MOTIF VATIONS

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ACTIVE MOTIF®

Tools to Analyze Nuclear Function

IN THIS ISSUE

NEW: Suspension Cell FACE™ – Quantify Phospho-proteins in Non-adherent Cells

Fast Activated Cell-based ELISA (FACE™) Kits provide a simple, sensitive method for detecting phosphorylation directly in the cell, without making extracts or running gels. The new Suspension Cell FACE module greatly improves results with suspension cells by providing greater flexibility for the study of over 20 different kinase and receptor targets.

FACE – the "in-cell" Western

FACE Kits have become a popular method for studying phosphorylation because they are fast, easy to use and the results generated are more specific, quantitative & reproducible than more traditionally used methods like radioactive kinase assays and Western blotting. Active Motif has developed FACE assays for over 20 different kinase and receptor targets (Table 1), and each is available in both chemiluminescent and colorimetric formats. However, until now, the method was not as effective when using suspension cells.

The FACE method

In FACE, cells are grown in a 96-well plate and stimulated to induce the pathway of interest. After stimulation, the cells are fixed to preserve protein activation and modification states. Each well is then incubated with a primary antibody specific to the protein of interest. An antibody that recognizes all forms of the protein is included as well as an antibody that recognizes the phosphorylated site of the target protein. This enables you to compare the effect of stimulation in your cells on both total and phosphorylated protein (Figure 1).

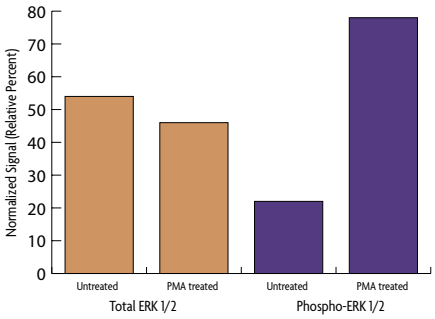

Figure 1: FACE ERK1/2 Chemi with Suspension Cell FACE. Suspension Cell FACE was used with the FACE ERK1/2 Chemi Kit to assay total and phosphorylated ERK1/2 using 50,000 Jurkat cells per well. The cells were serum-starved and treated with 100 ng/ml PMA (Phorbol 12-myristate 13-acetate) for 15 minutes. A three-fold increase in phospho-ERK1/2 was detected in the assay.

Capture & measure more cells

The Suspension Cell FACE module was designed to work with any of our FACE Kits. It provides 96-well filter plates that make it easier to perform washing & liquid handling steps through use of an appropriate vacuum manifold. This eliminates the loss of suspension cells that can occur when performing fixation and wash steps in normal 96-well plates. So, you're able to measure a greater number of cells, which makes the assay better able to measure small effects.

Suspension Cell FACE advantages

- Adapt any of the optimized colorimetric or chemiluminescent FACE Kits for use with suspension cells
- No cell extracts or immunoblotting
- Compare phosphorylated vs. total target with the 2 included antibodies
- Less than 2 hours of hands-on time

A variety of kits to choose from

FACE kits are available for over 20 different targets (Table 1). And, with the FACE Maker Kits, you can use your own primary and secondary antibodies to detect any target or modification state of interest. For complete information on the FACE product line, including the new Suspension Cell FACE, please give us a call or visit www.activemotif.com/face.

Product	Format	Catalog No.
Suspension Cell FACE™	2 x 96 rxns	48305
Suspension Cell FACE™ Chemi	2 x 96 rxns	48405

Table 1: The FACE™ Product Line

FACE™ AKT (S473)	FACE™ ATF-2 (T71)	FACE™ Bad (S112)	FACE™ c-Jun (S63)	FACE™ c-Jun (S73)	FACE™ c-Src (Y418)
FACE™ EGFR (Y845)	FACE™ EGFR (Y992)	FACE™ EGFR (Y1173)	FACE™ ErbB-2 (Y877)	FACE™ ErbB-2 (Y1248)	FACE™ ERK1/2 (T202/Y204 & T185/Y187)
FACE™ FAK (Y397)	FACE™ FKHR (T24)	FACE™ GSK3β (S9)	FACE™ HSP27 (S82)	FACE™ JAK1 (Y1022/Y1023)	FACE™ JNK (S473)
FACE™ Maker	FACE™ MEK1/2 (S217/S221)	FACE™ NFκB Profiler (S468 & S536)	FACE™ p38 (T180/Y182)	FACE™ P13 Kinase	FACE™ STAT2 (S473)
FACE™ STAT4 (Y693)	FACE™ STAT6 (Y641)				

NEW: Magnetic Beads Improve Co-IP of Both Nuclear & Whole-cell Complexes

The Universal Magnetic Co-IP Kit includes protein G-coated magnetic beads that improve co-immunoprecipitation (Co-IP) by simplifying the IP and wash steps, while providing lower background. The kit also includes reagents to prepare both nuclear and whole-cell extracts, so you can study intact protein complexes whether they were originally bound to DNA or in the cytoplasm.

Protein/protein interactions are often studied using Co-Immunoprecipitation (Co-IP), in which a single antibody is used to precipitate an entire protein complex. Additional antibodies are then used in Western blot to identify/verify other complex members (Figure 1).

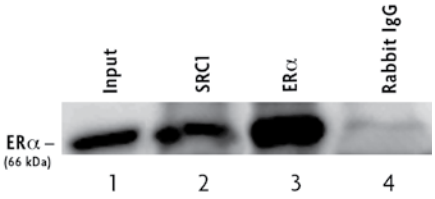

Figure 1: Nuclear Co-IP of SRC-1 and ERα.
The Universal Magnetic Co-IP Kit was used to make nuclear extract from MCF-7 cells induced 1 hour with 10 nM Estradiol. IP was performed on 300 µg samples using 2 µg of SRC-1 pAb, ERα pAb and rabbit IgG (as a negative control). Western blot was then performed using the ERα pAb on 10 µg Input Extract (Lane 1), SRC-1 IP (Lane 2), ERα IP (Lane 3) and the rabbit IgG IP (Lane 4).

Co-IP of cytoplasmic & nuclear proteins

While Co-IP is often used to study cytoplasmic protein complexes, traditional methods are not optimal for studying DNA-binding proteins because nuclear complexes are very fragile. This often causes them to be disrupted during extraction. For this reason, in addition to containing components for whole-cell extractions, the Universal Magnetic Co-IP Kit provides nuclear extraction reagents that have been optimized to preserve nuclear protein complexes. The kit's Enzymatic Shearing Cocktail uses DNA digestion to gently release the complexes from the DNA, so they are intact and ready for Co-IP.

