MOTIF VATIONS

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ACTIVE MOTIF®

Tools to Analyze Nuclear Function

Do Not Miss This!

Active Motif's new DNA Methyltransferase Assay changes *everything*....(see page 2)

IN THIS ISSUE

NEW: Non-radioactive Assay to Screen for DNA Methyltransferase Activity

Looking for a fast, non-radioactive alternative to screen purified enzymes or nuclear extracts for DNA methyltransferase activity or inhibition? Active Motif's DNMT Activity / Inhibition Assay utilizes the high affinity of methyl CpG binding domain (MBD) proteins towards methylated DNA to provide a highly sensitive assay capable of detecting DNA methyltransferase activity and/or inhibition from purified DNMT1, DNMT3a & DNMT3b enzymes or nuclear extracts. This simple colorimetric assay can be completed in less than 3 hours.

Get improved sensitivity

Active Motif's new DNMT Activity / Inhibition Assay is a fast, user-friendly assay to specifically detect DNA methyltransferase activity from recombinant DNMT enzymes or nuclear extract samples without the need for radioisotopes or expensive equipment. This 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 (Figure 1) or 0.5 µg of nuclear extract can be detected.

DNMT Assay advantages

- **Non-radioactive** – colorimetric assay is easily quantified by spectrophotometry on a microplate reader at 450 nm
- **Sensitive** – unique MBD protein approach enhances the sensitivity of detection from either purified proteins or nuclear extracts
- **Fast** – can be completed in less than 3 hours
- **Less effort required** – the kit is compatible with multi-channel pipettors to streamline wash steps
- **Flexible** – stripwell plate allows screening in low or high throughput

Active Motif also offers DNA methylation antibodies and enrichment kits. For more information, please call or visit us at www.activemotif.com/dnamt.

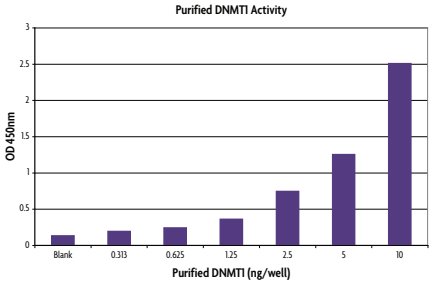


Figure 1: Purified DNMT1 activity.
The DNMT Activity / Inhibition Assay was used to generate a standard curve from 0.313 - 10 ng per well of the included DNMT1 control enzyme. The assay was able to detect DNMT1 activity from as little as 0.313 ng of DNMT1 with a 1.5 hour incubation time and a 3 minute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

What comes in the kit?

The DNMT Activity / Inhibition Assay contains a universal CpG-enriched DNA substrate coated to a 96-stripwell plate. Purified DNMTs or DNMT activities from nuclear extracts will catalyze the transfer of methyl groups from the provided AdoMet reagent to the coated DNA substrate. A His-tagged recombinant MBD2b protein is used to bind the newly methylated DNA substrate with high affinity. Addition of a polyHistidine antibody conjugated to horseradish peroxidase (HRP) and developing solutions provide a sensitive colorimetric readout that is easily quantified by spectrophotometry. A small sample of purified DNMT1 enzyme is included as a positive control.

Screen for inhibitors

Screen for DNA methyltransferase inhibitors with the DNMT Activity / Inhibition Assay. DNA methyltransferase inhibitors can provide key insight into understanding gene regulation, especially as it relates to hypermethylation of tumor suppressor genes. The stripwell plate offers the convenience to perform either low- or high-throughput screening. To demonstrate the effectiveness of screening for DNMT inhibitors, MCF-7 cells that were either untreated or treated with the DNA methyltransferase inhibitor procaine for 96 hours were tested in the DNMT Activity / Inhibition Assay (Figure 2).

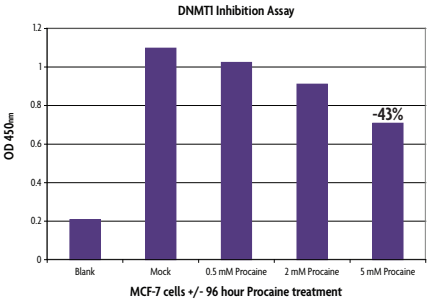


Figure 2: DNMT inhibition with procaine treatment.
The DNMT Activity / Inhibition Assay was used to screen for DNMT inhibition in MCF-7 cells that were either untreated or treated with procaine for 96 hours. Nuclear extracts were prepared using Active Motif's Nuclear Extract Kit (Catalog No. 40010); 5 µg of each treatment condition was tested in the assay with a 1.5 hour incubation time and a 3 minute developing time. The 5 mM procaine treatment showed a 43% inhibition of DNMT activity as compared to mock treated sample.

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

NEW: A Better Way to Screen for Protein:DNA Binding

The GelShift™ Chemiluminescent EMSA Kit simplifies the process of identifying protein:DNA interactions by providing an easy-to-use assay with proven reagents. In this electrophoretic mobility shift assay (EMSA), cell extracts or purified factors are incubated with biotin end-labeled probe containing the consensus binding site of interest; these binding reactions are then resolved 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).

- Non-radioactive assay
- Includes control DNA and extract
- Fast procedure can be completed in 5 hours
- Additional reagents supplied to help optimize conditions for your sample system

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

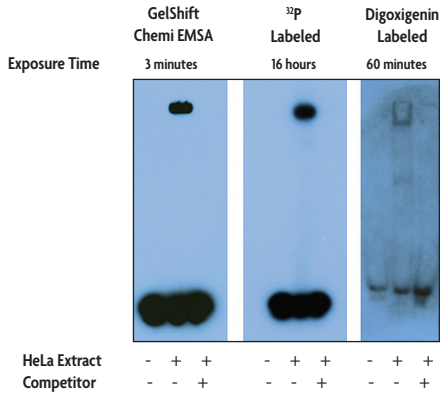


Figure 1: GelShift EMSA gives better results. Comparison of GelShift Chemiluminescent EMSA to radioactive and digoxigenin-based methods shows that detection of a 22 bp duplex probe specific for Oct-1 with HeLa nuclear extract with the chemiluminescent EMSA gives better results in less time.

