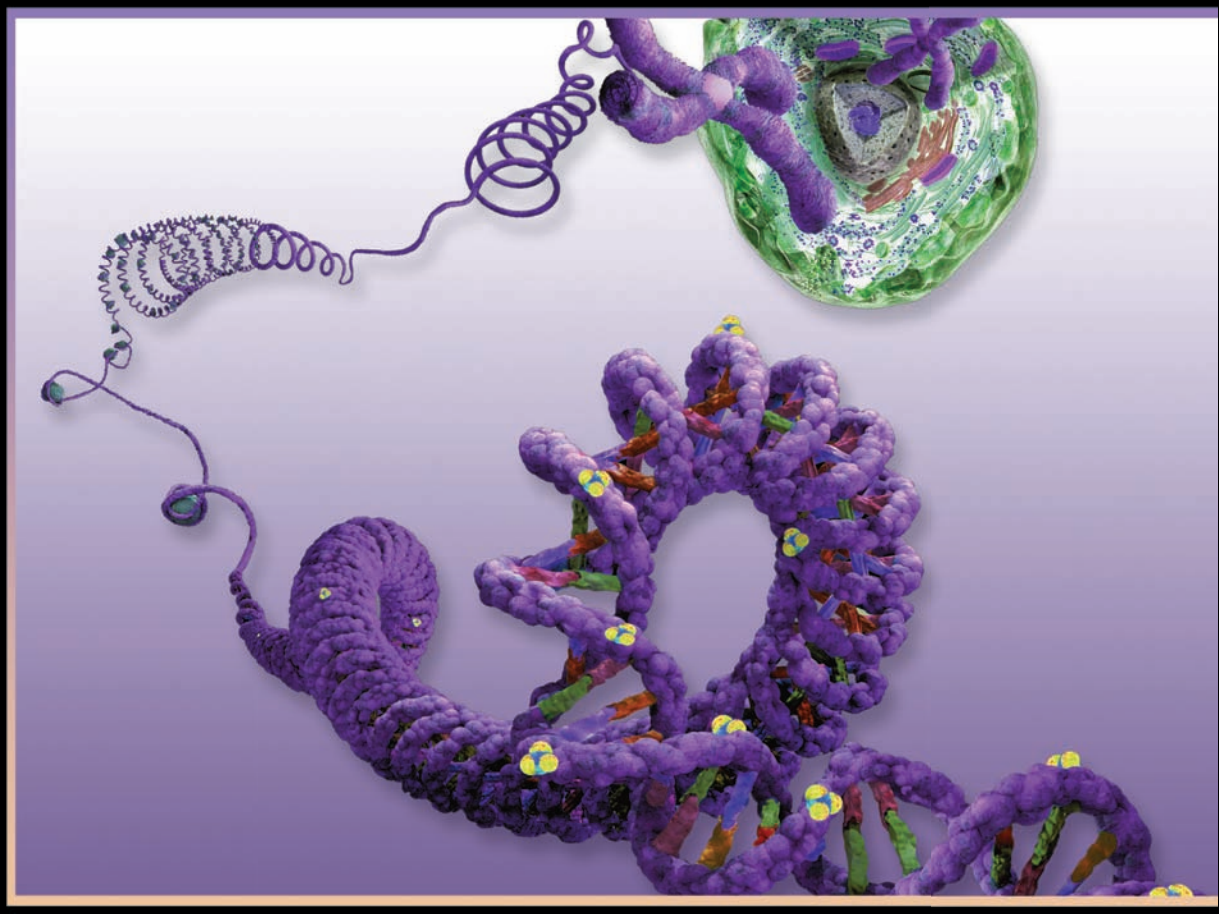
ACTIVE MOTIF®

MOTIFvations

The Newsletter of Active Motif October 2010 Volume 11 Number 3



IN THIS ISSUE

Epigenetics, Chromatin Biology & Cell Signaling

NEW: RNA ChIP-IT™ for Studying Chromatin-associated RNAs

Evidence is building that RNA-directed processes play a critical role in orchestrating chromatin architecture and epigenetic memory. Nucleic acids purified from chromatin are 2-5% RNA; these RNAs are non-coding sequences that play important roles in chromatin structure and transcriptional silencing. But, characterizing these RNAs by ChIP techniques is difficult due to the complexity of chromatin and the high amounts of DNA in chromatin.

Optimized RNA-ChIP

To make the characterization of the role of RNA in genome regulation possible, Active Motif has leveraged its expertise in ChIP to develop the first of its kind kit for RNA-ChIP. The RNA ChIP-IT™ Kit was designed to study RNA-protein interactions in a chromatin context, and optimized to recover RNA for RT-PCR analysis. It contains sufficient material for 25 assays and uses protocols that utilize magnetic beads, which improve results while reducing time and effort.