Simpler procedure, lower background

The Universal Magnetic Co-IP Kit includes protein G-coated magnetic beads, which simplify Co-IP by enabling IP and wash steps to be performed in seconds, rather than having to use centrifugation. Moreover, because the beads have very low non-specific binding, background is reduced even while using a low-salt Co-IP/Wash Buffer that helps maintain weaker complexes.

Universal Magnetic Co-IP Kit advantages

- Magnetic beads simplify procedure and reduce background
- Optimized extraction method maintains nuclear protein complexes
- Preserve protein modifications

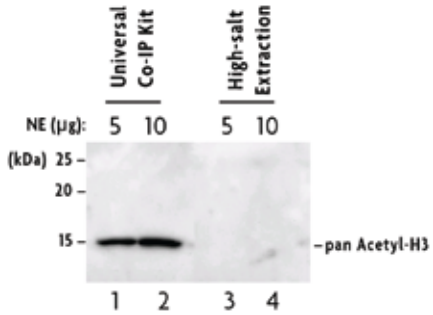

Figure 2: Detection of acetylated Histone H3.
HeLa nuclear extracts were made using the Universal Magnetic Co-IP Kit and a traditional high-salt extraction protocol, each supplemented with the 1 µM trichostatin A, a deacetylase inhibitor. Five and ten µg samples of each extract were used in Western blot with Histone H3 acetyl rabbit pAb (Cat. No. 39139). Protein was detected only in samples made using the kit's gentle nuclear extraction procedure.

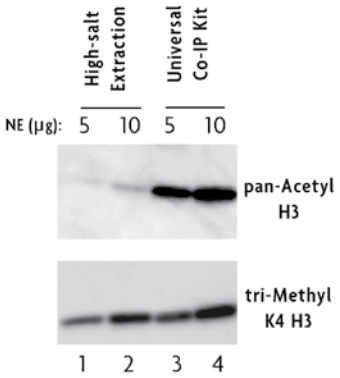

Figure 3: Preservation of acetylation and methylation.
Nuclear extracts were made from HeLa cells treated with 500 nM trichostatin A for 20 hours using either the Universal Magnetic Co-IP Kit (with its deacetylase inhibitor) or traditional high-salt extraction. Five and ten µg samples of these extracts were used in Western blot with Histone H3 acetyl pAb (Cat. No. 39139) and Histone H3 trimethyl Lys4 pAb (Cat. No. 39159). The acetylated protein was detected only in the sample made using the kit. Methylation was slightly better maintained in the sample made using the kit.

Complete kit for better results

The Universal Magnetic Co-IP Kit has both nuclear and whole-cell extraction reagents, so you can perform IP on all types of protein complexes. The kit also includes protein G-coated magnetic beads, a unique Co-IP/Wash Buffer as well as phosphatase, protease and deacetylase inhibitors that preserve the integrity of the proteins. Finally, the kit includes a strong bar magnet, so you can take advantage of the improved wash and IP steps enabled by the magnetic beads. This makes the Universal Magnetic Co-IP Kit a simple, flexible and complete solution for getting more from your Co-IP. To find out more, please give us a call or visit us on the web at www.activemotif.com.

Product	Format	Catalog No.
Universal Magnetic Co-IP Kit	25 rxns	54002

Rapid and Efficient Comparison of Methylation in Various DNA Samples

Active Motif's MethylCollector™ Kit provides users with a fast and efficient protocol for isolating and comparing CpG-methylated DNA from cell or tissue samples. MethylCollector uses a recombinant Methyl-binding protein (MBD2b) to capture DNA, rather than traditional antibody-based immunoprecipitations, which improves sensitivity.

MethylCollector advantages

- Fast and easy protocol completed in less than 4 hours
- Flexible – enables detection from 5 ng to 1 µg of DNA
- Suitable for use with DNA fragmented by sonication or enzymatic digestion
- Positive control DNA and PCR primers ensure success

The MethylCollector method

In the MethylCollector Kit method, His-tagged recombinant MBD2b protein specifically binds to CpG-methylated DNA fragments that have been prepared by enzymatic digestion or sonication. These protein-DNA complexes are captured with nickel-coated magnetic beads and subsequent wash steps are performed with a stringent high-salt buffer to remove DNA fragments that have little or no methylation. Ready-to-use methylated DNA is then eluted from the beads (Figure 1). MethylCollector is highly efficient, enabling analysis of the methylation state of any specific locus on genomic DNA isolated from less than 800 cells (~5 ng DNA).

Applications of MethylCollector

The highly specific isolation of methylated DNA by MethylCollector enables many powerful applications, including the rapid screening of the methylation status of multiple loci in tumor tissue or cells. It can also be used to detect changes in DNA methylation in other situations, such as normal cellular differentiation and aging. To find out more, please visit www.activemotif.com.

Fully Methylated Jurkat DNA Control

Fully Methylated Jurkat DNA is now available separately for use as a control in your DNA methylation applications. Whether you're performing bisulfite sequencing, methylation-specific PCR (MSP), MeDIP or using our MethylCollector or MethylDetector™ Kits (page 12), Fully Methylated Jurkat DNA is a convenient positive control for investigating CpG dinucleotide methylation (Figure 2). Fully Methylated Jurkat DNA is supplied with a BRCA1 primer set. As native Jurkat DNA is not methylated at the BRCA1 locus, this primer set is an ideal control for use in methylation-specific experiments with Fully Methylated Jurkat DNA.

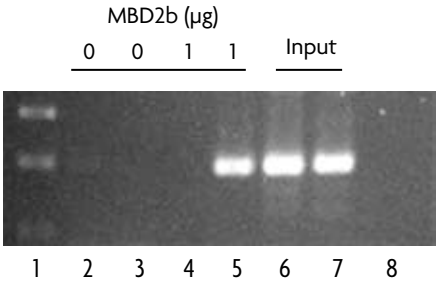

Figure 2: Fully Methylated Jurkat DNA is a control for MethylCollector.
100 ng genomic (lanes 2 & 4) and Fully Methylated Jurkat DNA (lanes 3 & 5) were assayed with (lanes 4 & 5) or without (lanes 2 & 3) inclusion of the MethylCollector His-tagged MBD2b protein. These samples and input genomic (lane 7) and Fully Methylated Jurkat DNA (lane 8) were then amplified by PCR with the BRCA1 primers. A positive result is observed only with Fully Methylated Jurkat DNA captured with the His-tagged MBD2b in the MethylCollector assay. Lane 8 is a water-only control.

