MOTIF VATIONS

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Tools to Analyze Nuclear Function

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The Impact of DNA Methylation and Chromatin Modification on Development and the Regulation of Gene Expression

IN THIS ISSUE

Epigenetics and Differences Between Monozygotic Twins and Cloned Animals

Monozygotic twins and cloned animals are considered genetically identical, but significant phenotypic discordances between them may exist. Epigenetic differences between individuals with identical DNA sequences can explain in part these discordances.

While monozygotic twins and cloned animals are considered to be genetically identical, significant phenotypic discordances between them may exist. These differences may in part account for discordant expression of disease in such pairs, including psychiatric diseases (*e.g.* schizophrenia, bipolar disease, depression, autism, eating disorder, alcoholism and other addictions, suicidal behavior and hyperactivity) and autoimmune diseases (*e.g.* juvenile diabetes, multiple sclerosis, systemic lupus and rheumatoid arthritis).[1-3] Also observed are increasing degrees of phenotypic discordances during aging of monozygotic twins or cloned animals.

The causes of incomplete concordance between individuals with identical DNA sequences are not clear, and this phenomenon represents one of the most challenging questions in complex, non-Mendelian diseases. These discordances can be attributable to environmental exposures,[4-6] to the quality of medical and spousal care, and to genetic changes. Indeed, point mutations and aberrant chromosomal segregations are known to have an impact upon the emergence of neoplastic diseases. Several subtypes of human endogenous retroviruses were also identified using representational difference analysis in monozygotic twins discordant for schizophrenia, but their role in the etiopathogenesis of the disease is not clear.[7] Epigenetic differences may be an important part of the solution to this puzzle. Moreover, epigenetic profiles may represent a link between an environmental factor and phenotypic differences between monozygotic twins or cloned animals.

Epigenetics refers to both heritable and transient changes in gene expression that do not entail a change in primary DNA sequence. These changes include DNA methylation and post-translational modifications of the histone proteins. Methylation of mammalian DNA has long been recognized to play a major role in different cellular functions, such as development and control of gene expression, and is generally associated with transcriptional repression. The DNA methyltransferases (DNMTs) catalyze the transfer of a methyl group from S-adenosyl methionine to the 5´ position of cytosines, mostly within CpG dinucleotide motifs (reviewed in 8). Reported histone modifications include acetylation, methylation, phosphorylation, ubiquitylation, glycosylation, ADP-ribosylation, carbonylation and SUMOylation. Many histone amino acids are modified, including lysine residues (acetylated, methylated or coupled to ubiquitin), arginine residues (methylated), and serine or threonine residues (phosphorylated). These modifications are positively or negatively correlated with specific transcriptional states, or the specific organization of repressive or open chromatin. Many modifications affect one another; collectively they constitute the "histone code".

A substantial degree of epigenetic discordance is accumulated over millions of mitotic cell divisions.

In this review, we will focus on the involvement of epigenetics in some discordances observed in genotypically identical individuals.

Epigenetic differences during the lifetime of monozygotic twins

Increasing degrees of phenotypic discordances are observed during the lifetime of monozygotic twins. Fraga *et al.* have shown by observing DNA methylation and histone acetylation of a large cohort of monozygotic twins that, although twins are epigenetically indistinguishable during the early years of life, older twins exhibit remarkable differences in their overall content and genomic distribution of 5-methylcytosine DNA and histone acetylation, affecting their gene-expression profile.

An interesting example is a report of twins, both of whom developed histologically confirmed dementia of the Alzheimer's type. But, one had the diagnosis in her late 60s and the other was not diagnosed until age 83.[9,10] It has also been suggested that epigenetic dysregulation may make an important contribution to the etiopathogenesis of major psychoses, which can affect one of the twins and not the other one.[11]

Discordant diseases in monozygotic twins

Beckwith-Wiedemann syndrome (BWS)