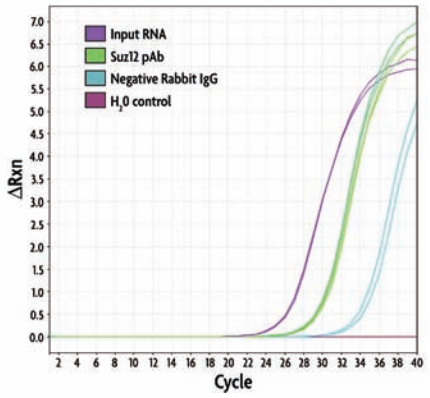
The RNA ChIP-IT method

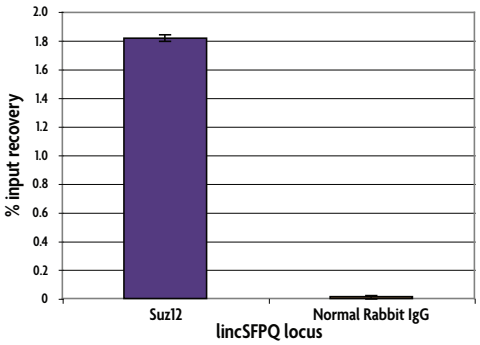
RNA ChIP-IT uses a modified ChIP protocol that has been optimized for RNA preservation and recovery. RNA-protein interactions are fixed with formaldehyde, and chromatin shearing is combined with DNase treatment to yield RNA/protein complexes that can be immunoprecipitated with antibodies to specific proteins. Cross-links are subsequently reversed; RNA is recovered and again treated with DNase to ensure the absence of DNA. The optimized method is quick and has been successfully used to study several non-coding RNAs in the chromatin context.

Advantages of the RNA ChIP-IT Kit

- Specifically tailored to study chromatin-associated RNA
- Designed to remove DNA while maintaining RNA integrity
- Step-by-step protocols for fixation of chromatin, sonication and immunoprecipitation, all optimized for RNA preservation
- Includes all RNase and protease inhibitors at precise concentrations
- Separate control kit available with control antibody and primers

In contrast to other kits designed to study RNA-protein interactions, called RIP kits, RNA ChIP-IT is designed specifically to extract and immunoprecipitate RNA from chromatin, and to solve the associated challenges in extracting chromatin while preserving RNA integrity, and removing all DNA, for a clean result that is attributable to RNA alone. The RNA ChIP-IT Kit is the solution optimized for the Epigeneticist studying RNA, rather than the RNA biologist.





Figures 1 & 2: Real-time RT-PCR analysis and % input recoveries of Suz12/normal rabbit IgG RNA-ChIP samples. The RNA ChIP-IT Kit was used on 10 µg samples of DNase I-treated HeLa chromatin with 10 µl of Suz12 antibody (Catalog No. 39357) and 2 µg of Normal rabbit IgG. Real-time RT-PCR was performed using primers for the lincRNA SFPQ locus. The amplification plot (left) and % input recoveries (right) are shown. Dividing the input recovery of the Suz12 antibody by that of the rabbit IgG indicates a 141-fold enrichment of the lincSFPQ region with the Suz12 antibody.

Take control of your RNA-ChIP

Also available is the RNA ChIP-IT Control Kit, which was designed to be used with RNA ChIP-IT. The kit contains positive & negative control antibodies and RT-PCR primers for the lincSFPQ locus. These are used to verify that the chromatin sample was prepared correctly, and to validate antibodies for use in RNA-ChIP.

Start investigating the role of RNA today

For more complete information, including a downloadable product manual, visit www.activemotif.com/rnachip.

Get Active Motif's New Histone Modifications Poster

Request your free copy of our new poster, which details histone modifications and their readers, writers and erasers, at: www.activemotif.com/poster.

Product	Format	Catalog No.
RNA ChIP-IT™	25 rxns	53024
RNA ChIP-IT™ Control Kit – Human	5 rxns	53025

NEW: Simple Formaldehyde Detection of Histone Demethylase Activity

The fluorescent Histone Demethylase Assay is a simple assay to analyze the efficiency of lysine specific demethylase enzyme (LSD1, also known as KDM1) samples, or to screen compounds for changes in histone demethylation activity. The Histone Demethylase Assay is designed to detect the formaldehyde released from the reaction of LSD1 with a methylated substrate. As the LSD1 enzyme demethylates the recombinant histone H3K4me2 substrate, formaldehyde is released as a by-product, which then reacts with the Detection Reagent to generate a fluorescent signal equivalent to the overall production of formaldehyde.

Histone Demethylase Assay advantages

- The recombinant histone H3K4me2 substrate used in the assay mimics a native histone substrate, providing you with results that more closely resemble *in vivo* conditions
- Complete assay includes a Demethylation Standard for formaldehyde quantification and an LSD1 enzyme as a positive control protein
- Simple fluorescent assay detects the formaldehyde by-product using an excitation wavelength of 410 nm and an emission wavelength of 480 nm

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

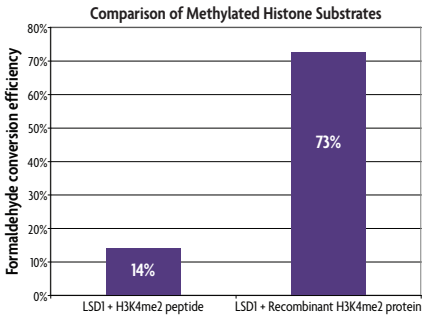


Figure 1: Comparison of different histone substrates and their effect on LSD1 demethylase efficiency. The positive control LSD1 enzyme from the Histone Demethylase Assay was used to evaluate demethylase activity using either a peptide or the kit's recombinant histone protein. One µg of LSD1 was tested with either 70 µM H3K4me2 peptide or with 13 µM recombinant histone H3K4me2 protein. LSD1 was able to convert 73% of the recombinant histone substrate into a formaldehyde by-product, yet it was only able to convert 14% of the peptide substrate into a formaldehyde by-product.