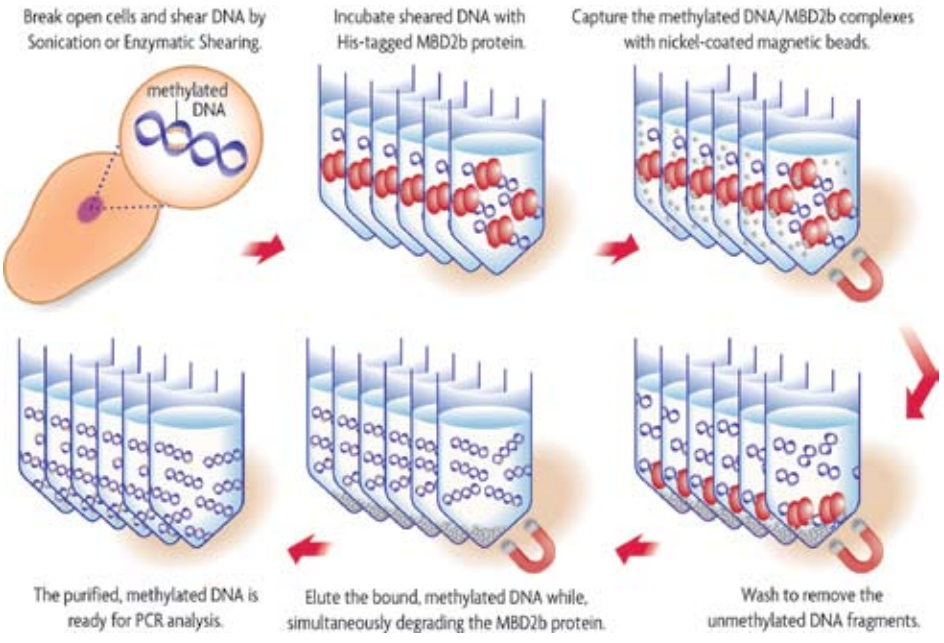

Figure 1: Flow chart of the MethylCollector process.

Product	Format	Catalog No.
MethylCollector™	25 rxns	55002
Fully Methylated Jurkat DNA	10 µg	55003

Upgrade your Chromatin IP Experiments using ChIP-IT™ Kits and Reagents

Active Motif's line of ChIP-IT™ Kits and reagents are a complete, flexible set of tools that make chromatin immunoprecipitation (ChIP) faster and more consistent. ChIP-IT Kits combine all of the critical components needed in a single kit, validated and proven to work in ChIP. Kits are available in either sonication or enzymatic shearing formats, with or without positive and negative controls.

Chromatin IP: The Nexus of Genomics and Proteomics

Chromatin immunoprecipitation involves enrichment of protein/DNA complexes using an antibody to a chromatin-associated protein. It is a versatile technique combining the specificity of immunoprecipitation, the sensitivity of PCR and the screening power of array profiling. However, ChIP can be technically challenging and difficult to validate without well-proven reagents. By providing proven ChIP-validated antibodies, reagents and controls, you will realize the best results possible.

Why use ChIP-IT?

- Complete solution – all reagents and controls supplied
- Direct measurement of protein/DNA interactions
- Choice of enzymatic or sonication shearing methods
- No messy phenol/chloroform extractions
- Compatible with genome-wide profiling or gene-specific PCR-based approaches
- No need to optimize reagents or protocols

ChIP-IT Control Kits

ChIP-IT Control Kits provide positive & negative control antibodies and species-specific PCR primers, PCR buffer and a convenient DNA loading dye that makes your PCR reactions ready for loading on an agarose gel straight from the thermocycler.

ChIP-IT Express: High-throughput ChIP is now a reality

Active Motif's innovative ChIP-IT Express Kits use protein G-coated magnetic beads, making it possible to simplify the ChIP protocol, optimize buffers and eliminate several time- and labor-intensive steps. The magnetic beads have much lower background than traditional agarose beads, so pre-clearing and blocking are no longer necessary. Washing is easier because the spin steps have been replaced by rapid magnetic pull-down. Additionally, our specialized ChIP Elution Buffer eliminates the need for DNA purification after the ChIP is complete. The result is that ChIP-IT Express makes ChIP more successful, more consistent and reduces your time and effort.

ChIP-IT Enzymatic Kits for simplified chromatin shearing

For successful ChIP experiments, chromatin must first be sheared to 200-1000 bp fragments. Traditionally, shearing is performed by subjecting chromatin to different pulses of sonication, which can be difficult to optimize and reproduce. To eliminate the problems associated with sonication, Active Motif has developed an enzymatic shearing method to quickly and easily shear chromatin into 200-1000 bp fragments (Figure 1), proving your ChIP results. These enzymatic shearing components are included in the ChIP-IT Enzymatic Kits (with or without controls) and the ChIP-IT Express Enzymatic Kit. They are also available separately as the Enzymatic Shearing Kit.

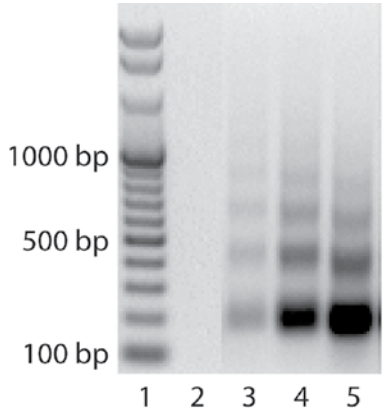

Figure 1: Analysis of DNA sheared using the Enzymatic Shearing Kit.
HeLa cells were fixed for 10 minutes with 1% formaldehyde and then chromatin was prepared using the Enzymatic Shearing Kit protocol. Chromatin was sheared with the Enzymatic Shearing Cocktail for 5, 10 or 15 minutes before the reaction was stopped.

Lane 1: 100 to 1000 bp ladder.
Lane 2: Unsheared HeLa DNA.
Lane 3: HeLa DNA treated for 5 minutes.
Lane 4: HeLa DNA treated for 10 minutes (optimized).
Lane 5: HeLa DNA treated for 15 minutes.

