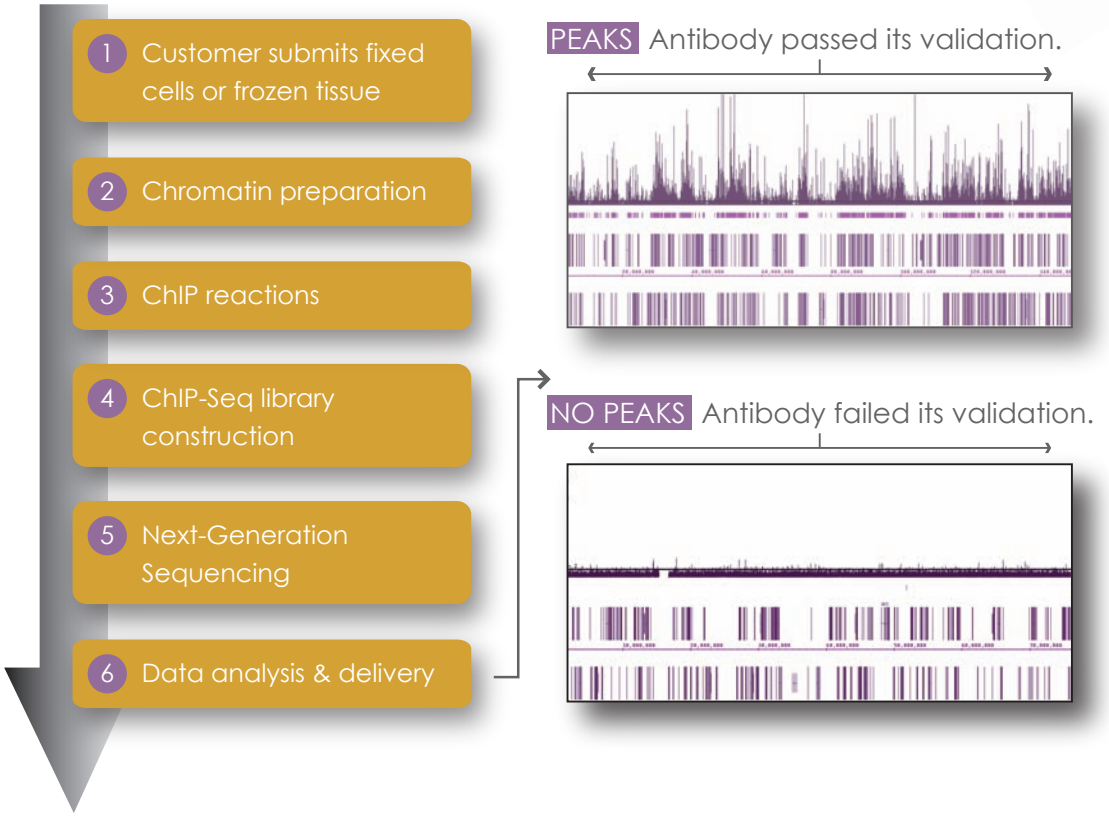
Let the *ChIP Experts*™ validate the suitability of your antibody for ChIP

One of the greatest challenges in ChIP experiments is the lack of available antibodies that can recognize fixed, target-bound proteins and that function in immunoprecipitation. Active Motif's ChIP Antibody Validation Service makes this process simple, fast, and convenient.

Only 30% of all antibodies work in ChIP-Seq. Therefore, identification of a good ChIP-Seq antibody presents a significant barrier to project initiation and completion. Our Epigenetic Services team has validated antibodies to over 350 targets. If your target of interest is on our list, we can start your project immediately. Otherwise, submit an antibody to us and our Antibody Validation Service can give you an answer in as little as 4 weeks.

- ✓ Submit any antibody for testing
- ✓ '*Yes*' or '*No*' results for ChIP-Seq antibody performance
- ✓ Fast turnaround time – results in 4 - 5 weeks.
- ✓ Hundreds of previously validated antibodies available

ChIP ANTIBODY VALIDATION SERVICES



GENE EXPRESSION SERVICES

Services for whole transcriptome sequencing & gene expression analysis

Active Motif's gene expression services include RNA-Seq for identification & quantitation of RNA transcripts and Pol II ChIP-Seq for measuring transcription rates across the genome. Combining our Gene Expression Services with ChIP-Seq data enables you to correlate transcriptional changes with transcription factor & histone modification occupancy.