A substantial degree of epigenetic discordance can be accumulated over millions of mitotic division of cells in genetically identical organisms, due to the partial stability of epigenetic regulation. In skin fibroblasts from five monozygotic twin pairs discordant for BWS, the affected twins had an imprinting defect at the *KCNQ1OT1* gene. The epigenetic defect is thought to arise from the unequal splitting of the inner cell mass, which contains the DNA methylation

enzymes, during the twinning; this results in differential maintenance of imprinting at *KCNQ1OT1*. In another study of twins, mapping of methylated cytosines revealed numerous subtle inter-individual epigenetic differences, which are likely to be a genome-wide phenomenon.[12]

Systemic Lupus Erythematosus (SLE)

Systemic Lupus Erythematosus is an archetypal systemic, autoimmune inflammatory disease, characterized by the production of autoantibodies to multiple nuclear antigens. Apoptotic defects and impaired removal of apoptotic cells contribute to an overload of autoantigens that initiate autoimmune response. The highest estimated concordance rate of this disease in twins is < 60%,[13, 14] so the involvement of non-genetic factors in the pathogenesis of SLE must be invoked. The high incidence of twin pairs in which only one of them has developed SLE supports the notion that environmental factors and their involvement in epigenetic modifications could have an affect on the disease. Indeed, as mentioned above, epigenetic alterations are accumulated during the individual's lifetime.[15]

The epigenetic dysregulation of genes can contribute to, or increase the activation of, apoptosis. Moreover, apoptotic-released material with a specific epigenetic pattern may act as an antigen.[16] Epigenetic alterations can also contribute to an exacerbated activation of T and B cells. T cells from patients with active lupus were shown to exhibit globally hypomethylated DNA,[17] apparently due to a decrease in the enzymatic activity of DNA methyltransferases (DNMTs).[18] The mechanism by which hypomethylated T cells induce SLE is poorly understood. Alterations of the histone code seem to be implied in the development of SLE. Indeed, the use of histone deacetylase inhibitors suggests that deacetylation is involved in the skewed expression of certain genes that are associated with the disease.[18]

Epigenetic differences between cloned animals during development

Epigenetic regulation of gene expression is recognized to be one of the key mechanisms governing embryonic development and cellular differentiation. The derivation of the cloned embryos from somatic cells, which contain quite different epigenetic profiles compared to the germline, generates abnormalities of development that can arise from inadequate or inappropriate nuclear programming.

The mice *agouti* gene is responsible for the coat color of wild-type mice, and isogenic *pseudoagouti* mice have a range of coat colors from yellow to black. The darkness of the *pseudoagouti* mice is proportional to the amount of DNA methylation in the *agouti* locus, with complete methylation found in black *pseudoagouti* mice and reduced methylation in yellow ones.[19] The obvious phenotype of this isogenic mouse strain is controlled by epigenetic factors. But these factors are only partially heritable, which can explain the phenotypic differences that exist between two cloned mice.

The first cloned cat was created in Texas from a calico cat, but was phenotypically different from her "genetic mother". This can be partly explained by an epigenetic phenomenon known as chromosome X-inactivation. Calico cats are always female. The gene responsible of the coat color is located on the X chromosome. One allele results in an orange coat color and another allele of this gene results in black fur. In female cats, one X chromosome is silenced due to DNA methylation and chromatin modifications. The process of inactivation is random, which is why cloning a calico cat will never produce the same pattern. During the development of the cloned cat, the developmental program reactivates both X chromosomes, and the process of inactivation recurs in a random manner. This results in an entirely different coat pattern even when two individuals are genetically identical.

Although monozygotic twins are epigenetically indistinguishable early of life, older twins exhibit remarkable differences in their DNA methylation.

All these studies show the importance of epigenetics in the discordances observed between individuals with an identical genotype.

References

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NEW: HiLite™ Binding Assays – Innovative Assay uses Fluorescence Polarization to Measure Binding of Chromatin-modifying Proteins to Histone Tails

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.