Ready-to-ChIP HeLa Chromatin

For your convenience, Active Motif offers Ready-to-ChIP HeLa Chromatin. Ready-to-ChIP HeLa Chromatin has been optimally sheared by sonication and validated in ChIP. As a result, you can more easily validate your own antibodies and primer sets. It can be used in conjunction with all of the ChIP-IT Kits and controls, so you can be certain the only variable in testing an antibody for ChIP is the antibody itself.

To upgrade your ChIP experiments, visit www.activemotif.com/chipit to learn more about the ChIP-IT line of kits, reagents and ChIP-validated antibodies.

NEW: High-quality Antibodies to Histones and Histone Modifications to Further your Chromatin Research

As part of the recent merger with Lake Placid Biologicals, Active Motif now offers high quality, well characterized antibodies to histones and histone modifications to facilitate your research into histones and chromatin biology. We have years of experience making antibodies to histone modifications and have developed methods to ensure that only the best possible antibodies reach your hands. We also work closely with researchers in the field to produce novel, cutting edge tools for your research.

Organization of DNA into chromatin

DNA is organized by its incorporation into chromatin, 147 base pairs of DNA coiled around an octamer of core histone proteins to form the basic subunit of chromatin, the nucleosome. The core histones are H2A, H2B, H3 and H4, while histone H1 associates with chromatin outside the nucleosome and regulates higher order chromatin structure. Specific functional groups (phospho-, methyl-, acetyl-, ubiquityl-) are added or subtracted from histone proteins, and these dynamic addition and subtraction events have profound effects on the function of chromatin. In fact, they are crucial to the regulation of all genome-based activity: transcription, chromosome packaging, DNA damage repair, DNA recombination, *etc*.

Rigorous specificity testing

Our standards for antibodies are just as high as those of our customers. When we make an antibody to a histone modification, it is crucial that the antibody recognize only the modification of interest, not a related modification or a modification at a site with a similar amino acid sequence. To this end, we employ dot blot analysis on every product we produce, and we make these data available for review on our web site and our product data sheets.

As most histone-modification antibodies are generated using synthetic peptides, we test ours against the immunogen, as well as the unmodified form of the peptide. This ensures that the signal you see in your experiments is indeed the modification we say it is. We also screen for reactivity against similar sites and sequences (Figure 1). Only after an antibody has passed the specificity test do we further characterize it by testing it in a variety of applications, including:

- Western Blot
- Chromatin Immunoprecipitation
- Immunofluorescence

These practices not only result in highly specific, high-quality antibodies, but they also enable us to virtually eliminate lot-to-lot variability. This reflects our philosophy that it is important to repeat your experiments, but not because your reagents failed.

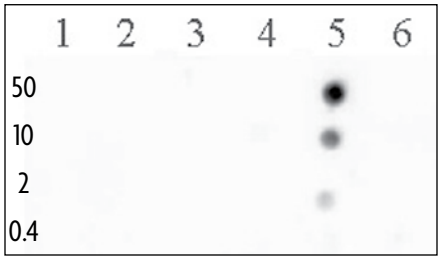

Figure 1: Dot blot analysis to confirm the specificity of Histone H2B acetyl Lys120 pAb.
Modified and unmodified peptides were spotted onto PVDF and probed with anti-acetyl Lys120 Histone H2B (Cat. No. 39119) at a 1:2,000 dilution. The amount of peptide spotted (in picomoles) is indicated next to each row. Lane 1: peptide acetylated at lysine 5 of H2B, Lane 2: unmodified lysine 5 peptide. Lane 3: peptide acetylated at lysine 16 of H2B, Lane 4: unmodified lysine 16 peptide, Lane 5: peptide acetylated at lysine 120 of H2B, Lane 6: unmodified lysine 120 peptide.

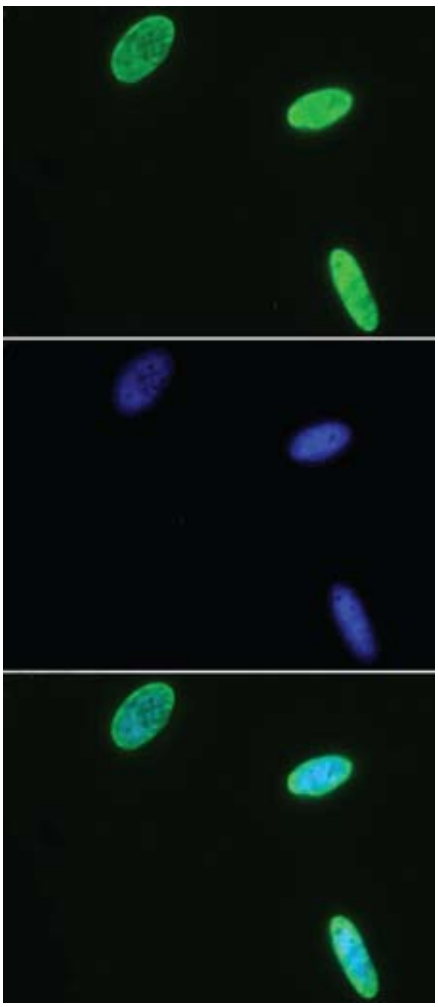

Figure 2: Immunofluorescence with HeLa cells.
HeLa cells stained with Histone H3 dimethyl Lys9 pAb (Catalog No. 39239) at a dilution of 1:1,000. **Top:** Histone H3 dimethyl Lys9 pAb. **Middle:** DAPI. **Bottom:** merge.

Please browse our complete line of histone and histone-modification antibodies by visiting us online at: www.activemotif.com/histonemods.

NEW: Sensitive HDAC Assay Kits in Colorimetric and Fluorescent Readouts

Active Motif's new HDAC Assay Kits are easy and sensitive assays that can be used to determine histone deacetylase activity as well as to screen potential inhibitor compounds in your cell or nuclear extracts, immunoprecipitates or purified enzymes.

What's in the kit?

HDAC (Histone Deacetylase) Assay Kits are 96-well plate-based assays for determining HDAC activity levels in your extracts or purified samples. HDAC Assay Kits are available in both colorimetric and fluorescent formats to suit your needs. Each HDAC Assay Kit contains positive control HeLa nuclear extract, deacetylated HDAC assay standard, HDAC peptide substrate, trichostatin A as a model inhibitor and all the essential buffers needed for a full 96-well plate assay.

How does it work?