RNA-SEQ SERVICE

Transcriptome profiling by RNA-Seq is a powerful tool that enables the discovery and characterization of novel and existing transcripts and the assessment of changes in gene expression in response to treatment or disease. Active Motif offers comprehensive RNA-Seq Services, from RNA isolation through data analysis, for any species or sample type. To learn more about our RNA-Seq Service, visit us at www.activemotif.com/rna-seq.

- ✓ Differential gene experession
- ✓ Changes in gene structure or splicing patterns
- ✓ Effects of TF binding on gene expression

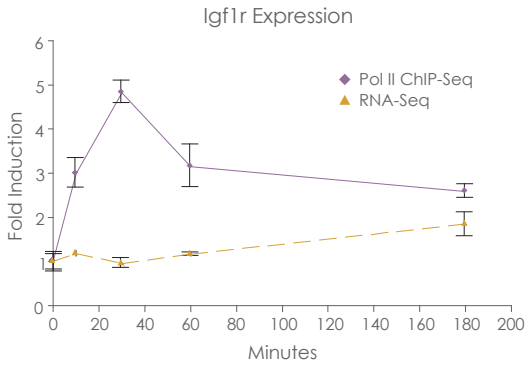
RNA-SEQ SERVICES

1. Prepare RNA from cells or tissues
2. Generate cDNA
3. Construct directional libraries
4. Perform Next-Generation Sequencing
5. Perform data analysis

POL II ChIP-SEQ SERVICE

Active Motif's TranscriptionPath™ is a chromatin IP-based assay to measure transcription rates as a function of genome-wide RNA Polymerase II occupancy. Unlike other RNA-based techniques, the method measures transcription rates independent of RNA half-life. The same sample material that is used in your transcription factor targeted ChIP experiments can be used for the Pol II ChIP-Seq assay to enable correlation of transcription factor occupancy with changes in gene expression within the same experiment. To learn more, visit us at www.activemotif.com/services-pol2.

- ✓ Measure transcription independent of RNA half-life
- ✓ Identify genes poised for transcriptional activation
- ✓ Detect alternate start sites and annotated genes
- ✓ Identify changes in gene expression at early time points
- ✓ Perform in parallel with targeted ChIP to correlate occupancy with changes in gene expression



▲ FIGURE: RNA-Seq and Pol II ChIP-Seq produce variant gene expression profiles.

Data for Igf1r was extracted from RNA–Seq and RNA Pol II ChIP-Seq data sets. Cell treatment resulted in induced gene expression that was measured at various time points. The cumulative data show that as measured by Pol II ChIP-Seq, transcription is induced immediately, while mRNA levels only accumulate over time.

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REDUCED REPRESENTATION BISULFITE SEQUENCING

DNA methylation patterns are cell-type specific, and alterations in these patterns can be indicative of disease. Reduced Representation Bisulfite Sequencing (RRBS) is a bisulfite dependent method that provides single base pair resolution of cytosine methylation at millions of locations and allowing for sample-to-sample comparisons of DNA methylation patterns. Comparing DNA methylation profiles from normal and diseased patient samples can facilitate novel biomarker discovery.

WHY IS RRBS THE RIGHT CHOICE?

RRBS is significantly less expensive than Whole Genome Bisulfite Sequencing, while still providing the methylation status of more than 4 million CpGs at biologically relevant positions such as promoters and CpG islands. You need only to provide 1 µg of purified DNA, or may also provide cells or tissues.