Improved Yields of Nuclear, Cytoplasmic or Whole-cell Extracts

Active Motif's Nuclear Extract Kit isolates high yields of active nuclear, cytoplasmic or whole-cell extract from mammalian cell or tissue samples. The simple protocol has been optimized to ensure that proteins remain segregated in their particular fraction, which eliminates cross-contamination that can cause misleading results.

Why use the Nuclear Extract Kit?

Extracts prepared using the Nuclear Extract Kit can be used in any procedure that requires nuclear, whole-cell or cytoplasmic extract, including Active Motif's TransAM Transcription Factor DNA-binding assays (Figure 1). The high yields and specificity of protein fractions help you save time and allow you to achieve results faster and more accurately.

Nuclear Extract Kit advantages

- Complete kit contains all required reagents, including protease and phosphatase inhibitors
- No need for optimization
- Quality controlled reagents ensure reproducibility
- Ability to prepare extracts from both cultured cells and tissue samples

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

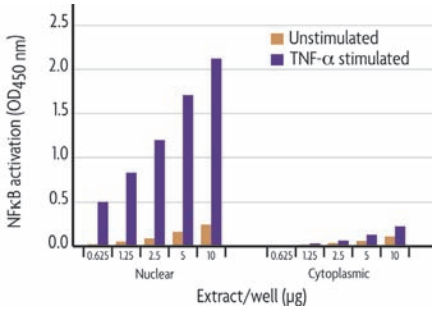


Figure 1: Specific nuclear and cytoplasmic extraction. Nuclear and cytoplasmic extracts were prepared using the Nuclear Extract Kit from HeLa samples unstimulated or stimulated with TNF-α for 30 minutes and assayed using the TransAM™ NFκB p50 Kit. Because activated NFκB translocates to the nucleus, only nuclear extract from stimulated cells should contain activated NFκB.

Special Offer:

Through the end of March 2010, purchase any of Active Motif's TransAM™ Transcription Factor DNA-binding Assays and receive a **FREE** Nuclear Extract Kit. Simply cite promo code SP2-2010 when you place your order. Visit www.activemotif.com for more details.

NEW: Simple Histone ELISAs to Detect Mitotic Markers

Active Motif has expanded its line of Histone Modification ELISAs to include assays that detect phosphorylation of serine 10 or serine 28 on histone H3, which serve as markers for cells undergoing mitosis. We have applied our expertise in making histone modification antibodies to produce optimal antibody pairs for the detection of specific histone modifications in an easy-to-use sandwich ELISA format.

How do the Histone ELISAs work?

The Histone Modification ELISA Kits include everything necessary to screen purified core histones or acid extracts for changes in phosphorylation levels. A 96-stripwell plate is coated with a histone H3 antibody to capture histone H3 from the sample, and an antibody that detects phosphorylation at either serine 10 or serine 28 is added for specific detection. An HRP-conjugated secondary antibody and developing solutions provide a sensitive colorimetric readout that is easily quantified by spectrophotometry (Figure 1). For added convenience, positive and negative control acid extracts are included.

For more information about our Histone Modification ELISAs, please call or visit www.activemotif.com/hiselisa.

Other Histone Modification ELISAs

Active Motif also offers quantitative Histone ELISA Kits to detect methylation events on histone H3. Methylation of lysine residues in nucleosomal histones is thought to mediate interactions with the protein complexes involved in regulating transcription, replication and DNA repair. These assays include our unique recombinant histone H3 proteins (page 5) with site- and degree-specific methylation for use as a reference standard curve.

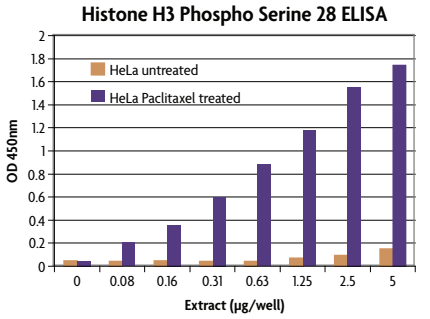


Figure 1: Histone H3 phospho Ser28 sensitivity. Untreated and paclitaxel treated HeLa cells were acid extracted (Catalog Nos. 36200 & 36201) and tested for changes in phosphorylation levels of serine 28 on histone H3 from as little as 78 ng of acid extract.

Product	Format	Catalog No.
Histone H3 phospho Ser10 ELISA	1 x 96 rxns	53111
Histone H3 phospho Ser28 ELISA	1 x 96 rxns	53100
Histone H3 trimethyl Lys27 ELISA	1 x 96 rxns	53106
Histone H3 dimethyl Lys9 ELISA	1 x 96 rxns	53108
Histone H3 trimethyl Lys9 ELISA	1 x 96 rxns	53109
Total Histone H3 ELISA	1 x 96 rxns	53110

HiLite™ Fluorescence Polarization Histone Binding Assays

Active Motif's new HiLite™ Binding Assay uses fluorescence polarization to provide researchers with an innovative, high-throughput tool for monitoring interactions between histone tails and chromatin-modifying proteins. The unique format of this assay enables homogenous quantitation of biologically important transcriptional regulation events, and can be used to determine the specificity of a particular protein for methylated histone tail peptides.