Identifying proteins that interact with modified histones is of great interest to the research community, as such proteins are often important regulators of genome function, transducing the histone modification to generate a specific cellular outcome. For example, HP1 and Polycomb proteins have each been shown to bind to histone H3 that has been methylated at either lysine 9 or lysine 27. Methylation of these residues is involved in specifying regions of the genome that are heterochromatic and transcriptionally silent, and both HP1 and Polycomb are known to be involved in maintaining heterochromatic regions. With Active Motif's HiLite Histone H3 Methyl-Lys9 / Lys27 Binding Assay, you can use fluorescence polarization (Figure 1) 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 (Figure 2).

HiLite Binding Assay advantages

- **Homogeneous** – no washing steps 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.*

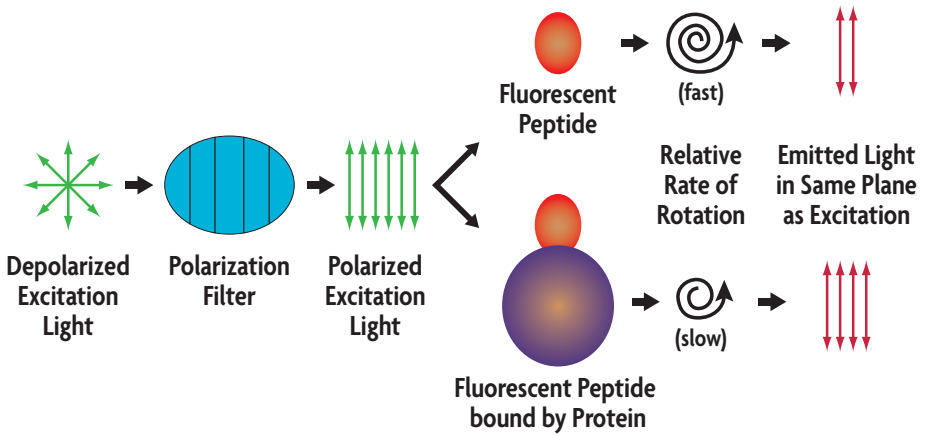

Figure 1: Schematic of the HighLite method that uses fluorescence polarization to measure protein binding. In HiLite, various fluorescent histone peptides with site-specific modifications are added to wells containing a recombinant protein (or binding domain), then excited with polarized light. If the peptide is bound by the protein, the rotation of the "bound complex" is much slower than the rotation of unbound peptide. Slower rotation causes the amount of polarized light that is emitted in the excitation plane to be greater for the complex than for the unbound peptide. This provides a quantitative measure of the histone-binding protein's affinity for the peptide's histone modification. With the peptides included in HiLite, it is possible to determine binding equilibria for proteins that range from 10 kDa to 100 kDa.

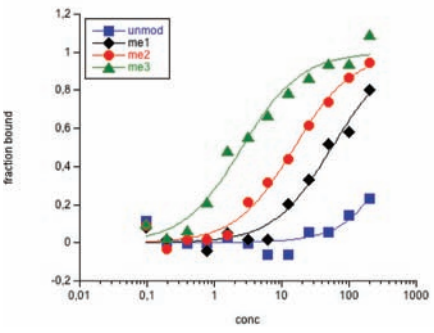

Figure 2: Binding of HP1 to histone H3 Methyl-Lys9. One µM of fluorescent histone H3 Lys 9 peptides, which are either unmodified or mono-, di- or trimethylated at K9, were added to a 96-well plate containing a serial dilution of HP1 protein (x axis) starting at a concentration of 200 µM. The plate was scanned on a Tecan Infinite F200 using 485 nm excitation and 535 nm emission filters with polarizers. A gain of 80 was used; 100 reads were taken of each well, then averaged.

What comes in the kit?

HiLite Histone H3 Methyl-Lys9 / Lys27 contains 8 fluorescently labeled peptides that correspond to the regions of histone H3 around lysine 9 and lysine 27 that are either unmodified, or mono-, di- or trimethylated. It also contains a positive control protein, assay buffer, calibration dye and five 96-well half area black polystyrene plates. One plate is for calibrating your microplate reader and performing a binding curve with the positive control protein; the remaining four plates are to assay your protein(s) of interest. 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

RECOMMENDED: Informally Informative Coverage of Epigenetics

EpiGenie is a website dedicated to keeping researchers in touch with the evolving field of epigenetics and spotlighting the innovative thinkers who are driving the field. It provides highlights on the latest epigenetics news, emerging methods and new products, as well as interviews with key researchers in the field in a casual and laid-back style that will have you soaking up key happenings effortlessly.