The HDAC Assay Kits utilize a peptide substrate that contains an acetylated lysine residue that can be deacetylated by Class I, II 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 and releases either the chromophore or the fluorophore from the substrate to result in either a colorimetric or fluorescent product. The colorimetric product absorbs maximally at 405 nm and the fluorescent product can be read with an excitation wavelength of 360 nm and emission wavelength of 460 nm (Figure 1).

Why not try it today?

Please visit www.activemotif.com or give us a call to find out more about the HDAC Assay Kits and related HDAC antibodies.

HDAC Assay Kit advantages

- Works with Class I, II and IV HDAC enzymes
- Easy to adapt to work with Class III or Sirtuin, HDAC enzymes
- Use nuclear extracts, immunoprecipitates, column fractions or purified proteins
- Calculate enzymatic activity and screen inhibitor compounds

Assay for activity or inhibition

With these simple HDAC Assay Kits, you can easily assay for HDAC activity or you can screen compounds for their inhibitory effects. Also, the provided deacetylated assay standard enables you to quantify HDAC effects with greater accuracy.

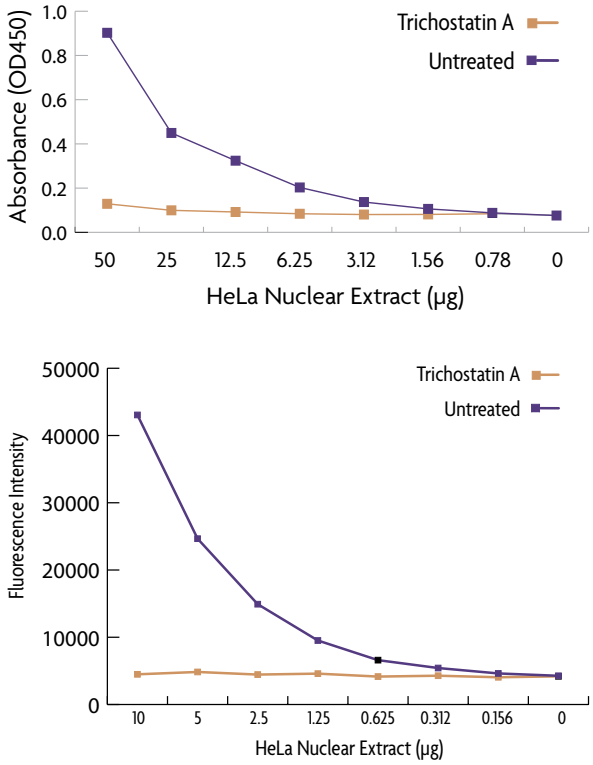

Figure 1: HDAC Assay Results.
Colorimetric (top) and Fluorescent (bottom) HDAC Assay results. HeLa Nuclear Extracts were assayed at 0 to 50 µg per well for the colorimetric assay and 0 to 10 µg per well for the fluorescent assay. Untreated extract results are shown with a purple line, and extracts inhibited with 1 mM Trichostatin A are shown with a copper line.

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

DNA Damage Assay – an Easy Assay for DNA Damage Signaling and Repair

Active Motif's DNA Damage Assay is a sensitive, accurate and simple 2-color fluorescent assay of H2AX phosphorylation, which occurs following double-stranded breaks in DNA. This early marker of DNA damage makes it easy to determine the level of DNA breaks in cultured cell samples in response to treatment conditions.

Overview of DNA damage

When cells are exposed to ionizing radiation or certain treatment compounds, double-stranded DNA breaks are created that initiate a rapid and highly coordinated series of signaling events. Phosphorylation of the histone variant H2AX at serine 139 to form γ-H2AX is one of the earliest of the DNA-damage signaling and repair events, and is crucial for the coordination of downstream signaling and repair activities. The active phosphorylation of H2AX has been shown to spread a distance of up to 1 to 2 mbp from the site of a double-strand break in DNA in mammalian cells, which makes phosphorylated H2AX (Ser 139) a useful marker for the level of DNA breaks in cells. With Active Motif's DNA Damage Assay it is easy to screen compounds and treatment conditions for their ability to induce DNA breaks, repair DNA breaks and study the timing of DNA breaks in cultured cells.

How does the assay work?

The DNA Damage Assay is a cell-based assay conducted in 96-well plates. Each kit includes all the reagents necessary for two 96-well plates. Cells are grown and treated in the plate, then fixed and incubated with the phospho-Histone H2AX (Ser 139) double-stranded DNA damage marker antibody. A short incubation with the included Chromeo™ 488 secondary antibody is performed for detection. The cells are then washed and stained with propidium iodide and read on a fluorescent plate reader.

What's in the kit?

The DNA Damage Assay includes the polyclonal phospho-Histone H2AX (Ser 139) antibody, Chromeo 488 Goat anti-Rabbit secondary antibody and the positive control compound etoposide, which can induce >98% DNA damage (Figure 1).

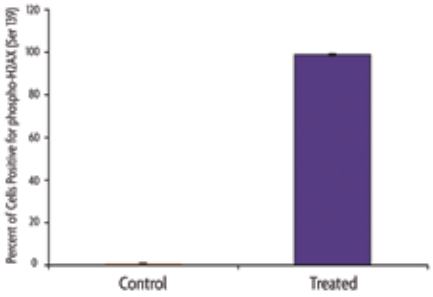

Figure 1: Percent induction of apoptosis in HeLa cells. Levels of phospho-H2AX measured in untreated (control) and cells treated with etoposide. Averages of quadruplicates are shown.

Propidium iodide is included to provide a total cell count. This simple detection method utilizing the superior Chromeo 488 fluorescence and propidium iodide stain makes the kit ideal for both high-throughput and high-content screening in all cells (Figure 2).