NEW: Pre-made HeLa Mononucleosomes Simplify Substrate Analysis

Histone analysis can be complicated when working with nucleosomal arrays, due to the number of potential binding interactions that are possible on the histone tails, histone globular domain and the nucleosomal DNA. Active Motif's new pre-made HeLa Mononucleosomes reduce the complexity of potential interactions, enabling simplified substrate analysis with histone methyltransferases, histone demethylases, histone acetyltransferases and histone deacetylases. To view a list of Active Motif's histone modifying enzymes, please visit www.activemotif.com/hismodenz.

Product	Format	Catalog No.
HeLa Mononucleosomes	10 µg	53300

New Recombinant Histones Available

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

For an up-to-date list of our more than 20 recombinant histones, please visit www.activemotif.com/recombhis.

NEW: MODified™ Array Labeling Kit and Free Software for Analysis

The MODified™ Histone Peptide Array* is a useful research tool that can be used to screen antibodies, proteins and enzymes for interactions with histones and their post-translational modifications. Each array contains 384 different histone modification combinations in duplicate. Modifications include acetylation, methylation, phosphorylation and citrullination on the N-terminal tails of histones H2A, H2B, H3 and H4. Active Motif's new MODified™ Array Labeling Kit contains all the necessary buffers and reagents for ECL-based detection of the MODified Histone Peptide Arrays.

How do the MODified Arrays work?

The MODified Histone Peptide arrays contain 384 unique histone peptides spotted in duplicate. Each 19mer peptide contains up to four separate modifications to allow researchers to study not only individual sites, but also to determine if neighboring modifications alter site recognition and binding.

The simple array protocol works like a Western blot, using either ECL or colorimetric detection systems. The image is then captured using film or a CCD camera; no special equipment is needed (Figure 1). For analysis, Active Motif's free Array Analyse Software can be used.

The MODified Histone Peptide Arrays are available individually, or in packs of five. For a complete solution, Active Motif's new MODified Array Labeling Kit provides all the reagents needed for ECL-based detection.

What's in the box?

The MODified Array Labeling Kit contains blocking buffer, wash buffer and ECL reagents for reliable and reproducible chemiluminescent detection. For added convenience, an antibody to recognize the arrays' control c-Myc peptide and HRP-conjugated secondary antibodies are also included.

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

Free software for analysis

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

MODified Array advantages

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

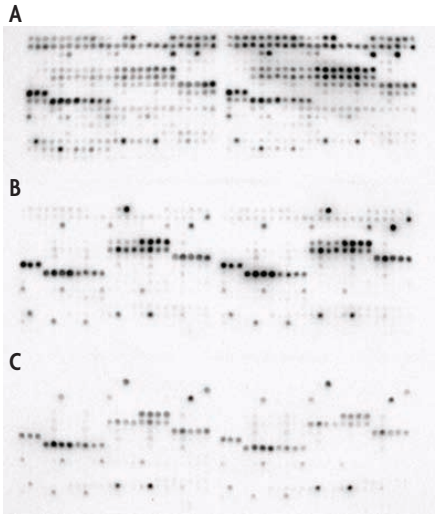


Figure 1: Images of ECL detection of MODified Histone Peptide Arrays treated with HMT G9a.
MODified histone peptide Arrays were treated with A) 25 µM G9a methyltransferase (Catalog No. 31327), B) 25 µM G9a mutant H904K (Catalog No. 31328), C) no enzyme control, overnight in the presence of 1 mM AdoMet.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

Visit www.activemotif.com/modified to view a complete list of the peptides available on the MODified™ Histone Peptide Array, to learn more about the MODified™ Array Labeling Kit, or to download the free Array Analyse Software and instruction manual.

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

Explore Chromatin Biology with Recombinant Histone Modifying Enzymes

The eukaryotic genome is packaged into the nucleus through the compaction afforded by the incorporation of DNA into chromatin. The primary structural components of chromatin are the highly conserved histone proteins, around which DNA is wrapped and organized. Histones are subject to a variety of reversible post-translational modifications that are tightly regulated; these include phosphorylation, acetylation, methylation and ubiquitylation. These modifications represent important regulatory events that govern the accessibility and function of the genome.

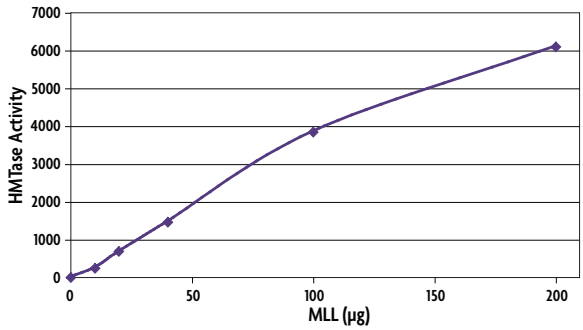


Figure 1: Recombinant MLL activity measured using a fluorescent histone H3 lysine 4 methyltransferase assay.
Increasing amounts of MLL protein (Catalog No. 31338) were incubated with the substrate and then the reaction was developed. MLL activity is measured in relative fluorescence units.

Histone modifications are dynamically regulated and are deposited and removed by enzymes that are generally part of large multi-subunit protein complexes recruited to chromatin by sequence-specific DNA binding proteins. These histone modifying enzymes are important regulators of genome function, and studying their function offers insight into the mechanisms that regulate processes dependent upon the genome.

Active Motif offers a variety of recombinant histone modifying enzymes, such as **Acetyltransferases, Deacetylases, Demethylases & Methyltransferases**, for use in your exploration of chromatin biology. To see our complete offering, please visit www.activemotif.com/hismodenz. To get them at **15% off**, please visit www.activemotif.com/promo.