It's not easy to keep up-to-date with the avalanche of information out there. That's why EpiGenie created the most informative site on the Internet (voted two years in a row; by...them!) dedicated to epigenetics. So, if you're tired of not getting the coverage of epigenetics you want, check out EpiGenie's:

- Daily **Doses of Epigenetics News** with Real-time PubMed Feeds
- Weekly **Featured Publications** You Won't Want to Miss
- Monthly Technical Tidbits in **Product Reviews**, **Featured Products**, and **Method Spotlights**
- **Product Database** Packed with Epigenetics Research Products
- Comprehensive Listings of **Epigenetics Conferences**, **Meetings**, and **Symposiums**

The EpiGenie website.
EpiGenie features daily news, monthly features, a database showing kits and antibodies from a wide variety of companies, and many epigenetic protocols.

So the next time your research grind starts to get the better of you, take a break and spend a few minutes at EpiGenie. Check them out at www.epigenie.com, or sign up for their Newsletter and they'll come to you!

Detect Transcription Factor DNA-binding with TransAM™ ELISAs

Looking for a fast and simple alternative to gelshift assays? Active Motif's TransAM™ Kits are highly sensitive ELISA-based assays capable of detecting small changes in transcription factor activity without the need for gels or radioactivity. TransAM Kits are 10-fold more sensitive than conventional gelshift assays, while TransAM Chemi Kits utilize chemiluminescent detection to improve sensitivity up to 100-fold, enabling the use of only 40 ng of extract.

TransAM advantages

- **Faster procedure** – ELISA-based assay can be completed in 5 hours, yet provides more quantitative results than gelshift assays (Figure 1)
- **Sensitive** – up to 100-fold greater sensitivity than gelshift assays
- **Less effort required** – our kits are compatible with multi-channel pipettors to streamline wash steps
- **Non-radioactive** – colorimetric and chemiluminescent formats available

Flexible ordering options

Active Motif offers a large selection of TransAM™ Kits for over 40 different transcription factor targets including NFκB, AP-1, STAT, and PPARγ to name a few. For a complete product listing and additional details, please visit us at www.activemotif.com/transam.

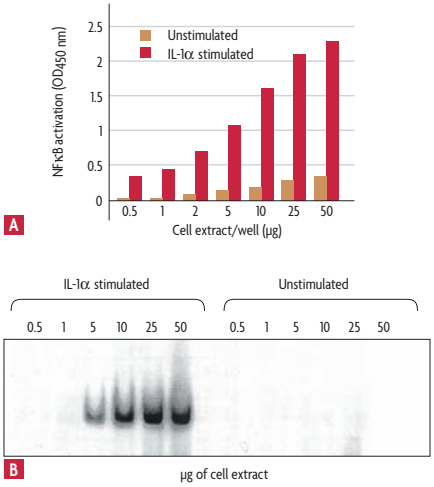

Figure 1: TransAM Kits are more sensitive than gelshift. Human fibroblast WI-38 cells were stimulated with IL-1α for 30 minutes. Increasing amounts of whole-cell extract are assayed using the TransAM NFκB p50 Kit (A) or gel retardation (B). The TransAM method is 10-fold more sensitive and provides quantitative results.

NEW: Histone Modification ELISAs to Quantitate Methylation Levels

Active Motif has made it easier to screen for changes in histone H3 methylation levels with the introduction of our Histone Modification ELISA kits. 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. Each kit also includes a site- and degree-specific methylated recombinant histone for use as a quantitative reference.

Histones & methylation

Methylation of lysine residues in nucleosomal histones is thought to mediate interactions with the protein complexes involved in regulating transcription, replication and DNA repair. Methylation of lysine 9 and lysine 27 of histone H3 has been correlated with heterochromatin formation and transcriptional repression, while methylation of lysine 4 on histone H3 is associated with active gene transcription in eukaryotes.