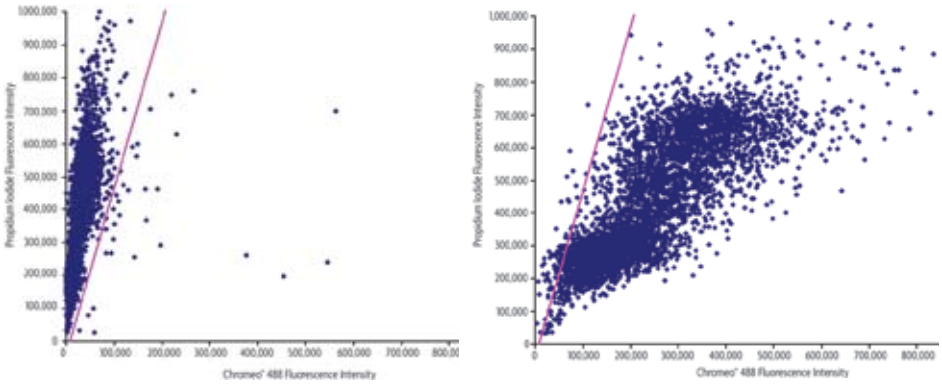

Figure 2: IsoCyte™ single cell analysis of phospho-H2AX detected with Chromeo 488 secondary in HeLa cells. Untreated HeLa cells (left panel) are detected mostly in the propidium iodide channel while etoposide-treated cells are seen in the green (488 nm) channel indicating a high degree of DNA damage.

Specificity for low background

The assay relies on a high-quality rabbit polyclonal phospho-Histone H2AX (Ser 139) antibody that is subsequently detected with the Chromeo 488 Goat anti-Rabbit secondary. Our Chromeo fluorescent secondary antibodies are highly specific with extremely low background, so your results are always accurate.

Start screening compounds today!

Screening treatments for the induction of DNA damage and repair has never been easier with our DNA Damage Assay. For more information about this assay and our other DNA Damage and Repair assays, please give us a call.

Product	Format	Catalog No.
DNA Damage Assay (Fluorescent)	2 x 96 rxns	18030
Histone H2AX phospho Ser139 pAb	200 µg	39117

NEW: MAX Stain™ Immunofluorescence Products for Better IF Experiments

Active Motif products for immunofluorescence are the result of our experience with standard, confocal and super-resolution microscopy and are actively employed in our own research efforts. Whatever your microscopy method may be, we are certain you will benefit from the time we have spent optimizing and standardizing the protocols involved and creating these products.

Immunofluorescence experiments require high-quality reagents

As biological research causes us to focus on continually smaller and more complex interactions within the cellular environment, fluorescence microscopy using antibodies to detect specific proteins (indirect immunofluorescence) is employed to answer questions with regards to protein localization, abundance and coincident staining. While technical advances in equipment and optics have helped to improve image quality, immunofluorescence experiments attempting to elucidate the different properties of proteins ultimately depend upon the quality of the reagents used.

MAX Stain™ Universal Immunofluorescence System

Active Motif eliminates the guesswork and challenge from immunofluorescence experiments with the new MAX Stain Universal Immunofluorescence System. It includes all components required to perform high-quality IF experiments and a detailed protocol – just add cells and a primary antibody directed against your target protein. MAX Stain includes:

- Two sterile 6-well plates with coverslips to grow your cells
- MAXblock™ Blocking Medium
- MAXbind™ Staining Medium
- MAXwash™ Washing Medium
- MAXfluor™ Trio Mounting Media Kit
- MAXseal™ Coverslip Sealing System
- 24 MAX Stain slides
- Chromeo™ 488 anti-rabbit secondary antibody

MAXblock™ – an highly effective blocking reagent for immunodetection experiments

MAXblock is a protein-based non-mammalian blocking agent for immunofluorescence and immunodetection assays. Superior blocking is achieved utilizing a protein blend that demonstrates no cross-reactivity with primary or secondary antibodies (Figure 1). MAXblock is utilized in all Active Motif immunofluorescence experiments, enabling us to provide the best possible testing data.

Improve your IF experiments today

Visit www.activemotif.com/maxstain to see all the IF kits and reagents that will make your IF experiments easier and more reproducible.

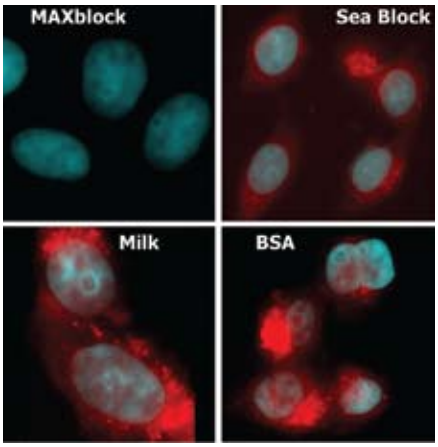

Figure 1: Negative controls pushed to their limits. Methanol-fixed cells were incubated with the indicated blocking agent, then with a fluorescent secondary antibody (pseudo-colored red) at twice the normal dilution. Any red observed in the figures represents non-specific binding of the secondary due to incomplete blocking. Note that no staining is observed in the MAXblock panel, demonstrating its effectiveness.

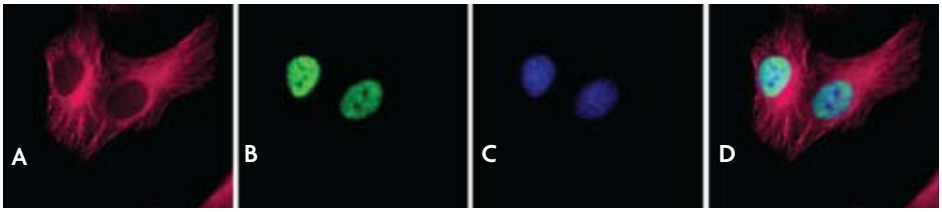

Figure 2: Immunofluorescence performed using the MAX Stain Universal Immunofluorescence System. **Image A:** alpha Tubulin mAb (Cat. No. 39527). **Image B:** Histone H3 acetyl Lys23 pAb (Cat. No. 39131). **Image C:** DAPI staining using MAXfluor™ DAPI Mounting Media. **Image D:** Merge of all three channels.

Product	Format	Catalog No.
MAX Stain™ Universal Immunofluorescence System	1 kit	15250
MAXblock™ Blocking Medium	150 ml	15252
MAXbind™ Staining Medium	250 ml	15253
MAXwash™ Washing Medium	1000 ml	15254
MAXpack™ Immunostaining Media Kit (incl. 15252, 15253, & 15254)	1 kit	15251
MAXfluor™ Mounting Medium	2 ml	15256
MAXfluor™ DAPI Mounting Medium	2 ml	15257
MAXfluor™ PI Mounting Medium	2 ml	15258
MAXfluor™ Trio Mounting Media Kit (incl. 15256, 15257, & 15258)	1 kit	15255

Most Published Transcription Factor Assay

TransAM™ Kits are DNA-binding ELISAs that have quickly become the assay of choice for transcription factor researchers. The combination of a fast, user-friendly format with high sensitivity and specificity makes it easy to see why over 640 citations are available for TransAM Kits (Figure 1).