HiLite Binding Assay advantages

- **Homogeneous** – no washing steps are required
- **Fast** – reactions reach equilibrium in just seconds to minutes
- **Reproducible** – kit reagents are stable and prepared at one time, resulting in high reproducibility
- **Robust** – resistant to changes in pH, temperature, salt concentration, *etc.*

With Active Motif's HiLite Histone H3 Methyl-Lys9 / Lys27 Binding Assay, you can use fluorescence polarization to determine if your specific protein of interest binds histone H3 that is methylated at either lysine 9 or 27. You can also measure the affinity of the binding interactions between your protein of interest and specific histone methylation states, which in turn enables fast and efficient inhibitor screening studies.

For additional details, please visit us at www.activemotif.com/hilite.

Product	Format	Catalog No.
HiLite™ Histone H3 Methyl-Lys9 / Lys27 Binding Assay	1 kit	57001

Easily Reconstitute Chromatin with the Chromatin Assembly Kit

Active Motif's Chromatin Assembly Kit makes it easy for you to assemble chromatin on your linear or supercoiled DNA of interest to investigate gene regulation in a context that closely resembles *in vivo* chromatin. The kit contains proven reagents that provide an easy and complete solution for chromatin generation.

Chromatin Assembly Kit advantages

- Generate chromatin from linear or supercoiled DNA
- ATP-dependent method results in an extended array of regularly spaced nucleosomes
- Easy protocol – simply incubate the kit components with your DNA
- Produces an excellent substrate for various gene regulation experiments

Why assemble chromatin?

Research has indicated that the transcriptional regulation of a promoter on naked DNA differs from that of a promoter in chromatin. Thus, using DNA not assembled into chromatin for testing can generate misleading results.

With the Chromatin Assembly Kit, the ATP-dependent method utilizes purified recombinant human chromatin assembly complex ACF and histone chaperone NAP-1 with purified HeLa core histones for *in vitro* assembly of extended, regularly ordered, periodic arrays of nucleosomes (Figure 1). This enables you to mimic the chromatin environment, so you can study regulation under conditions that are closer to real life.

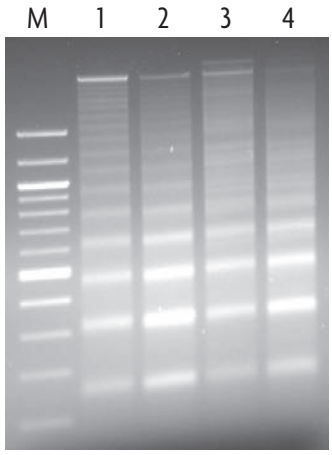


Figure 1: Enzymatic digestion of assembled chromatin. Limited micrococcal nuclease digestion was carried out on chromatin assembled using 1 µg samples of circular DNA (Lanes 1 & 2) or linear DNA (Lanes 3 & 4), resulting in regularly spaced nucleosomes.

Product	Format	Catalog No.
Chromatin Assembly Kit	10 rxns	53500
HeLa Core Histones	36 µg	53501

NEW: Recombinant Core Histone Proteins with Site-specific Methylation

Post-translational modifications on histone tails have been shown to correlate very closely with the nuclear processes of transcription, chromosome packaging and DNA damage repair. Active Motif offers recombinant histones with specific methylated lysine residues to better investigate how methylation patterns impact regulatory processes.

How are they made?

Recombinant methylated histones are created using a patented approach in which an analog of methyl lysine is incorporated into the histone via a chemical alkylation reaction. This enables the site and degree of methylation to be carefully controlled. Because the engineered methylation state closely mimics natural methylation, these recombinant proteins are ideal for use in functional assays.

For more complete information, please visit www.activemotif.com/mehisprots.

Product	Format
Recombinant Histone H2A **NEW**	50 µg
Recombinant Histone H2B **COMING SOON**	50 µg
Recombinant Histone H3 (C110A)	50 µg
Recombinant Histone H3 Lys4 (mono-, di-, or trimethyl)	50 µg
Recombinant Histone H3 Lys9 (mono-, di-, or trimethyl)	50 µg
Recombinant Histone H3 Lys27 (mono-, di-, or trimethyl)	50 µg
Recombinant Histone H3 Lys36 (mono-, di-, or trimethyl)	50 µg
Recombinant Histone H3 Lys79 (mono-, di-, or trimethyl)	50 µg
Recombinant Histone H4	50 µg
Recombinant Histone H4 Lys20 (mono-, di-, trimethyl)	50 µg

Ensure Your Research Success with Antibodies Proven to Work in ChIP

ChIP can be technically challenging and difficult to validate without well-proven reagents. By providing proven ChIP-validated antibodies, reagents and controls, Active Motif can help you achieve the best results possible.

Chromatin immunoprecipitation (ChIP) is a powerful and important technique that allows researchers to identify the *in vivo* localization sites of a chromatin protein or transcription factor, or to find regions of the genome associated with a particular histone modification or histone variant. In combination with microarray methods or next generation DNA sequencing, ChIP allows some very important questions to be asked on a genome-wide scale.

While the number of commercially available antibodies is growing on a daily basis, few companies can validate their antibodies for use in ChIP. This means that scientists often spend considerable amounts of time and money validating antibodies. Active Motif specializes in manufacturing high-quality ChIP-validated antibodies to histones, histone modifications, chromatin proteins and transcription factors. When you buy one of our ChIP-validated antibodies, you can be confident that it will work for you because our in-house scientists have validated it (Figures 1-2).