Rigorously Tested Antibodies to Chromatin Proteins

The proteins associated with chromatin are important to the organization of genomic DNA and the regulation of its activity and accessibility. Active Motif is committed to providing high-quality reagents for the study of epigenetics and chromatin biology. We specialize in developing antibodies to study histones, chromatin proteins and transcription factors, which are then validated for use in the applications you need them for: Western blot, chromatin immunoprecipitation (ChIP) and immunofluorescence.

Chromatin Protein Antibody Categories

- Non-histone chromatin proteins
- Histone modifying enzymes
- Polycomb group proteins
- Chromatin remodeling proteins

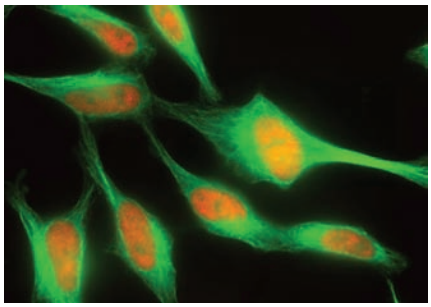


Figure 1: HDAC2 detected by immunofluorescence.
HeLa cells were stained with HDAC2 monoclonal antibody (Clone 3F3, Catalog No. 39533) at a dilution of 1:1,000. Red: HDAC2 staining. Green: alpha-Tubulin mouse monoclonal (Clone 5-B-1-2, Catalog No. 39527) conjugated to Chromeo™ 488.

To see our complete offering of antibodies to chromatin-associated proteins, visit www.activemotif.com/chromabs. For our complete list of antibodies to histone and histone modifications, visit www.activemotif.com/histoneabs. To find out more about our antibody development and validation process, visit www.activemotif.com/abdevel.

NEW: Study Both Types of DNA Methylation with the Proper Controls

Methylation of DNA at the 5´ position of cytosines is an important regulator of cellular function, influencing gene expression, cellular identity and disease development. With the recent discovery of the importance of a novel form of DNA methylation, 5-hydroxycytosine methylation, it is crucial to have the right tools for your experiments. No matter which type of DNA methylation you are studying, Active Motif's new Methylated DNA Standard Kit can help.

The importance of DNA methylation

In mammals, DNA is methylated at the 5´ position of cytosines and is found primarily at clusters of CpG residues that reside in the promoter region of half of all human genes. Non-CpG methylation, however, is also very abundant in certain cell types such as stem cells. DNA methylation is involved in many cellular functions including embryonic development, genetic imprinting, X chromosome inactivation and control of gene expression. DNA methylation and chromatin modifications interact to bring about transcriptional silencing. Aberrant DNA methylation patterns are also associated with certain cancers as well as developmental abnormalities.

A novel type of DNA methylation, 5-hmC

In addition to cytosine methylation, several papers have been published recently describing the relative abundance of 5-hydroxymethylcytosine (5-hmC) in specific cell types. 5-methylcytosine (5-mC) is converted into 5-hmC by the TET family of iron-dependant oxygenases. 5-hmC can be replaced by cytosine by DNA repair proteins, thus conversion of 5-mC into 5-hmC may represent a pathway by which DNA is demethylated.

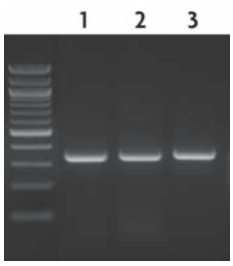


Figure 1: Agarose gel analysis of samples supplied in the Methylated DNA Standard Kit.
Five hundred ng of each DNA standard was loaded on a 2.5% agarose gel.

Lane 1: unmethylated DNA.
Lane 2: 5-methylcytosine containing DNA.
Lane 3: 5-hydroxymethylcytosine containing DNA.

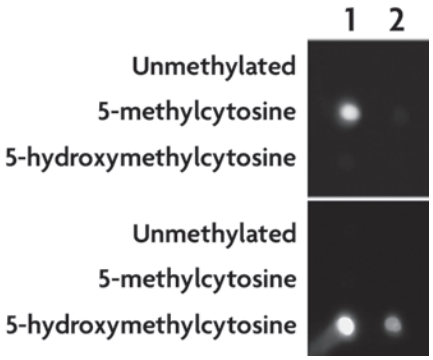


Figure 2: Dot blot analysis of Methylated DNA Standard Kit samples.
DNA (50 ng) from the three different samples was spotted onto a positively charged nylon membrane.
Top panel: Dot blot probed with 5-Methylcytidine antibody (Clone 33D3) (Cat. No. 39649, 1:1,000 dilution).
Bottom panel: Dot blot probed with 5-Hydroxymethylcytidine antibody (Cat. No. 39769, 1:5,000 dilution). **Lane 1:** Single-stranded DNA. **Lane 2:** Double-stranded DNA.

Active Motif's Methylated DNA Standard Kit includes three recombinant DNA standards derived from the APC gene regulatory region: unmethylated DNA, 5-methylcytosine methylated DNA and 5-hydroxymethylcytosine methylated DNA. This kit (which also includes PCR primers specific to the APC gene) can be used to provide controls for experiments studying the different types of DNA methylation. It can be used in the study of either 5-methylcytosine or 5-hydroxymethylcytosine methylation. It has been validated for use in MeDIP and dot blot and may be useful in gel shift-type experiments studying proteins that bind 5-mC or 5-hmC methylated DNA.