Why TransAM is best

The DNA-binding function of activated transcription factors is often studied using EMSAs/gelshifts. But, EMSAs are time consuming and, at best, provide only semi-quantitative results. Moreover, they are low throughput, require radioactive probes and tend to lack sensitivity and reproducibility (Table 1). TransAM Kits, however, use a unique plate-based format to capture activated transcription factors, which are analyzed using antibody specific to your isoform of interest. TransAM Kits are also non-radioactive and offer up to 100-fold more sensitivity than traditional gelshift techniques (Figure 2). This enables you to detect even the smallest changes in transcription factor levels.

Widest selection of targets and formats

Not only is TransAM the most cited transcription factor ELISA, but Active Motif is proud to offer the broadest selection of targets, including kits for NFκB, AP-1, STAT, PPARγ, HIF-1, NFATc1, C/EBP and many others. TransAM NFκB Kits are also available in a colorimetric or ultra-sensitive chemiluminescent readout. And, for the ultimate in flexibility, TransAM Flexi NFκB Kits let you use any capture oligo you choose. Please visit our website for a complete product listing, and be sure to check for upcoming new releases!

Table 1: Common Methods and Their Properties

Method	Non-Radioactive	Sample Versatility	Functional Assay	Quantitative	Convenience
Western Blot	+	+	-	-	-
EMSA	-	+	+	-	-
Reporter Assay	+/-	-	+	+/-	-
TransAM	+	+	+	+	+

A complete solution

TransAM's quantitative and flexible format has made it an invaluable tool for transcription factor researchers. Furthermore, its sensitivity has been proven with various cell lines and primary material such as tissues and peripheral blood mononuclear cells (PBMCs). Researchers have used the kits to screen drugs, monitor transcriptional activity regulation, study protein structure/function relationships and more. Consequently, TransAM is the most widely cited DNA-binding ELISA available (Figure 1). So, why not visit www.activemotif.com/transam or give us a call to find out more?

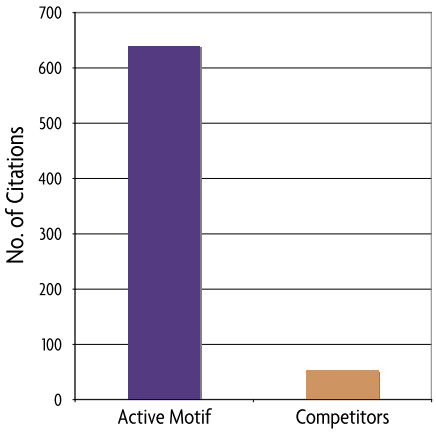

Figure 1: TransAM Kit citations.
Using HighWire Press, http://highwire.stanford.edu, a comparison was made by searching for citations of TransAM from Active Motif versus the tradenames of all competitor kits combined. TransAM is clearly the leader.

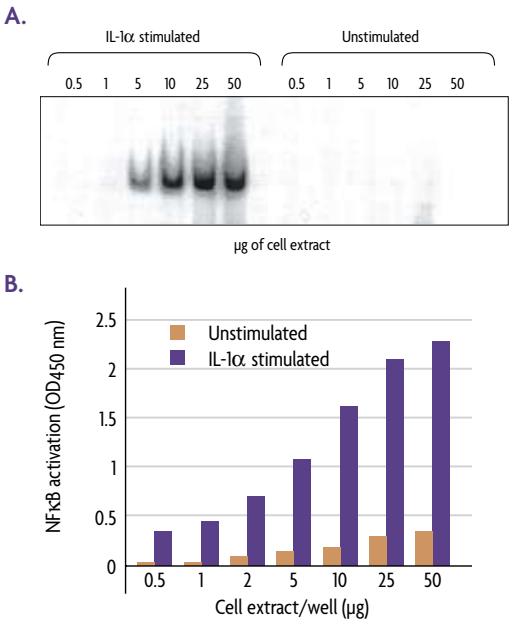

Figure 2: TransAM NFκB is more sensitive than gelshift.
Human fibroblast WI-38 cells are stimulated with IL-1α for 30 minutes. Increasing amounts of whole-cell extract are assayed using the TransAM NFκB p50 Kit (B) or gel retardation (A).

Isolate Pure, Separate Fractions of Histone H2A/H2B and H3/H4 While Preserving Post-translational Modifications

Now you can easily purify histones and further separate the fractions of core histones from any cell culture or tissue sample while maintaining post-translational modifications like acetylation, methylation and phosphorylation states.

Histone purification made easy

Active Motif's Histone Purification Kit enables you to isolate core histones from any cell culture or tissue sample (Figure 1). The core histones may be purified as one total population containing H2A, H2B, H3 and H4, or fractionated into separate pools of H2A/H2B dimers and H3/H4 tetramers (Figure 2).

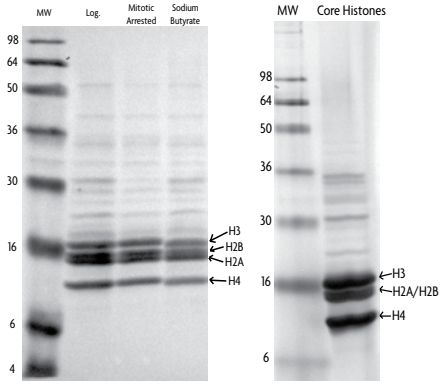

Figure 1: Core histones isolated from cells and tissue. Total core histones were isolated from HeLa cells (left) and rat brain tissue (right).

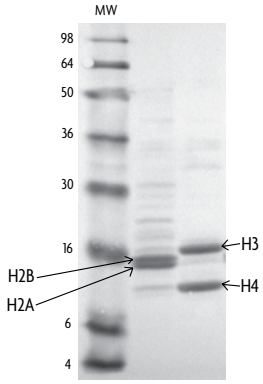

Figure 2: Separate H2A/H2B and H3/H4 fractions. H2A/H2B and H3/H4 fractions isolated from HeLa cells.

Preserve important modifications

Preserving post-translational modifications like acetylation, methylation and phosphorylation is critical when investigating the role of histones in transcription or chromatin biology. Our proprietary buffer system is optimized to maintain these modifications while separating the histones into pure fractions (Figure 3).