Competitive Testing

Our antibodies stack up against the competition, and we can prove it. To see how some of our antibodies to important histone modifications compared to those of our competitors, we put them through side-by-side comparison testing. Identical ChIP experiments were performed using the amount of antibody recommended by each manufacturer. Real-time quantitative PCR was carried out using primers specific for the gene indicated, and the results were plotted as "Fold Enrichment" relative to an IgG negative control (Figures 3-5).

The table below list some of Active Motif's ChIP-validated antibodies directed against histone modifications. For a complete list of all of antibodies validated for use in ChIP, please visit us at www.activemotif.com/chipabs.

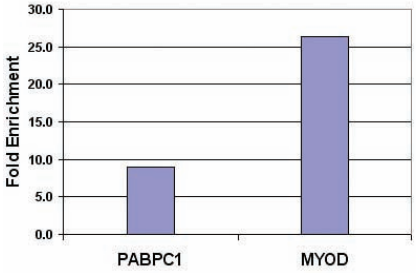


Figure 1: ChIP analysis of Histone H3 dimethyl Lys9 pAb. Chromatin IP performed using the ChIP-IT™ Express Kit (Catalog No. 53008) and HeLa Chromatin (1.5 x 10^6 cell equivalents per ChIP) using 10 µl of Histone H3 dimethyl Lys9 pAb (Catalog No. 39375) or the equivalent amount of rabbit IgG as a negative control. Real time, quantitative PCR (RT-qPCR) was performed on DNA purified from each of the ChIP reactions using a primer pair specific for either the PABPC1 gene or the MyoD gene.

Figure 2: Histone H3 trimethyl Lys27 pAb tested by ChIP. ChIP performed using the ChIP-IT™ Express Kit and 50 µl of Ready-to-ChIP HeLa Chromatin (Catalog No. 53015) per ChIP. Following the ChIP reaction, DNA was purified from the immunoprecipitated chromatin and a region approximately 1000 base pairs 5´ of the transcriptional start site of the HoxD13 gene was amplified by PCR. **Lane 1:** ChIP using negative control rabbit IgG. **Lane 2:** PCR input control. **Lane 3:** ChIP using 10 µl of Histone H3 trimethyl Lys27 pAb (Catalog No. 39155).

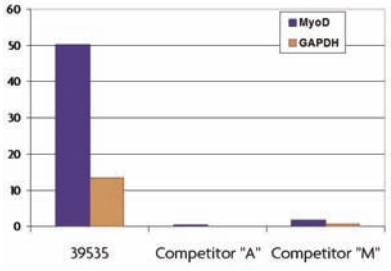


Figure 3: Competitive testing of ChIP-validated antibodies against Histone H3 trimethyl Lys27.

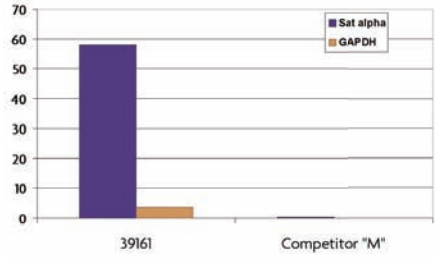


Figure 4: Competitive testing of ChIP-validated antibodies against Histone H3 trimethyl Lys9.

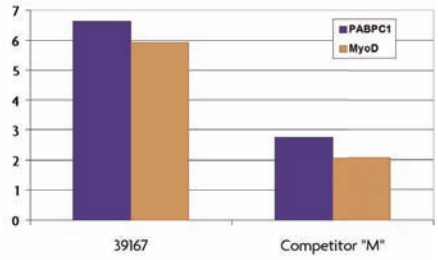


Figure 5: Competitive testing of ChIP-validated antibodies against Histone H4 acetyl Lys16.

Product	Format	Catalog No.
Histone H3 monomethyl Lys4 mAb (Clone MABI 0302)	100 µg	39635
Histone H3 dimethyl Lys4 mAb (Clone MABI 0303)	100 µg	39679
Histone H3 monomethyl Lys9 mAb (Clone MABI 0306)	100 µg	39681
Histone H3 dimethyl Lys9 mAb (Clone MABI 0307)	100 µg	39683
Histone H3 acetyl Lys27 mAb (Clone MABI 0309)	100 µg	39685
Histone H3 trimethyl Lys27 mAb	100 µg	39535
Histone H4 trimethyl Lys20 mAb (Clone 6F8-D9)	100 µg	39671

Our Antibody Development Process Helps Ensure that Our Products Perform as Specified

At Active Motif, antibody development and testing is a very important and tightly controlled process, with many steps along the way. From the design of the immunogen to the specificity screening and application validation, our years of expertise in antibody development ensures that only the highest quality antibodies are offered for use in your research.

Immunogen selection

Immunogens are selected to decrease the likelihood of cross-reactivity with related proteins, and to maximize detection of the protein in its native context. Immunogens for modification-specific antibodies are selected to ensure that the antibody recognizes only the modified version of the protein, and does not cross-react with the same modification on different proteins.

Specificity screening

The first test performed on every antibody is dot blot analysis, which ensures its specificity for the desired protein or modification (Figure 1). Antibodies that do not exhibit a greater than 25-fold selectivity for the desired modification are failed.