Methylated DNA Standard advantages

- Your experiments can now utilize controls for both forms of DNA methylation – 5-methylcytosine and 5-hydroxymethylcytosine
- Includes primers that work in endpoint and real-time PCR
- Validated for use in MeDIP and dot blot experiments
- DNA is methylated at both CpG and non-CpG sites

Full line of DNA Methylation products

Due to the importance of DNA methylation in development and disease, much of today's epigenetic research depends on the ability to accurately detect and quantify DNA methylation. Active Motif offers a broad range of products for DNA methylation analysis, from methylation enrichment kits to recombinant proteins and antibodies against proteins involved in DNA methylation. For a complete list of products available for the study of DNA methylation, please visit www.activemotif.com/dnamt.

Product	Format	Catalog No.
Methylated DNA Standard Kit	3 x 2.5 µg	55008
5-Hydroxymethylcytidine antibody (rabbit IgG)	100 µg	39791
5-Hydroxymethylcytidine antibody (rabbit serum)	100 µl	39679
5-Methylcytidine antibody (mouse IgG)	50 µg	39649
Fully Methylated Jurkat DNA	10 µg	55003
Jurkat genomic DNA	10 µg	55007

Reproducible Bisulfite Conversion for Accurate Analysis of DNA Methylation

Bisulfite conversion is a useful tool to obtain single nucleotide resolution information about the methylation status of a particular region of DNA. The MethylDetector™ Bisulfite Modification Kit makes DNA methylation analysis fast and efficient by providing optimized reagents, time-saving DNA purification columns and positive control PCR primers for assay validation.

Proven controls verify your success

DNA methylation analysis often uses bisulfite to convert unmethylated cytosines to uracils, leaving methylated cytosines unchanged. The DNA is then PCR amplified and analyzed by sequencing or restriction digest, which can be costly and time-consuming. Thus, confirming that the conversion was successful before analyzing the samples is a big benefit. To that end, the MethylDetector Kit provides positive control PCR primers specific for bisulfite-converted DNA, so you can confirm the conversion worked before starting the analysis (Figure 1).

For additional information about bisulfite conversion, or to see a complete list of Active Motif's DNA methylation antibodies and assay kits, please visit www.activemotif.com/dnamt.

> "MethylDetector's reliable and consistent results improved my analysis of imprinted genes, and worked well in combination with the Sequenom EpiTYPER system."
> *– Benedetta Izzi, Katholieke Universiteit Leuven, Belgium*

Purchase a MethylDetector™ Kit by November 30th and **save 10%**. Simply cite code AUP2 at the time of the order. For complete promotional details, please visit www.activemotif.com/promo.

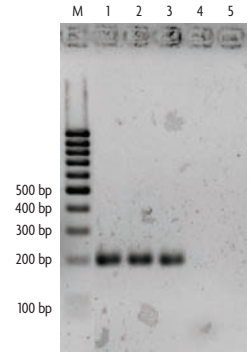


Figure 1: Reproducible conversion by MethylDetector. MethylDetector was used for bisulfite conversion of 3 different DNA samples (Lanes: 1-3) and a control with no DNA (Lane 4). PCR was performed on these samples and an unconverted DNA control (Lane 5) using the kit's control PCR primers. The presence of PCR product in only the converted samples demonstrates the efficiency and reproducibility of the MethylDetector Kit.

Product	Format	Catalog No.
MethylDetector™	50 rxns	55001

Simple Screen for DNMT Activity or Inhibition

Active Motif's DNMT Activity / Inhibition Assay is a fast, user-friendly assay to specifically detect DNA methyltransferase (DNMT) activity from purified proteins or nuclear extract samples, without the need for radioisotopes.

Unique method enhances sensitivity

The DNMT Activity / Inhibition Assay is unique in that it utilizes a methyl CpG binding domain protein (MBD) to detect methyltransferase activity. MBD proteins are capable of binding methylated DNA with a higher affinity than antibody approaches, which increases the sensitivity of the assay. With this method, as little as 0.5 ng of purified enzyme or 0. 5 µg of nuclear extract can be detected.

Learn more at www.activemotif.com/dnmt.

DNMT Assay advantages

- **Non-radioactive** – colorimetric assay is easily quantified at 450 nm
- **Sensitive** – unique MBD protein approach enhances the sensitivity of detection (Figure 1)
- **Fast** – assay can be completed in less than 3 hours

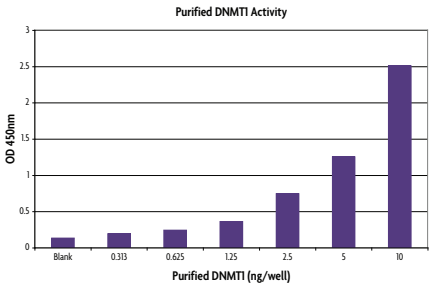


Figure 1: Purified DNMT1 activity. The DNMT Activity / Inhibition Assay was used to generate a standard curve from 0.313 - 10 ng per well of the included DNMT1 control enzyme. The assay was able to detect DNMT1 activity from as little as 0.313 ng of DNMT1 with a 1.5 hour incubation time and a 3 minute developing time.

Product	Format	Catalog No.
DNMT Activity / Inhibition Assay	1 x 96 rxns	55006
Recombinant DNMT1 protein, active	10 µg	31335

Get Pure Fractions with Every Sample Preparation

To obtain results you can rely on, it is important to know that your starting material is pure. With Active Motif's Nuclear Extract Kit and Mitochondrial Fractionation Kit, it is possible to have confidence in your sample preparation and know that your extract is not contaminated with proteins from other cellular compartments (Figure 1).

The **Nuclear Extract Kit** is ideal for the preparation of nuclear, cytoplasmic and whole-cell extracts from mammalian cells and tissues. The included phosphatase and protease inhibitors help to preserve protein modifications.