FEATURES

- Low starting material requirements
- Data provided on millions of CpGs
- Data from biologically relevant regions
  - promoters
  - CpG Islands

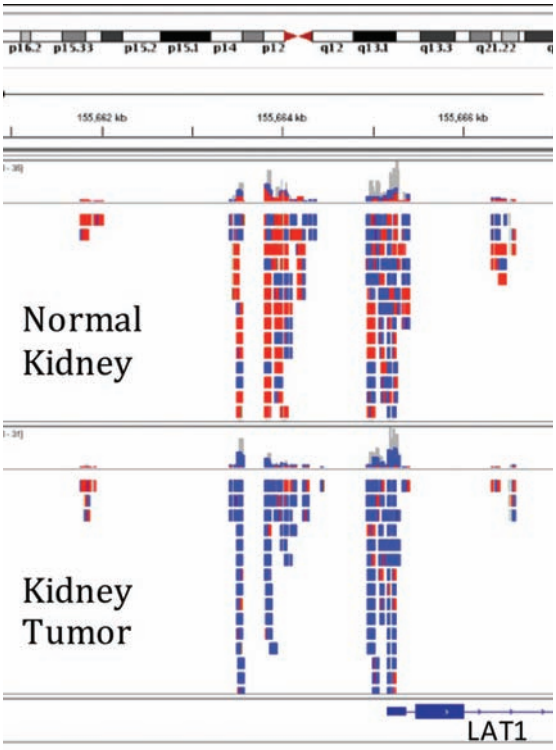
WHY USE RRBS FOR BIOMARKER IDENTIFICATION?

- Single base resolution
- Quantitation at each base
- Data at millions of locations across the genome
- Data enriched at promoters and CpG islands
- Dramatically less expensive than Whole Genome Bisulfite Sequencing

RRBS SERVICE

Customers submit DNA, cell pellets or frozen tissue then we perform

1. DNA purification
2. DNA digestion
3. Sequencing adaptor ligation
4. Bisulfite conversion
5. PCR amplification
6. Sequencing on Illumina platform
7. Bioinformatic analysis



▲ **FIGURE:** RRBS data using biopsied human kidney tumor and adjacent normal kidney. The displayed region is a representative region from the genome wide data set and shows differential DNA methylation at the promoter of the LAT1 gene. Each block is a separate data point with red representing a methylated cytosine and blue representing the unmethylated base.

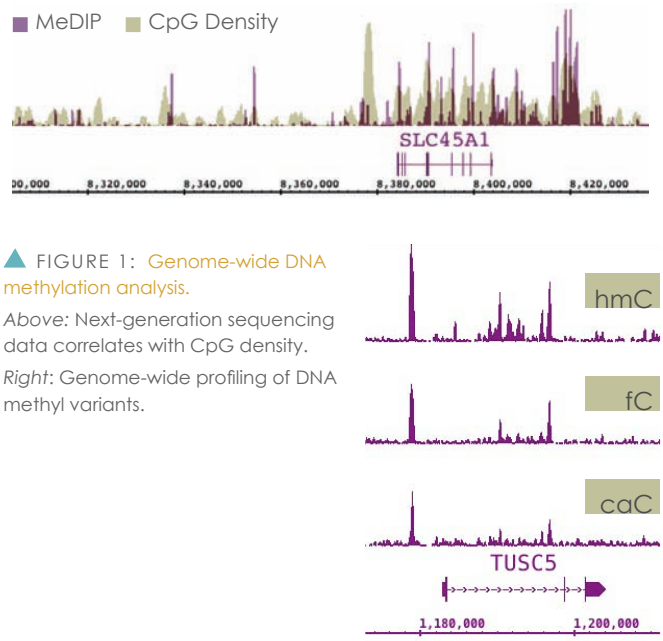
DNA METHYLATION ANALYSIS SERVICES

Genome-wide DNA methylation profiling

Active Motif provides comprehensive services for DNA enrichment and profiling of genomic regions marked by DNA methylation or DNA methyl variants. DNA is enriched using our highly validated collection of DNA methyl or methyl variant specific antibodies followed by sequencing on an Illumina Next-generation sequencing platform for genome-wide analysis of these modifications.

Genome-wide services are available for:

- ✓ 5-Methylcytosine (5-mC)
- ✓ 5-Hydroxymethylcytosine (5-hmC)
- ✓ 5-Formylcytosine (5-fC)
- ✓ 5-Carboxylcytosine (5-caC)



▲ FIGURE 1: Genome-wide DNA methylation analysis.