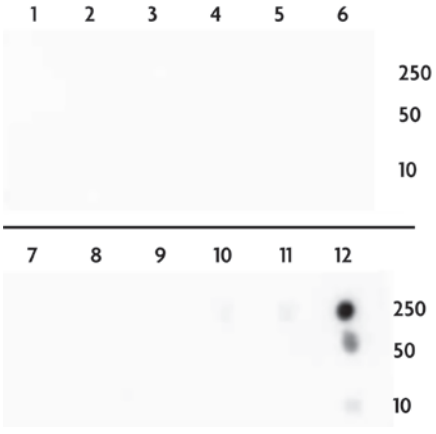


Figure 1: Dot blot analysis confirms specificity. 10, 50 and 250 picomole aliquots of synthetic peptides corresponding to histone H3 that is unmodified, mono-, di- or trimethylated at Lys4 (Lanes 1-4), Lys9 (Lanes 5-8) or Lys27 (Lanes 9-12) were spotted and probed with Histone H3 trimethyl Lys27 pAb (Catalog No. 39155), confirming the antibody's specificity for histone H3 trimethyl Lys27 (Lane 12). No cross-reactivity is observed for the unmodified Lys27 peptide (Lane 9) and only a hint of signal is observed at the 250 picomole spots for mono- (Lane 10) and dimethyl Lys27 (Lane 11).

Western blot

Western blotting is performed to verify that the antibody recognizes a protein of the correct molecular weight and does not cross-react with other proteins. Commonly used chemical treatments that stimulate a specific modification (*e.g.* HDAC inhibitors and acetylation) are used to better detect the modification (Figure 2).

Application validation

Each antibody is validated for use in important applications such as IF (Figure 3) and ChIP (Figures 1 and 2, page 6), so you can have confidence when using them in your experiments.

Additional specificity testing

Antibodies to histone modifications that exist in budding yeast (*S. cerevisiae*) can be further screened for specificity by testing strains of yeast that contain a point mutation at the position of the modified amino acid (Figure 4).

For a complete listing of all of our high-quality antibodies, please visit us at www.activemotif.com/abs.

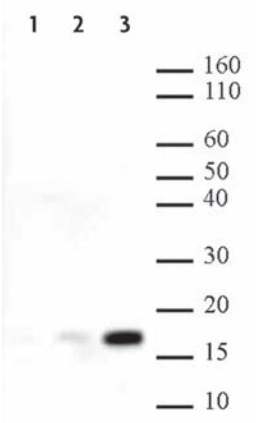


Figure 2: Western blot confirming size and modification. Histone H2B acetyl Lys120 pAb (Catalog No. 39119) was tested by Western blot. No reactivity is observed on recombinant human histone H2B (Lane 1, 200 ng protein), as it is not acetylated. Lanes 2 and 3 contain 5 µg each of HeLa cell acid extract. Cells that were treated with a histone deacetylase inhibitor (sodium butyrate) prior to extraction have an increased signal (Lane 3), which is to be expected for an antibody to a site of acetylation.

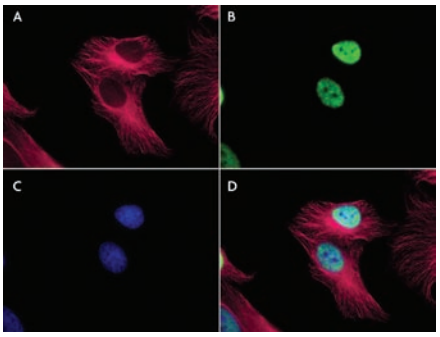


Figure 3: Immunofluorescence testing. HeLa cells stained with alpha Tubulin mAb in red (Catalog No. 39527, panels A and D), Histone H4 monomethyl Lys20 pAb in green (Catalog No. 39175, panels B and D) and DAPI in blue (panels C and D). Note the strong nuclear staining and absence of cytoplasmic staining in panel B, which is to be expected of an antibody directed against a histone modification.

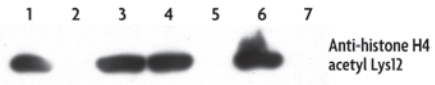


Figure 4: Mutational analysis of yeast modifications. Histone H4 acetyl Lys12 pAb (Catalog No. 39165) was tested by Western blot using extracts from wild-type yeast or yeast containing a mutated histone H4 gene.

Lane 1: Yeast with wild-type histone H4.
Lane 2: H4 with the amino-terminal tail deleted.
Lane 3: H4 with an arginine instead of lysine 5.
Lane 4: H4 with an arginine instead of lysine 8.
Lane 5: H4 with an arginine instead of lysine 12.
Lane 6: H4 with an arginine instead of lysine 16.
Lane 7: H4 with arginines instead of lysines 5, 8 & 12.

Note the absence of reactivity in yeast extracts in which lysine 12 is either missing or mutated (Lanes 2, 5 & 7).

Chromeo™ Py-Dyes Eliminate Background in Fluorescent Protein Labeling

Py-Dyes represent a new class of fluorescent pyrylium molecules that undergo a shortwave spectral shift and a large increase in quantum yield when they react with amine groups on proteins. As a result, their fluorescence is only detectable when the dye is conjugated to protein, which eliminates background. In addition, Py-Dyes have a number of other properties that make them ideal for labeling proteins. Five different Py-Dyes are available, and several staining kits that utilize Py-Dyes are also offered.

Advantages of Py-Dye protein labeling

- Py-Dyes are supplied ready to use, with no need for activation
- Conjugation is a simple, one-step, room-temperature incubation
- Labeled protein can be used immediately after conjugation
- Because unreacted Py-Dye is hydrolyzed during conjugation, there is no need for purification, and background is eliminated

Figure 1: Spectral shift following conjugation.
The emission of Chromeo P503 in its unbound, free state is blue. Following conjugation to protein, the color of the dye changes to red.

Better chemistry yields better results

A useful property of Py-Dyes is the large spectral shift they undergo upon conjugation, which greatly changes the wavelength of their emission (Figure 1). As shown in Figure 2, unbound Py-Dye P503 has negligible emission. However, when conjugated to protein, it absorbs at a much shorter wavelength and its quantum yield increases dramatically. When excited in the 488 nm range, only conjugated P503 fluoresces, and its peak fluorescence is easily distinguished from that of Chromeo 488 and other 488-excitable dyes.