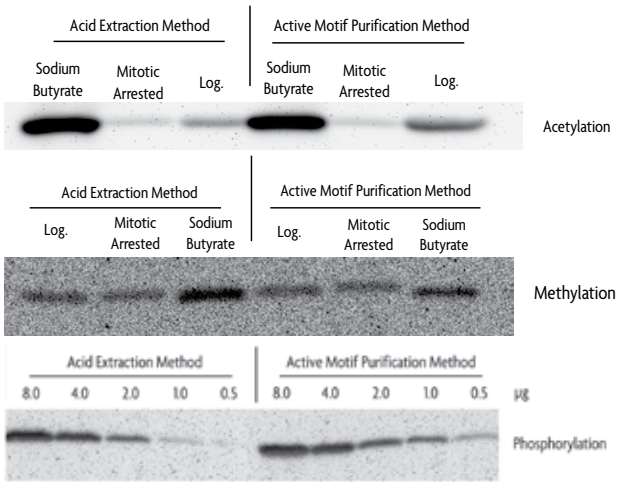

Figure 3: Post-translational modifications preserved. Acetylation, methylation and phosphorylation states are preserved as well or better with the Histone Purification Kit vs. a straight acid precipitation method.

How does it work?

Unlike histone purification by acid precipitation, our method utilizes a unique purification resin and a series of proprietary elution buffers to isolate very pure histone fractions. The resin has a high binding capacity for histones, so core histones can be isolated from small cell culture samples or from grams of tissue. Sequential elution steps let you collect the histones in either one step containing all core histones or into two separate populations: one enriched for H2A and H2B and a second fraction containing >90% pure H3 and H4.

What's in the kit?

Each kit contains all the necessary equilibration, neutralization and elution buffers plus a reusable purification column for 10 histone purifications.

Better substrate for downstream assays

Core histones isolated by the Histone Purification Kit method are highly pure and suitable as substrates for downstream assays. Purified histones can be used with the Active Motif's Chromatin Assembly Kit (Catalog No. 53500) to enable the generation of chromatin that very closely resembles native chromatin for functional assays (Figure 4).

Figure 4: Chromatin assembled with purified histones. Histones were purified from HeLa cells and used in the Chromatin Assembly Kit. The ordered spacing of nucleosomes was confirmed and analyzed by agarose gel.

Try it today!

Call or visit us at www.activemotif.com to find out more about the Histone Purification Kit and related products that will benefit your research.

Product	Format	Catalog No.
Histone Purification Kit	10 rxns	40025

Investigate SUMOylation with Efficient SUMOlink™

Active Motif's SUMOlink™ Kits provide a simple, effective method for generating SUMOylated proteins *in vitro*. These fast, efficient and robust assays contain all the necessary reagents for SUMOylation of target proteins, and include positive and negative controls that help ensure your success.

The SUMOlink method

SUMOlink™ Kits enable you to easily perform and detect post-translational modifications by SUMO (small ubiquitin-like modifier). With SUMOlink, you 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 (Figure 1). With the kit's p53 antibody and either SUMO-1 or SUMO-2/3 antibodies, you can easily see the extent to which your target protein has been SUMOylated.

Everything you need to study SUMO

The kits contain E1 activating and E2 conjugating enzymes along with wild-type and mutant SUMO-1 (SUMO-1 Kit) or SUMO-2 and -3 proteins (SUMO-2/3 Kit). Antibodies for SUMO-1 or SUMO-2/3 modifications, as well as control p53 protein and antibody, are included. For complete information, please give us a call or visit us at www.activemotif.com.

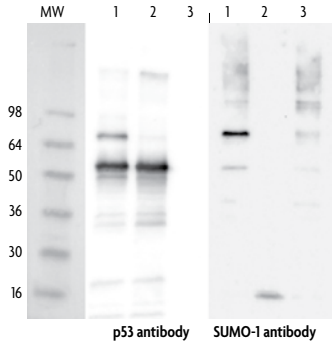

Figure 1: Specific SUMO-1 labeling of p53 by SUMOlink. Western blot analysis of *in vitro* SUMOylation of p53 protein by wild-type and mutated isoforms of SUMO-1: The 2 Western blots were incubated with p53 antibody (1:5000 dilution) and SUMO-1 antibody (1 :4000 dilution).

Lane 1: Wild-type SUMO protein conjugation reaction.
Lane 2: Mutated SUMO protein conjugation reaction.
Lane 3: No p53 control protein used in conjugation.

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

MethylDetector™ – Fast, Reproducible DNA Methylation Analysis

Active Motif's MethylDetector™ Bisulfite Modification Kit makes DNA methylation analysis fast and efficient by combining optimized reagents for performing DNA conversion with time-saving DNA purification columns and positive control PCR primers to help you validate your results.

Proven controls ensure success

DNA methylation analysis typically involves using bisulfite to convert unmethylated cytosines to uracils, while leaving methylated cytosines unchanged. The DNA is then amplified by PCR and analyzed by sequencing or restriction digest. However, bisulfite conversion can be technically challenging, and confirming the process was successful before sample analysis is preferred. MethylDetector provides positive control PCR primers that are specific for bisulfite-converted DNA, so you can confirm the procedure worked before starting further costly analysis (Figure 1).

Advantages

- **Flexible** – use high G/C content sequences and uncut DNA
- **Reproducible** – 99% conversion efficiency
- **Efficient** – reagents and protocol with proven controls
- **Easy** – DNA purification columns mean no more precipitations

Try MethylDetector today

MethylDetector will speed and simplify your methylation analysis. Learn more at www.activemotif.com.

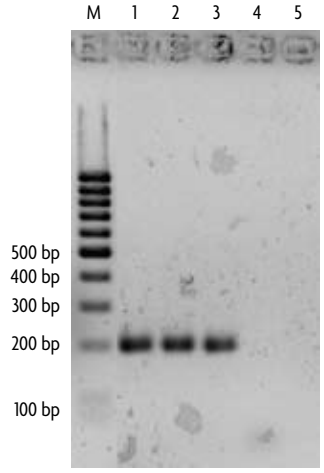

Figure 1: Agarose gel analysis of PCR products generated with MethylDetector.
Three different DNA conversions were performed (Lanes: 1-3) and compared to an unconverted DNA control (Lane: 5) and to a no DNA control (Lane: 4). The presence of PCR product in only the converted samples demonstrates the conversion efficiency and reproducibility of the MethylDetector Kit.

Product	Format	Catalog No.
MethylDetector™	50 rxns	55001