Above: Next-generation sequencing data correlates with CpG density.

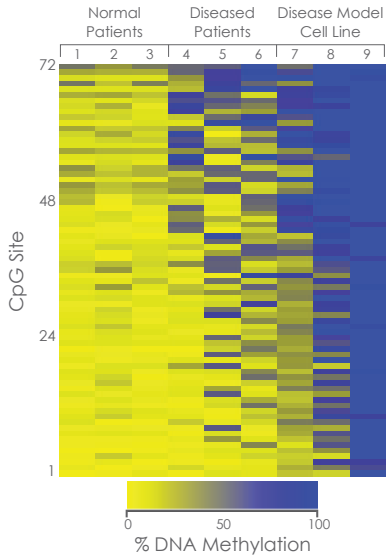
Right: Genome-wide profiling of DNA methyl variants.

Targeted Bisulfite Sequencing

Once differentially methylated regions are identified as potential biomarker candidates or regions of interest, these regions require further validation across larger populations. Active Motif's Targeted Next-generation Bisulfite Sequencing Service offers a single base-pair, high-throughput solution for targeted validation of these regions.

Services include:

- ✓ Bisulfite conversion
- ✓ Primer design and testing
- ✓ PCR amplification
- ✓ Barcoded library generation
- ✓ DNA sequencing
- ✓ Data analysis



▲ FIGURE 2: Single base-pair validation of differentially methylated sites identified by MethylCollector Ultra-Seq. Heat map represents targeted Next-generation bisulfite sequencing data of 72 CpGs from nine samples. Differential methylation is observed across the samples.

For a complete list of services, visit www.activemotif.com/servicemethylation.

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MOD SPEC®

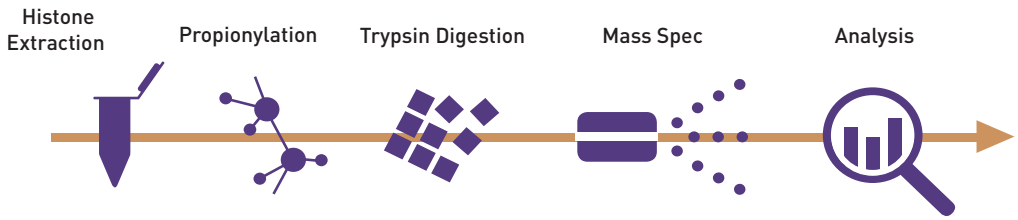
Histone modification detection service

Total nuclear levels of histone post-translational modifications (PTM) may differ under varying conditions – disease vs normal, DMSO vs inhibitor, or WT vs KO. Active Motif's Mod Spec® service can verify expected differences, and more importantly, identify unexpected changes in histone PTM levels. This service uses mass spectrometry for relative quantitation of over 80 histone modifications.

QUANTIFY HISTONE MODIFICATIONS USING MASS SPEC

- Optimized to detect over 80 different histone states
- Measure acetylation, methylation, ubiquitination, and unmodified peptides
- Analyze histone modifications on H1, H2, H3.1, H3.3, and H4
- More quantitative and comprehensive than Western blots or ELISA
- **No hassle.** Send your cells to Active Motif and receive data

HOW DOES MOD SPEC® WORK?



Cell pellets or tissues are sent to Active Motif and processed.

1. Histones are acid extracted
2. Lysines are blocked to prevent trypsin cleavage at all lysine amino acids
3. Histones are digested using trypsin
4. Peptide masses are measured using mass spectrometry
5. Data is analyzed to determine modifications on each histone peptide