Variety of Py-Dyes available

Table 1 shows the Py-Dyes available and their physical properties. In every case, the conjugated dye is the sole emitter at the specified wavelength, ensuring the lack of background described above. For complete information on each Py-Dye, go to www.activemotif.com/pydye.

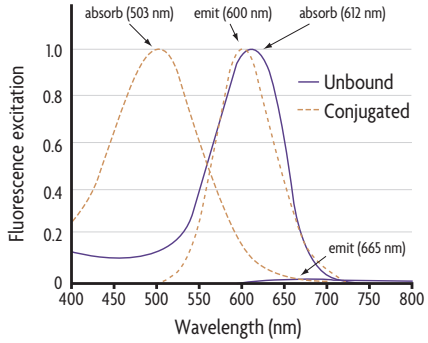


Figure 2: Absorption and emission spectra of free vs. bound Chromeo P503.
Normalized absorption and emission spectra of Chromeo P503 when the dye is free (solid, purple lines) and conjugated (dotted, copper lines). As free dye and bound dye absorb at different wavelengths (612 nm vs. 503 nm) and the quantum yield of bound dye is 50-fold greater than that of free dye, background from any free dye that is not hydrolyzed is effectively eliminated.

Convenient staining kits

Active Motif has also utilized Py-Dyes as the basis for several useful staining kits:

- The Py-Dye in the **Chromeo™ Live Cell Mitochondrial Staining Kit** diffuses passively through the membrane of living cells, where it localizes at the mitochondria. This enables mitochondrial staining without fixation or washes.
- The **Chromeo™ Red Fluorescent Fixed Cell Staining Kit** stains the nuclear membrane, mitochondria, fibers and nucleoli in fixed cells; it can also be used as a counterstain in immunofluorescence experiments with green dyes such as FITC or other 488-excitable dyes.
- The Py-Dye in the **ProStain™ Protein Quantification Kit** reacts broadly with all proteins, so its fluorescence intensity is directly proportional to protein concentration.

For complete details on these kits, please visit us at www.activemotif.com.

Py-Dye	Dye State	Absorption	Emission	ε L/(mol-cm)	Quantum Yield*
Chromeo™ P465	Free	655 nm	732 nm	25,000	< 1%
	Conjugated	465 nm	630 nm	25,000	~14%
Chromeo™ P503	Free	612 nm	665 nm	60,000	< 1%
	Conjugated	503 nm	600 nm	24,000	~50%
Chromeo™ P540	Free	587 nm	—	80,000	0%
	Conjugated	503 nm	627 nm	50,000	~20%
Chromeo™ P429	Free	457 nm	—	65,000	0%
	Conjugated	429 nm	536 nm	75,000	~10%
Chromeo™ P543	Free	570 nm	—	110,000	0%
	Conjugated	543 nm	590 nm	57,000	~15%

Table 1: Properties of Chromeo Py-Dyes
*The Quantum Yield will depend on the Dye-to-Protein Ratio (DPR) of the conjugated protein.

Fluorescent Secondary Antibodies for Enhanced Detection

Active Motif offers an extensive number of high-quality fluorescent dyes, including the Chromeo™ line of fluorescent dyes, giving you a variety of choices for fluorescent detection. Our proprietary dyes exhibit equivalent or superior fluorescent performance compared to existing fluorophores. They have been developed specifically to be compatible with all light sources, and match the most commonly used wavelengths in fluorescent microscopes. For the utmost in convenience, the dyes are available as conjugates to goat anti-mouse and goat anti-rabbit secondary antibodies, for immediate use in fluorescent experiments. In addition to our pre-conjugated secondary antibodies, we also offer complete, optimized labeling kits to simplify conjugation of Chromeo Dyes to primary antibodies and other proteins.

Chromeo Secondary Fluorescent Antibody Conjugates enable sensitive and specific detection in immunofluorescence, high-content screening, ELISA, FRET applications and flow cytometry. They are compatible with most excitation sources including diode lasers, LEDs, tungsten lamps and xenon arc lamps. The dyes have been conjugated to high-quality secondary antibodies by an optimized conjugation method, including subsequent purification from interfering substances.

Why use Chromeo Secondaries?

- **Brightness** – high fluorescent intensity improves sensitivity
- **Limited photobleaching** – enables multiple exposures and increased exposure times
- **pH stability** – great for biological assays
- **Flexible formats** – in addition to secondary antibody conjugates, the dyes are available as NHS-Esters, Carboxylic Acids, and as Biotin & Streptavidin conjugates