Active Motif's unique **Mitochondrial Fractionation Kit** isolates highly enriched, segregated mitochondrial and cytosolic fractions from mammalian cell lines using a simple procedure that does not require ultracentrifugation or the use of toxic chemicals.

Get 10% off the purchase of any Nuclear Extract or Mitochondrial Fractionation Kit between now and November 30th. Simply cite code AUP1 at the time of the order. For complete details, please visit www.activemotif.com/promo.

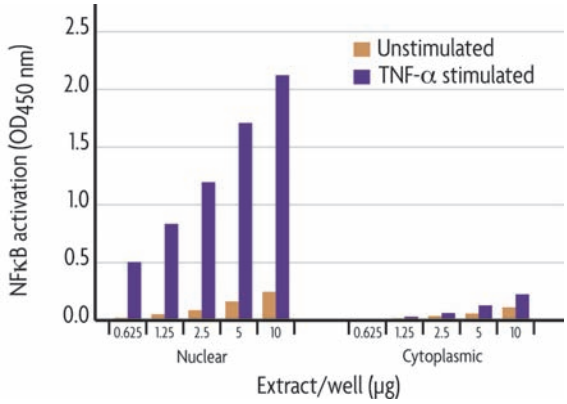


Figure 1: Specific extraction of nuclear and cytoplasmic extracts.
Nuclear and cytoplasmic extracts were isolated using the Nuclear Extract Kit. NFκB activation was then assayed with the TransAM™ NFκB p50 Kit using increasing amounts of each extract. This data demonstrates the kit's high specificity; because activated NFκB translocates into the nucleus, only nuclear extract from stimulated cells should contain activated NFκB.

Product	Format	Catalog No.
Nuclear Extract Kit	100 rxns	40010
	400 rxns	40410
Mitochondrial Fractionation Kit	100 rxns	40015

Non-radioactive Evaluation of Protein:DNA Binding

Looking for an easier way to assess protein:DNA interactions? Active Motif's Gelshift™ Chemiluminescent Kit is a simple, non-radioactive electrophoretic mobility shift assay (EMSA) that can be used to screen for protein binding to DNA. Simply incubate cell extracts or purified factors with a biotin end-labeled probe containing the consensus binding site of interest; then resolve these reactions on a native polyacrylamide gel. Samples in which the protein of interest bound the target DNA will migrate slower than DNA alone resulting in a "shift" of the labeled DNA band. The non-radioactive format does not sacrifice sensitivity when compared to ^{32}P or digoxigenin-labeled methods (Figure 1).

Product	Format	Catalog No.
Gelshift™ Chemiluminescent EMSA	100 rxns	37341

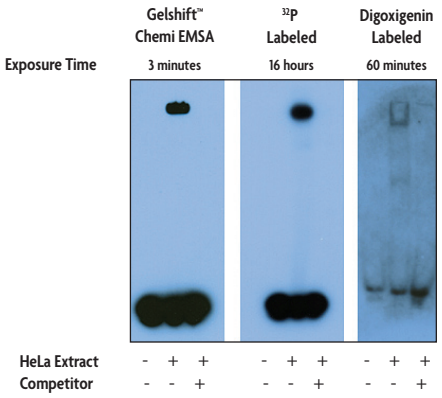


Figure 1: Gelshift EMSA gives better results.
Comparison of Gelshift Chemiluminescent EMSA to radioactive and digoxigenin-based methods show that detection of a 22 bp duplex probe specific for Oct-1 with HeLa nuclear extract using the Gelshift Chemiluminescent EMSA gives better results in less time.

Signaling Pathway Readouts by In-Cell ELISA: Simple, Quick, and Scalable

Active Motif's FACE™ Kits provide a simple, sensitive, cell-based method for monitoring the status of signaling pathways by In-Cell ELISA.

Is AKT active in my cell system? How about ERK, or p38? Does this have an effect on what is seen in the nucleus? Study signaling pathways with FACE Kits.

The regulation of chromatin and other aspects of the nucleus is greatly dependent on the context of signaling pathways active in a particular cell type. As you study the nucleus, take some time to look at the signaling pathways active in your cell type, and make new associations between signaling and the nucleus. Rather than the laborious process of making extracts, running gels, and Western blots, why not try our Fast Activated in-Cell ELISA (FACE) Kits? We've done all the validation of antibodies and protocols, ensuring that only the cognate protein is recognized. You only need to grow your cells (in a 96-well dish), treat as appropriate, fix, stain and detect. In this way you can learn important aspects of cell regulation with a minimum of effort, to dovetail with discoveries about the status of the nucleus in those cells.