HISTONE PTM QUANTITATION SERVICE

High-throughput multiplex screening of histone modification levels

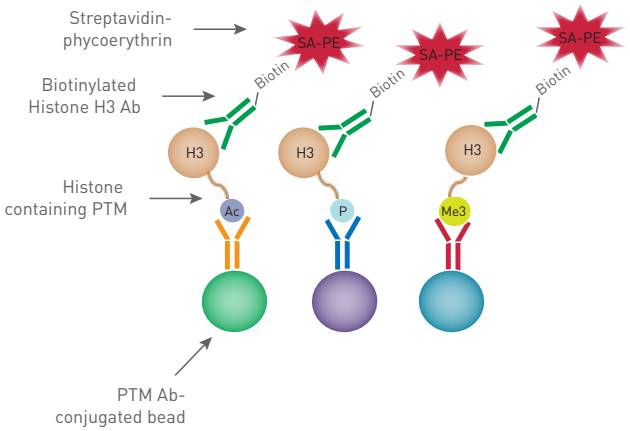
Active Motif's Histone PTM Quantitation Service gives you access to ground-breaking Luminex® xMAP® magnetic bead-based multiplexing technology (page 43) to enable simultaneous measurement of multiple histone modifications in a single reaction. This first-of-its-kind multiplex epigenetic assay generates more data using less sample material than traditional Western blot, mass spectrometry or ELISA methods and enables you to analyze both specific and off-target effects in your sample simultaneously.

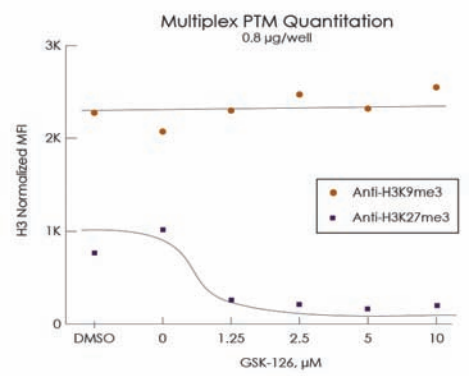
SERVICE FEATURES

- ✓ MULTIPLEX – analyze multiple histone modifications in a single assay
- ✓ EFFICIENT – use less input amounts than WB or ELISAs
- ✓ SENSITIVE – 250K cells is sufficient to quantify 13 histone modifications
- ✓ HIGH CONTENT – simultaneously measure specific & off-target effects

HISTONE PTM MULTIPLEX ASSAY WORKFLOW

Histone-specific modifications on the N-terminus are captured using antibodies conjugated to fluorescent-labeled magnetic beads. Each bead emits a unique fluorescent signal to enable detection of multiple targets within the same well. A biotinylated H3 C-terminal antibody is used to capture histones. Streptavidin-phycoerythrin is then added to bind biotin and produce a signal. A Luminex instrument is used to read signals and decipher both the bead identity and the number of binding events pertaining to each histone modification.





◀ FIGURE: Histone PTM quantitation data showing EZH2 inhibitor GSK-126 reduction of H3K27me3 but not H3K9me3 levels.

Acid extracts of HeLa cells treated for 10 days with 0 - 10 µM GSK-126 were used either in a three-bead multiplex assay containing H3-Total, H3K9me3 and H3K27me3 beads demonstrate reduction in H3K27me3 levels.

AVAILABLE TARGETS	
H3K4me3	H3 pan-ac
H3K9ac	H3S10ph
H3K9me1	H3T11ph
H3K9me2	H3K27ac
H3K9me3	H3K27me2
H3K9me3	H3K27me3
H3K36me3	

For more detailed information on our Histone PTM Quantitation Service, visit www.activemotif.com/services-luminex.

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RIME

Mass spectrometry identifies co-factor recruitment into transcriptional complexes

Active Motif's RIME (Rapid Immunoprecipitation Mass Spectrometry of Endogenous Proteins) Service sheds light on the complex process of gene regulation by enabling capture and identification of chromatin associated proteins that interact with an endogenous protein of interest.

WHY RIME? Gene regulation is often oversimplified when the focus is on one particular transcription factor in any given cell model. In reality, differential gene expression is greatly influenced by co-factors and other protein interactions within chromatin. RIME clarifies this complexity by providing a means to identify the protein interactions that are important for gene regulation.