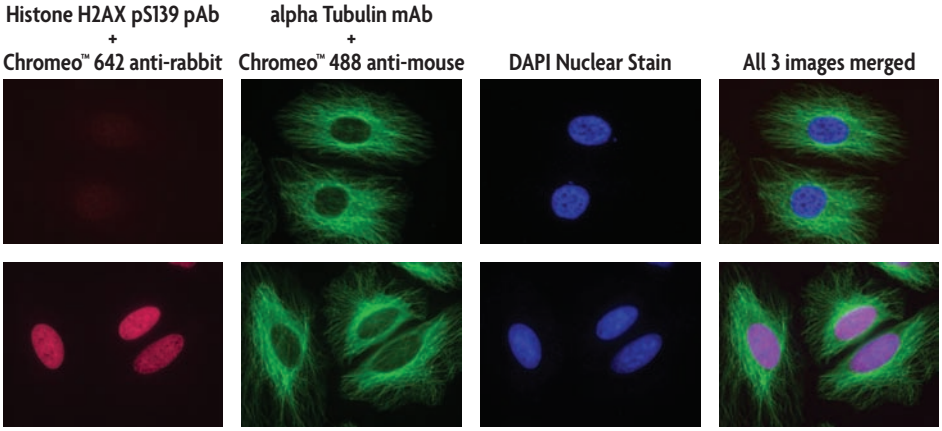


Figure 1: Concurrent staining using Chromeo 488 and Chromeo 642 fluorescent secondaries HeLa cells.
HeLa cells were left untreated (top row) or treated with 100 µM etoposide for 6 hours (bottom row) and methanol fixed. The histone variant H2AX was then stained by Histone H2AX phospho Ser139 rabbit pAb (Cat. No. 39117) and Chromeo 642 goat anti-rabbit IgG (Cat. No. 15044), while tubulin was visualized using alpha Tubulin mouse mAb (Cat. No. 39527) and Chromeo 488 goat anti-mouse IgG (Cat. No. 15031). The nuclei were stained using DAPI and the 3 separate images merged.

Even better when used together

As shown in Figure 1, Chromeo 488 and Chromeo 642 secondary antibody conjugates produce intense fluorescence that is resistant to fading, facilitating multiple exposures and superior quality images. Table 1 shows the properties of the various conjugates. As many of their emission spectra are well separated, they can be used together for multi-color staining experiments. The large Stokes shift of Chromeo 494 (Figure 2) enables it to be used even with Chromeo 488 and other 488-excitable dyes that have similar excitation peaks.

Get all the information today

For complete details on our secondary antibody conjugates, dyes and labeling kits, go to www.activemotif.com/fluor.

Secondary Antibody	Absorption	Emission	ε L/(mol-cm)	Stokes Shift
Chromeo™ 488 IgG	488 nm	517 nm	73,000	29 nm
Chromeo™ 494 IgG	494 nm	628 nm	55,000	124 nm
Chromeo™ 505 IgG	505 nm	526 nm	70,000	21 nm
Chromeo™ 546 IgG	545 nm	561 nm	98,800	16 nm
Chromeo™ 642 IgG	642 nm	660 nm	180,000	18 nm

Table 1: Properties of Chromeo Secondary Antibody Conjugates.

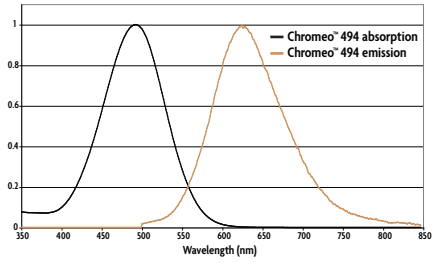


Figure 2: The absorption and emission spectra of Chromeo 494 have peaks separated by 124 nm.

NEW: Amplify Genomic DNA Samples Without Representational Bias

Active Motif's new GenoMatrix™ Whole Genome Amplification Kit allows you to accurately duplicate DNA from your experiments for downstream analysis techniques that require more DNA than is yielded initially.

With the advent of techniques that allow important questions to be asked on a genome-wide scale, experiments do not always yield sufficient quantities of DNA. The GenoMatrix Kit can be used as a stand-alone product to amplify unsheared DNA, or with pre-sheared samples generated through use of Active Motif's ChIP-IT™ Express, MethylCollector™ or UnMethylCollector™ Kits. The kit utilizes a new approach that virtually eliminates amplification bias, so the amplified material has the same sequence representation as the material that you started with (Figure 1).

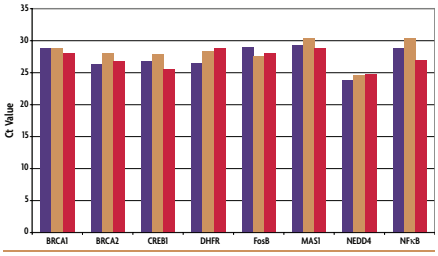


Figure 1: Representative and quantitative DNA amplification without sequence bias.
Quantitative real-time PCR was carried out on DNA samples using primers indicated below each data set and the Ct value for each sample plotted. The DNA was derived from an original DNA sample amplified using the GenoMatrix Whole Genome Amplification Kit (red bars), amplified with a competitor's whole genome amplification kit (copper bars) or not subjected to whole genome amplification (purple bars).

Product	Format	Catalog No.
GenoMatrix™ Whole Genome Amplification Kit	50 rxns	58001

GenoMatrix Kit advantages

- **Bias Free Amplification** of genomic DNA, even from small amounts of sample
- **Preserve the Original Sequence** representation of your starting material
- **Obtain Enough DNA** for genome-wide applications that require large amounts of DNA

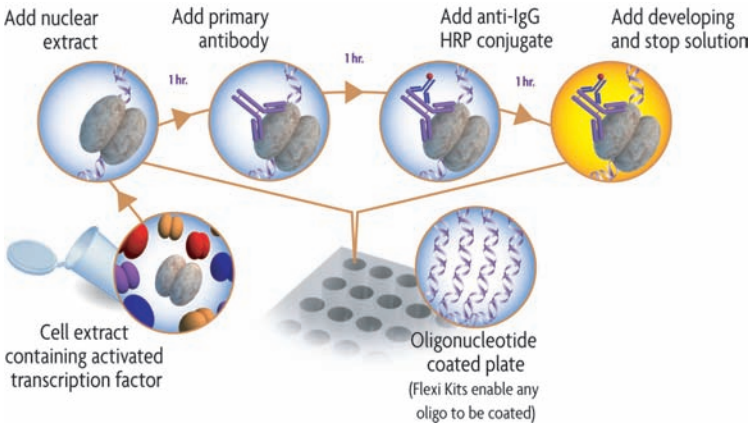
The GenoMatrix™ Kit provides up to a 500-fold amplification starting from as little as 10 ng of any type of genomic DNA. GenoMatrix Kits provide all reagents needed to quickly and easily perform genome-representative amplifications. For more information, please visit www.activemotif.com/wga.