Histone Modification ELISA advantages

- **Sensitive** – works with purified core histones and acid extracts
- **Specific** – each modification antibody is tested to ensure low background and no cross-reactivity with other site- and degree-specific modifications (Figure 1)
- **Faster procedure** – the convenient ELISA format saves valuable time over immunoblotting methods
- **Controls ensure success** – includes positive control recombinant protein as a reference standard curve

Quantitate methylation levels

Our unique Recombinant Histone H3 proteins with site- and degree-specific methylation states are included for use as a reference standard curve (Figure 2). A comparison of sample results with known protein amounts will provide quantitative data on methylation levels. Active Motif's site- and degree-specific methylated lysine histones are also available separately (see page 7).

How do the Histone ELISAs work?

The Histone Modification ELISA kits include everything necessary to screen purified core histones (see page 7), or acid extracts for changes in methylation levels. A 96-stripwell plate is coated with a monoclonal histone H3 antibody to capture histone H3 from the sample. Active Motif's site- and degree-specific histone modification antibodies are used for detection. An HRP-conjugated secondary antibody and developing solutions provide a sensitive colorimetric readout that is easily quantified by spectrophotometry.

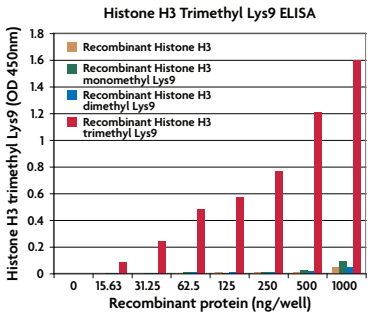

Figure 1: Histone H3 trimethyl Lys9 specificity. Recombinant Histone H3, mono-, di- and trimethyl Lys9 proteins were assayed from 15 ng - 1 µg per well using the Histone H3 Trimethyl Lys9 ELISA Kit. The results demonstrate the specificity of the assay. There is extremely low background from unmodified histone H3 and little cross-reactivity for mono- or dimethylated Lys9. This means that small, specific changes in trimethyl Lys9 levels can be detected with the ELISA.

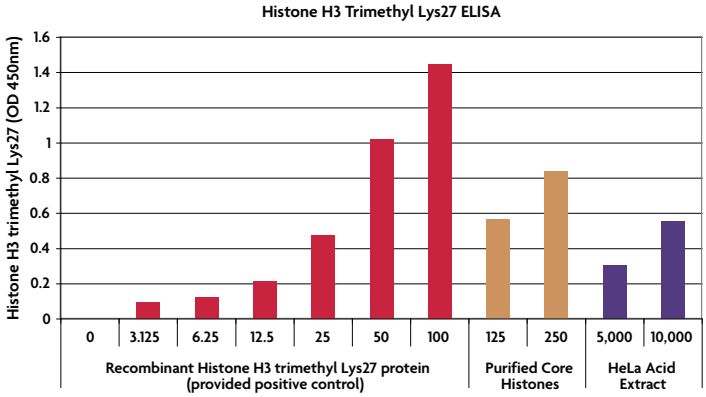

Figure 2: Histone H3 Trimethyl Lys27 detection with reference standard curve. The Histone H3 Trimethyl Lys27 ELISA was used to assay purified HeLa core histones (125-250 ng) and HeLa acid extract (5-10 µg). The provided recombinant Histone H3 trimethyl Lys27 protein was assayed from 3.125-100 ng per well as a reference standard curve. The results show the sensitivity of the assay in detecting Histone H3 trimethyl Lys27.

Product	Format	Catalog No.
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

Easily Purify Core Histones from Any Cell Culture or Tissue Sample

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. Active Motif has two versions of our Histone Purification Kit to meet your experimental needs.

How does it work?