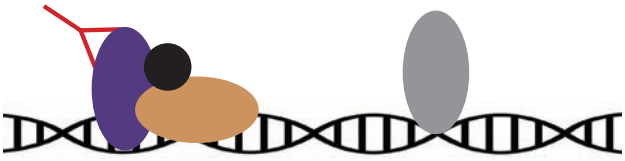
The RIME advantage

- ✓ Targeting only DNA and chromatin associated proteins
- ✓ Enabling capture of low affinity interactions
- ✓ Allows more stringent wash conditions resulting in less non-specific interactions

EXPERIMENTAL DESIGN

1. Antibody validation is performed on a single sample to show that the target protein is detected
2. IP-mass spec using the target antibody is performed in duplicate
3. IP-mass spec using anti-IgG is performed in duplicate
4. IgG interactions are removed from the target antibody specific interaction list

RIME identifies interacting TFs and cofactors



- Targeted TF
- DNA binding direct interactor
- Non-DNA binding direct interactor
- Indirect interactor

Ligand 1	Ligand 2
Estrogen Receptor	Estrogen Receptor
Nuclear receptor co-activator 3	Vang-like protein 1
Nuclear receptor interacting protein 1	Pericentriolar material 1 protein
Pericentriolar material 1 protein	Centrosomal protein of 131 kDa
Centrosomal protein of 131 kDa	Protein GREB1
CREB-binding protein	E3 ubiquitin-protein ligase TRIM33
E3 ubiquitin-protein ligase TRIM33	Nuclear receptor interacting protein 1

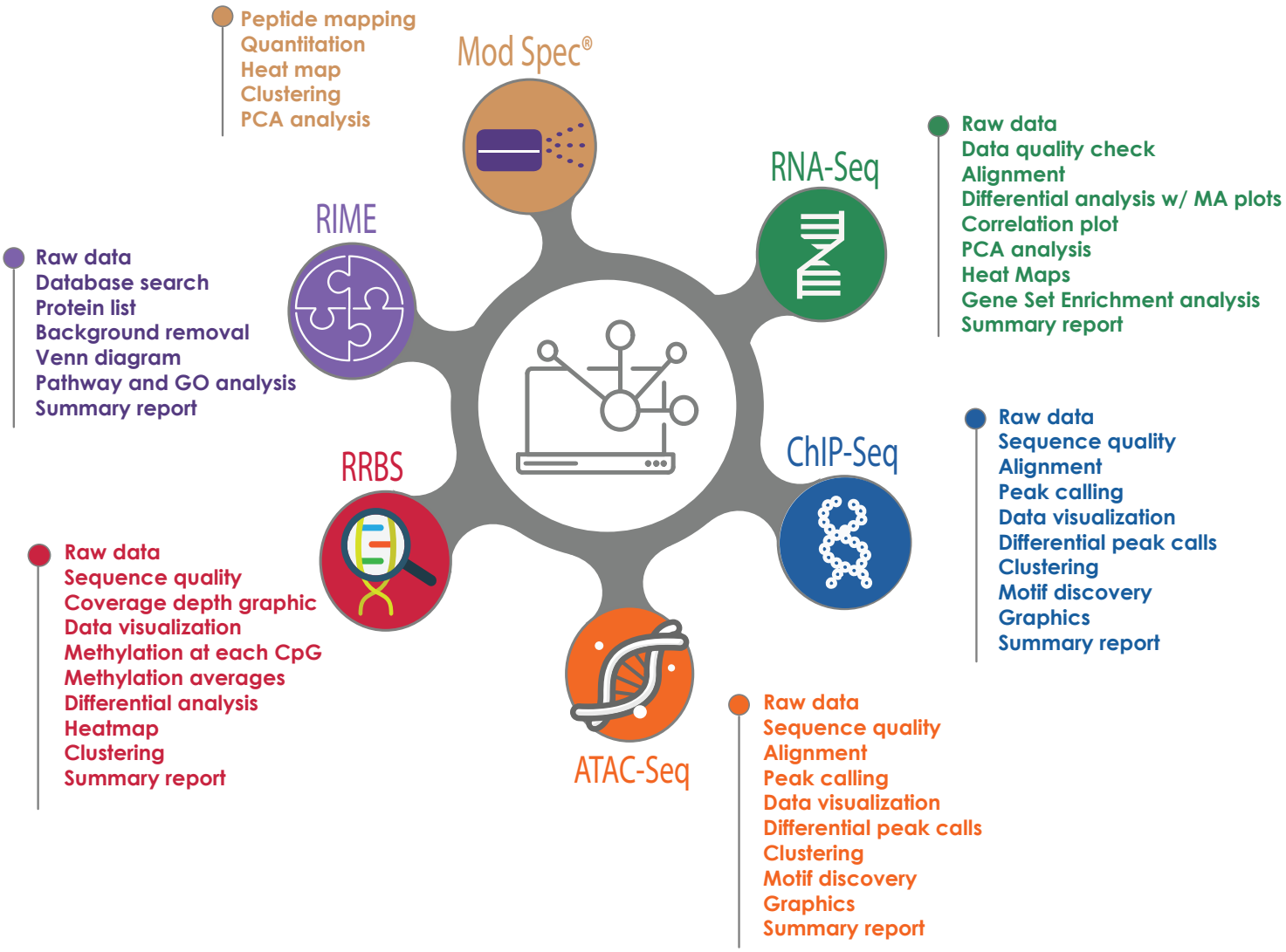
▲ **EXAMPLE DATA FROM RIME:** Different Estrogen Receptor (ER) binding profiles have been observed depending on the ligand used to stimulate ER binding. Our RIME data shows differential recruitment of co-factors to DNA bound estrogen receptor after ligand 1 and ligand 2 treatment. Grey indicates recruited proteins with similar rank order for both ligands. Red indicates common proteins detected, but with change in order. Purple indicates unique interacting proteins.