Recombinant Transcription Factors for the Study of Regulatory Mechanisms

When studying transcriptional regulation, because it is useful to study the function of a specific transcription factor in reconstitution assays, it is of great value to have available high-quality recombinant transcription factor proteins. Recombinant transcription factors can be used in many biological applications, including as substrates in biochemical assays, as many of them are also substrates for important regulatory enzymes. Active Motif offers a complete line of recombinant transcription factors for your research needs. Many of them, including NFκB p50, NFκB p65, p53, c-Fos, c-Jun, c-Myc, CREB and Sp1 proteins are validated for use as standards in Active Motif's TransAM™ DNA-binding ELISAs.

Active Motif Transcription Factor Categories

- General Transcription Factors
- Nuclear Receptors
- NFκB Family
- Transcriptional Activators



Flow chart of the TransAM process.

A complete list of available recombinant transcription factors can be found at www.activemotif.com/tfprot.

ELISA Method for More Accurate Quantification of Activated Ras GTPase

Active Motif's Ras GTPase Chemi ELISA Kit is the first ELISA-based kit designed to detect and quantify activated Ras GTPase. The method offers several advantages over other commercially available kits, which require you to perform immunoprecipitation of Ras, followed by Western blotting.

In contrast, the Ras GTPase Chemi ELISA uses Raf-RBD protein and antibodies in a 96-well format to capture and quantify the activated Ras in your sample. This faster, more sensitive alternative enables you to use less of your precious sample, yet still detect low-level events. In addition, because ELISAs provide more quantitative results than Westerns, the data generated are more meaningful.

The Ras GTPase Chemi ELISA method

Because activated Ras binds the Ras-binding domain (RBD) of the Raf effector protein, Raf-RBD is used as a probe to isolate activated Ras. The kit contains a Raf-RBD protein fused to GST and a 96-well, glutathione-coated assay plate. GST-Raf-RBD is first incubated on the plate for one hour to immobilize this capture protein. Addition of sample to the plate results in the binding of activated Ras to the Raf-RBD. Primary antibody specific for Ras and an HRP-conjugated secondary are then added, followed by developing reagent. The plate is then read on a luminometer, providing a sensitive, quantitative readout of activated Ras GTPase (Figure 1).

Ras GTPase ELISA advantages

- **More sensitive** – assay uses only 25 µg of extract, or 20-fold less than pull-down/Western methods
- **Better results** – quantitative data makes it easier to compare results
- **Less effort** – no need to perform IP, run gels or develop Western blots

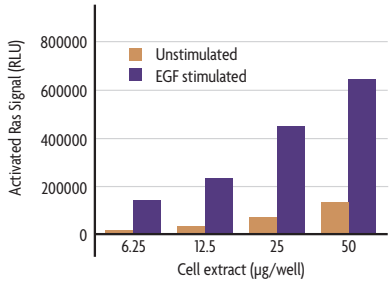


Figure 1: Quantification of activated Ras GTPase. Increasing amounts of whole-cell extract from HeLa cells either unstimulated or stimulated 2 minutes with 5 ng/ml EGF are assayed using the Ras GTPase ELISA Kit.

Product	Format	Catalog No.
Ras GTPase Chemi ELISA Kit	1 x 96-well plate	52097

Method to Measure the Activity of DNA Repair Proteins

DNA Repair Protein Kits are DNA-binding ELISAs that detect DNA repair protein activity in both mammalian tissue and cell culture extracts. The sensitive, specific assay is 10-fold more sensitive than gelshift, without the need for gels or radioactivity. Quantitative results are achieved in under 5 hours.

DNA Repair Protein Kit advantages

- ELISA format eliminates gels, blotting and radioactivity
- Up to 10-fold greater sensitivity than gelshift assays
- Ability to assay both cultured cells and tissue samples
- Quantitative results in < 5 hours

The DNA Repair Protein Kit method

DNA Repair Protein Kits are better than Western for detection of DNA repair proteins. Each kit contains a 96-well plate with multiple copies of an immobilized oligonucleotide containing a DNA lesion. When cell extract is added to each well, the repair protein of interest binds specifically to the oligonucleotide on the plate. Addition of primary antibody, HRP-conjugated secondary and developing solution yields an easily quantified colorimetric readout (Figure 1).

Product	Format	Catalog No.
GTBP DNA Repair Kit	1 x 96-well plate 5 x 96-well plates	51096 51596
Ku70/86 DNA Repair Kit	1 x 96-well plate 5 x 96-well plates	51196 51696
RPA DNA Repair Kit	1 x 96-well plate 5 x 96-well plates	51296 51796

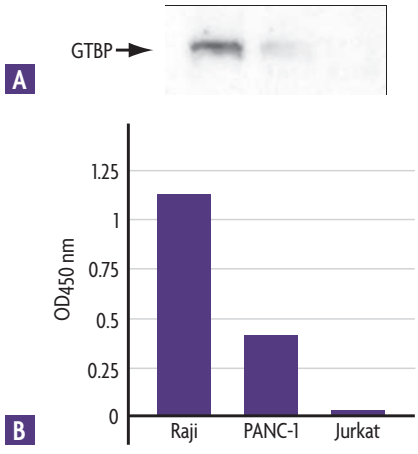


Figure 1: Measuring GTBP activity in several cell lines. Jurkat, PANC-1 and Raji nuclear extracts were assessed for GTBP activity using Western blot (A) and the GTBP DNA Repair Protein Kit (B). The DNA Repair Protein Kit is clearly more quantitative and sensitive.