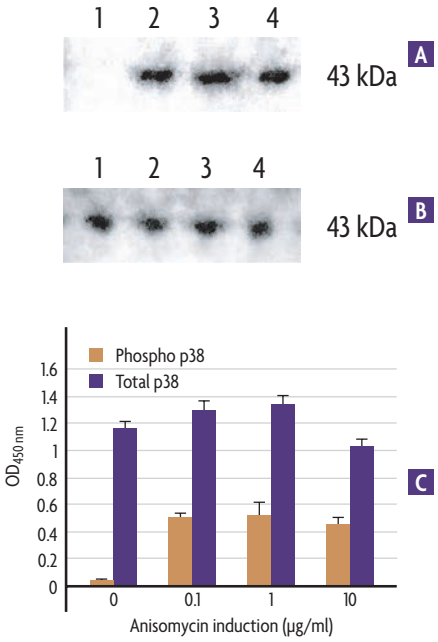
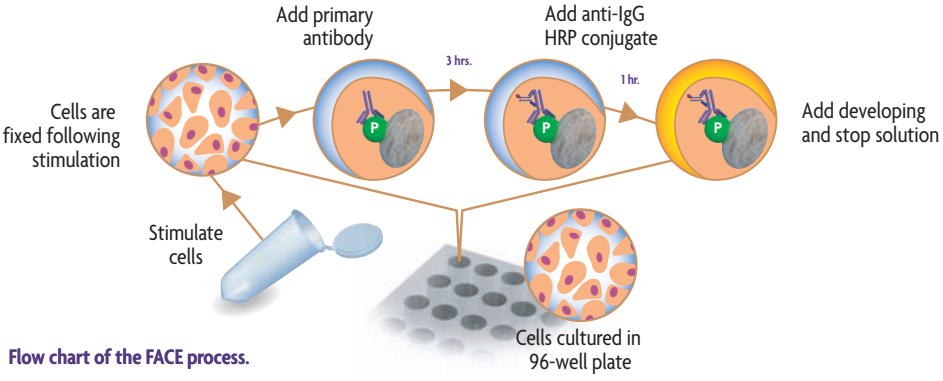


Figure 1: Phospho and total p38 MAPK assays. Macrophage 4/4 cells were grown in 10 cm dishes to 80% confluency, serum-starved for 16 hours and stimulated with anisomycin for 15 minutes. Cell lysates were made and Western blots performed using phospho- (A) and total-p38 antibodies (B). For FACE, 4/4 cells were grown in 96-well plates, stimulated as above, fixed and then assayed in triplicate using the FACE p38 Kit (C). Data were corrected for cell number through use of the kit's Crystal Violet Dye. Western blot data provided courtesy of Dr. Henri H. Versteeg and Dr. Maikel P. Peppelenbosch.

In-cell ELISAs: Cell Signaling made easy

Active Motif's In-cell ELISAs can be completed with under 3 hours of hands-on time. Cells are grown in 96-well culture plates, stimulated, fixed with formaldehyde, and probed in parallel with a phospho-state independent antibody to the protein of interest, and a phospho-specific antibody to a regulatory site on the same protein. Detection is colorimetric or luminescent; we provide both formats. When you compare the two signals, you see total protein versus phospho-protein, readily giving you the relative phosphorylation state, and a readout of the activation state of that signaling pathway (Figure 1).



Flow chart of the FACE process.

FACE advantages

- **Quick** – less than 3 hours of hands-on time
- **High Information Yield** – works on 96-well plates
- **Easy** – it's cell based, so it stays on the plate
- **Biologically Relevant** – phosphorylation state of regulatory sites is quickly characterized

A variety of kits to choose from

FACE Kits are available for over 20 different targets covering the major signaling pathways (see web address below). The Suspension Cell FACE module works with all FACE Kits; it improves results when working with suspension cells by providing 96-well filter plates that make it easier to perform washing & liquid handling steps. And, with FACE Maker Kits, you can use your own primary and secondary antibodies to detect any target or modification state of interest.

- With FACE™ NFκB p65 Profiler, distinguish between p65 that is phosphorylated at Ser468 vs. Ser536
- Detect the anti-apoptotic phosphorylation of Bad at Ser112
- Characterize the activation of the MAP kinases ERK1 and ERK2
- Profile the JAK-STAT pathway: JAK1, STAT2, STAT4, STAT6
- Study the receptor tyrosine kinases EGFR and HER-2
- Monitor c-Jun phosphorylation at Ser63 or Ser73

Simplify your phospho-assays today!

For complete information on FACE, please visit www.activemotif.com/face. To learn how to get FACE Kits at **30% off**, visit www.activemotif.com/promo.

SUMOlink™ Enables Efficient Analysis of the Effects of SUMOylation

SUMOlink™ Kits provide a fast, simple method for generating SUMOylated proteins *in vitro*. SUMO (small ubiquitin-like modifier) shares degrees of sequence identity with ubiquitin, a post-translational modification that serves a variety of functions, such as protein sorting, transcriptional activation and DNA repair. The ability to investigate the effects of SUMOylation on enzyme function may help in understanding the role of SUMOylation in the regulation of cellular processes and the identification of novel proteins as targets for SUMO.

The SUMOlink method

With SUMOlink, simply add the assay components to a microcentrifuge tube with your protein of interest. After a 3-hour incubation, the reaction is stopped and results can be analyzed by Western blot. With the kit's SUMO-1 or SUMO-2/3 antibodies, you can easily see the extent to which your target protein has been SUMOylated. The included p53 protein and antibody serve as a positive control, while mutant SUMO proteins are included as a negative control.

To learn more, please call or visit us at www.activemotif.com/sumolink.

Why use SUMOlink?

- Simple, effective method for SUMO conjugation and detection of SUMOylated proteins
- Positive control p53 protein and antibody provided to ensure success
- Wild-type and mutant SUMO proteins are included
- Versatile – study SUMOylation of cell or tissue extracts, or recombinant proteins

What's in the box?

Each kit contains E1 activating and E2 conjugating enzymes along with wild-type and mutant SUMO-1 (SUMO-1 Kit) or SUMO-2 and SUMO-3 proteins (SUMO-2/3 Kit). Antibodies for SUMO-1 or SUMO-2/3 modifications, as well as control p53 protein and antibody. Enough reagents are provided to perform 20 *in vitro* SUMOylations with the wild-type protein and 20 *in vitro* SUMOylations with the mutant protein.