For more information about RIME Services, visit www.activemotif.com/rime.

BIOINFORMATIC SOLUTIONS

Comprehensive and customizable data analysis & support from our expert team of scientists

To help our clients interpret large and highly complex whole-genome data sets, our Services team provides complete bioinformatic analysis and support for all services. We leverage our extensive experience and background to extract relevant information embedded within complex data sets and provide you with the most meaningful and highest quality data analysis.



For more information, please visit us at www.activemotif.com/services.

LIGHTSWITCH™ CUSTOM SERVICES

Custom Cloning and Mutagenesis Service

assess the activity of regulatory elements using the LightSwitch system enginered to provide industry-leading sensitivity and dynamic range

In addition to the 30,000 human regulatory elements available as pre-cloned LightSwitch reporter vectors, Active Motif offers custom services to clone and/or mutate any human, mouse or rat genomic element into any LightSwitch reporter vector. Services include cloning and sequence validation of every reporter construct. LightSwitch vectors are engineered to provide optimal kinetics from your reporter assays. For more on LightSwitch™ Custom Services, visit www.activemotif.com/ls-services.

SERVICE FEATURES

- Clone any genomic element of interest
- Promoter, long-range, 3´UTR, and 5´UTR reporter vectors available
- Ideal for gene regulation studies or validation of ChIP binding events
- Create sequence variants of any genomic sequence
- Custom vectors ready in 6-8 weeks

INSERT ANY ELEMENT...	FROM...	INTO ANY LIGHTSWITCH VECTOR
Promoters	Rat	Promoter
Enhancers	Mouse	Long-range
UTRs	Human	3'UTR
Motifs		5'UTR
SNP variants		

For more on Custom Cloning & Mutagenesis Services or to place an order, please visit www.activemotif.com/ls-services.

IDENTIFY SEQUENCE MOTIFS NECESSARY FOR FUNCTION

	WT seed seq	Mutant seed seq
RIMS1	ACACTCC	AGTCTCC
GNPDA2	ACACTCCA	AGTCTCCA
ANKRD13C	ACACTCC	ACAGACC
G6PC3	ACACTCCA	ACAGACCA
G3BP2	ACACTCC	GGTCTCC
ALDOA	ACACTCCA	AGGTCTCCA