Our unique kits use patented methods with proprietary binding columns and elution buffers to isolate very pure fractions of histones. Using the Histone Purification Mini Kit, core histones can be purified from as few as 8 x 10^5 cultured cells or tissue samples as a single population containing H2A, H2B, H3 and H4. Core histone isolated with the Mini Kit can be used in the Histone Modification ELISA Kits to screen for changes in the methylation level of specific modifications of histone H3 (see page 6).

The original Histone Purification Kit enables purification of several milligrams of core histones using either a spin-column or gravity-flow format, eluted as one total population or fractionated into separate pools of H2A/H2B dimers and H3/H4 tetramers (Figure 1). Core histones isolated using the Histone Purification Kit are highly pure and suitable as substrates for downstream assays, including Active Motif's Chromatin Assembly Kit, which generates chromatin *in vitro* that very closely resembles native chromatin for use in functional assays.

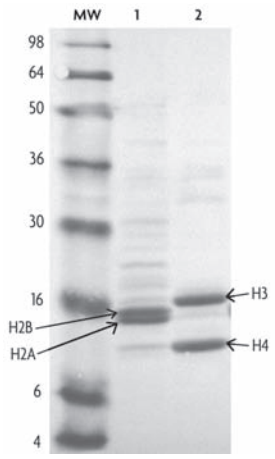

Figure 1: Histone Purification Kit.
The Histone Purification Kit was used to purify separate H2A/H2B (Lane 1) and H3/H4 (Lane 2) fractions from HeLa cells.

Product	Format	Catalog No.
Histone Purification Mini Kit	20 rxns	40026
Histone Purification Kit	10 rxns	40025
Chromatin Assembly Kit	10 rxns	53500

NEW: Histones Containing Specific Methylated Lysine Residue Modifications

To further investigate the influence of specific lysine methylation on the binding of associated chromatin proteins and transcriptional regulation, Active Motif offers recombinant histones that contain site-specific mono-, di- and trimethylated lysines. In addition to our previously released proteins, we now offer methylated H3 Lys36 proteins.

How are they made?

Recombinant histone H3 proteins are created using a patented approach in which an analog of methyl lysine is installed in the histone via a chemical alkylation reaction. This enables the site and degree of methylation to be carefully controlled. Each methylation reaction is over 99% complete and is verified by high-resolution mass spectrometry. The recombinant histones are also analyzed by dot blot or immunoblot to confirm identity. 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	Catalog No.
Recombinant Histone H3 (C110A)	50 µg	31207
Recombinant Histone H3 Lys4 (mono-, di-, or trimethyl)	50 µg	31208, 31209 or 31210
Recombinant Histone H3 Lys9 (mono-, di-, or trimethyl)	50 µg	31211, 31212 or 31213
Recombinant Histone H3 Lys27 (mono-, di-, or trimethyl)	50 µg	31214, 31215 or 31216
Recombinant Histone H3 Lys36 (mono-, di-, or trimethyl)	50 µg	31217, 31218 or 31219
Recombinant Histone H3 Lys79 (mono-, di-, or trimethyl)	50 µg	31220, 31221 or 31222
Recombinant Histone H4	50 µg	31223
Recombinant Histone H4 Lys20 (mono-, di-, or trimethyl)	50 µg	31224, 31225 or 31226

Improve Methyl-CpG Enrichment with MethylCollector™ Ultra

The MethylCollector™ Ultra Kit* improves the enrichment of CpG-methylated DNA by incorporating the Methylated CpG Island Recovery Assay (MIRA), which uses a combination of methyl-binding proteins (MBD2b and MBD3L1) to increase the affinity for methylated DNA fragments.[1] This unique protein complex provides greater specificity for methylated CpG dinucleotides than antibody immunoprecipitation (MeDIP) methods, and in less than half the time.