Product	Format	Catalog No.
SUMOlink™ SUMO-1 Kit	20 rxns	40120
SUMOlink™ SUMO-2/3 Kit	20 rxns	40220

Sensitive Reporter Assay for Better Screening Results

Active Motif's patented RapidReporter® gene assays provide a faster, more pronounced response to stimulation and repression than other systems. RapidReporter vectors include double destabilization elements for both the luciferase protein and its mRNA, so the assay yields more accurate kinetic and drug concentration-dependent responses and enables researchers to detect smaller changes in activity than is possible with non-destabilized reporter gene assays.

Why use RapidReporter?

- Stronger fold induction reduces false positives
- Faster response enables detection of transient effects, and drugs that decompose rapidly (Figure 1)
- Your choice of stringency – pRR-High for best response to changes in transcription and pRR-Low for a stronger basal signal
- "Empty" vectors available for cloning your own elements in, or use pre-made vectors containing widely studied promoter sites

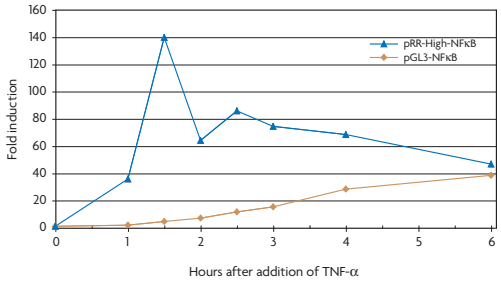


Figure 1: RapidReporter "unmasks" hidden effects. HeLa cells were transiently transfected with pRR-High NFκB or pGL3 vector containing NFκB, then plated in 96-well plates. Twenty-four hours post-transfection, the cells were stimulated with 10 ng/ml TNF-α and measured for *Gaussia* Luciferase (pRR) and firefly luciferase (pGL3) at the indicated time points. Because RapidReporter has double destabilizing elements that reduce background, relatively small and transient events, like the natural oscillation of NFκB from the cytosol to the nucleus during its activation, can be observed.

RapidReporter is available in a variety of formats to suit your research needs. You can purchase either luciferase assay reagents, vectors or the vector and assay reagents together. For complete information about RapidReporter and a list of available products, please visit www.activemotif.com/rapidreporter.

Efficient, Extremely Affordable Competent *E. coli* in a Convenient Format

RapidTrans™ are high-efficiency competent *E. coli* supplied in a convenient 96-tube tray. Ideal for cloning, plasmid preparation and library construction, RapidTrans cells are affordably priced and packaged in a format that provides maximum flexibility while eliminating waste. Each tube contains 50 µl of cells for one transformation reaction. This enables the use of as few or as many reactions as needed, without thawing the other cells. This eliminates wasted cells and the reduced efficiencies caused by repeated freeze/thaw cycles.

Use only what you need

Unlike other 96-well formats, which force you to thaw all 96 wells, RapidTrans is a tray of 12 x 8 tubes that can be used singly or with multi-channel pipettors. So, you can thaw only what you'll use.

Don't pay for unused capacity

Compared to other suppliers, RapidTrans are very affordable, primarily because the transformation efficiency of TAM1 *E. coli* is slightly less, at > 1 x 10^8 cfu/µg supercoiled pUC19 DNA. However, this is more than adequate for most uses. So, don't pay more for super competency cells that impact your lab's budget, but not your results. You wouldn't buy a Ferrari to go to the supermarket, would you? Spend wisely; try RapidTrans today!

TAM1 genotype and contents

*mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80lacZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G

For your convenience, cells include SOC media, pUC19 DNA and sterile reservoirs for use with multi-channel pipettors.

Product	Format	Catalog No.
RapidTrans™ TAM1 Competent *E. coli*	1 x 96 rxns	11096
	5 x 96 rxns	11596

Chariot™ Delivers Functional Protein Directly into Living Cells

The Chariot™ delivery reagent efficiently transports biologically active proteins, peptides and antibodies directly into cultured mammalian cells. Delivery is complete in less than two hours and provides efficiencies of 65-95%. After delivery, living cells can be assayed immediately to determine the effects of the introduced material. These features make Chariot an ideal tool for a variety of functional studies.

Targeted delivery of functional protein

Chariot is a peptide that forms a non-covalent complex when incubated with purified protein, peptide or antibody for 30 minutes at room temperature. Adding the complex to cells results in its rapid internalization. Once inside the cell, the complex dissociates and Chariot is transported to the nucleus, while the delivered protein is biologically active and free to proceed to its cellular target.

Non-covalent delivery

Many protein delivery systems require that you fuse a carrier protein to your macromolecule. However, this can change the folding characteristics of your protein and, ultimately, its function. Because Chariot forms a non-covalent bond with the protein, it does not affect the delivered protein's folding or function.

What can Chariot do for you?

Direct delivery of active protein makes it easy to perform many studies that are not possible using DNA transfection and expression. Chariot results have been extensively published; successful delivery of proteins, peptides and antibodies has been shown in a wide range of cell lines, including hard-to-transfect neuronal, primary and plant cells. The method has proven to be effective on both adherent and suspension cells, as well as *in vivo*. For a more complete information, including a list of papers citing Chariot, go to www.activemotif.com/chariot.

Product	Format	Catalog No.
Chariot™	25 rxns	30025
	100 rxns	30100

Histone Modifications

Trying to solve the puzzle of Histone Modifications?

Why not send for our New Poster? (see page 2)

ACTIVE MOTIF®