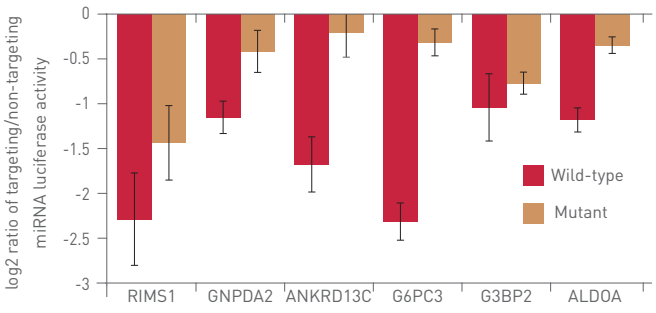


FIGURE 1: LightSwitch 3´UTR Reporter constructs containing the 3´UTRs of 6 genes known to be miR-122 target sites were subjected to site-directed mutagenesis. Constructs were co-transfected in cells with an miR-122 miRNA mimic or non-targeting control and then assayed to identify seed sequences necessary for miR-122 function. Wild-type miRNAs reduce luciferase expression through 3´UTR interactions. miRNA mutations in the seed sequence disrupt miRNA targeting and reduce the functional effect.

LIGHTSWITCH™ SYSTEM APPLICATIONS

TRANSCRIPTIONAL REGULATION

- Assess promoter and enhancer response to transcription factor (TF) modulation
- Confirm functionality of binding sites identified in ChIP-Seq experiments
- Measure functional effects of mutated motifs
- Validate computational predictions and add biological relevance to microarray or NGS data

POST-TRANSCRIPTIONAL REGULATION

- Validate the 3´UTR targets of any miRNA, Figure 1
- Measure the effect of miRNA or siRNA on transcript stability or translation efficiency
- Quantify the impact of a 3´UTR in post-transcriptional gene regulation
- Assess miRNA function in response to various stimuli

HIGH-THROUGHPUT SCREENING (HTS)

- Primary screens using the LightSwitch stable cell lines (agonist or antagonist mode)
- Secondary screens using our collection of over 18,000 promoters, TF response elements, and validated biomarkers
- Investigate MOA, specificity, and off-target effects
- Dose response analysis

VALIDATED PATHWAY REPORTER CELL LINES

Target and Pathway Panel	
HIF-1a	Hypoxia
NFkB	Inflammation
AhR	Dioxin, Toxicology
HSF1	Heat shock
p53	DNA damage, apoptosis
STAT	Interferon
SREBP	Cholesterol biosynthesis
GR	Glucocorticoid
BMP	Bone Morphogenesis
Control	Basal factors
Custom	Any pathway or target

To learn more, visit www. activemotif.com/services

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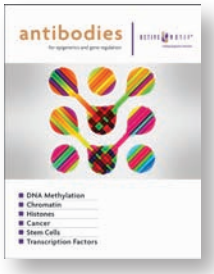
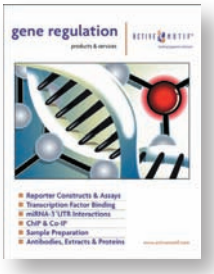
More Tools for Epigenetics Research

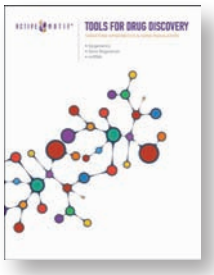


Antibodies for Epigenetics and Gene Regulation



Products for Gene Regulation

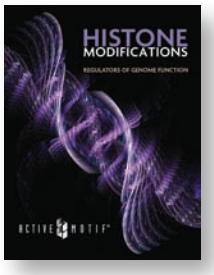
Custom Services



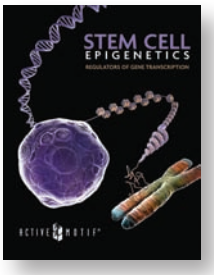
Tools for Drug Discovery

Tools for Disease Research

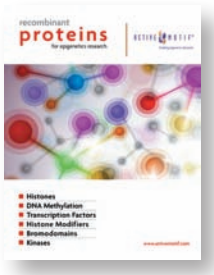
Products for Sonication



Histone Modifications Guide



Stem Cell Epigenetics



Recombinant Proteins for Epigenetics Research

Get any (or all) of our product information by mail or by download

In addition to this product profile, Active Motif has created profiles that describe our products in other areas of epigenetics and gene regulation. These detailed brochures can be downloaded or requested by mail at www.activemotif.com/info. Product manuals and technical data sheets are also available on our website.

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