MethylCollector Ultra advantages

- **Improved efficiency** – high-affinity binding provides greater enrichment than antibody immunoprecipitation (MeDIP) methods
- **Faster procedure** – the convenient magnetic protocol can be completed in less than 3 hours
- **Uses minimal sample material** – requires as little as 1 ng of DNA (~200 cells), fragmented by sonication or enzymatic digestion
- **Controls ensure success** – includes positive control DNA and methylation specific PCR primers
- **Versatility** – the eluted DNA is suitable for use in endpoint or real time PCR, bisulfite conversion, or amplification and labeling for microarray analysis

"I compared MethylCollector Ultra to a commercial magnetic MeDIP. qPCR analysis showed that MethylCollector Ultra gave a much better enrichment....it was also a quicker and simpler procedure." – *Irina Bogdarina, Queen Mary University of London*

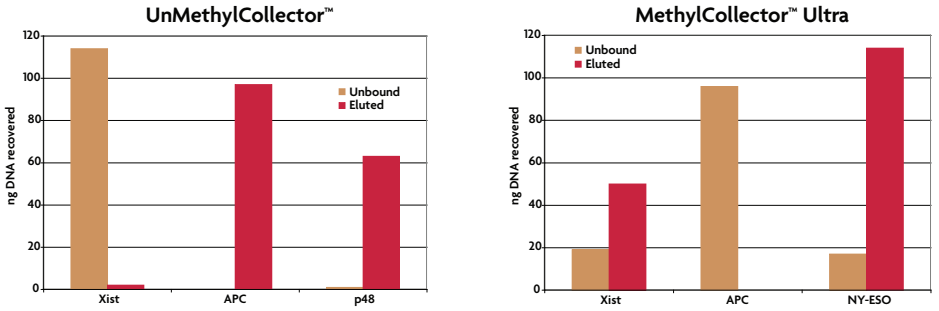

Figure 1: Direct comparison of UnMethylCollector and MethylCollector™ Ultra illustrates specificity.
Both UnMethylCollector and MethylCollector™ Ultra were run according to the protocols using either 200 ng or 100 ng respectively of the provided *Mse* I digested human, male genomic DNA. Real time PCR analysis was run across multiple loci on both the unbound and eluted fractions. UnMethylCollector clearly captures the unmethylated loci (APC and p48), while MethylCollector Ultra enriches for the methylated loci (Xist and NY-ESO).

Novel UnMethylCollector™ Assay Isolates Unmethylated CpG Islands

UnMethylCollector™** is the first commercially available kit for the specific isolation and enrichment of unmethylated CpG islands. The kit utilizes the specificity of the CXXC binding domain towards unmethylated CpGs to capture and enrich for DNA fragments that lack methylation.

How does it work?

UnMethylCollector uses a recombinant CXXC protein to specifically bind unmethylated DNA fragments containing as few as one CpG dinucleotide. The kit provides two buffers: a low-salt buffer for use with samples containing less than 5 CpGs per fragment and a higher salt buffer for efficient binding of fragments with more than 5 CpGs. Nickel-coated magnetic beads capture the protein:DNA complexes, then the unmethylated DNA is eluted from the beads. Following clean up, the eluted DNA is ready for use.

A side-by-side comparison of fractions obtained from both UnMethylCollector and MethylCollector Ultra Kits illustrates the specificity of each technique at binding and enriching for the appropriate methylation status across multiple loci (Figure 1).

Product	Format	Catalog No.
MethylCollector™ Ultra	30 rxns	55005
UnMethylCollector™	30 rxns	55004
Fully Methylated Jurkat DNA	10 µg	55003

* MethylCollector™ Ultra is covered under U.S. Patent No. 7,425,415.

** Patent Pending

REFERENCE
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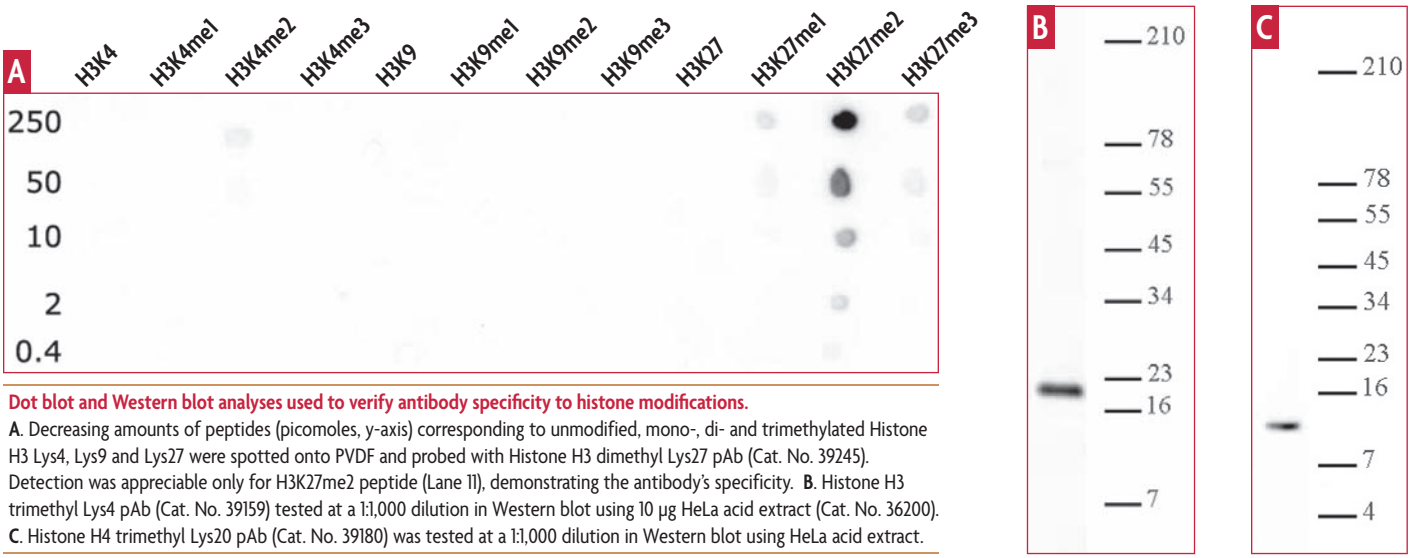
New: Histone Antibodies with Supplies that You Can Rely On

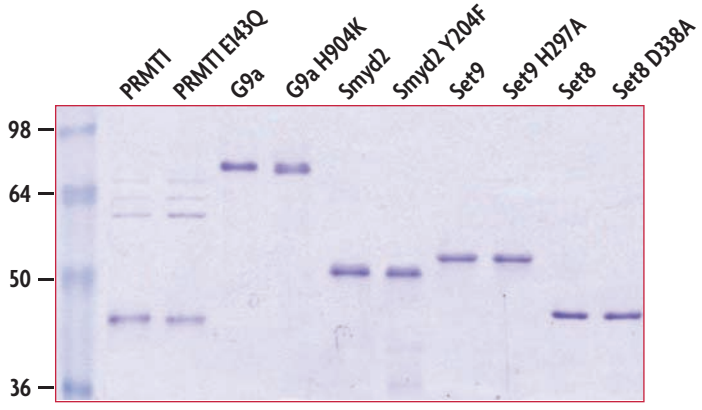
We know that having the highest quality antibodies available when you want them is critical to accomplishing your research goals. That's why Active Motif is proud to be able to produce and test all its own antibodies, unlike many other companies that "source" their antibodies. This means that we can guarantee our customers not only the quality they desire, but also the availability. So, if you're having problems getting hold of important antibodies from your usual antibody "supplier", take the time to visit www.activemotif.com/abs today. New antibodies are released every day, and because we make them ourselves, we'll have them available tomorrow, or whenever you need to re-order.



Dot blot and Western blot analyses used to verify antibody specificity to histone modifications.
A. Decreasing amounts of peptides (picomoles, y-axis) corresponding to unmodified, mono-, di- and trimethylated Histone H3 Lys4, Lys9 and Lys27 were spotted onto PVDF and probed with Histone H3 dimethyl Lys27 pAb (Cat. No. 39245). Detection was appreciable only for H3K27me2 peptide (Lane 11), demonstrating the antibody's specificity. **B**. Histone H3 trimethyl Lys4 pAb (Cat. No. 39159) tested at a 1:1,000 dilution in Western blot using 10 µg HeLa acid extract (Cat. No. 36200). **C**. Histone H4 trimethyl Lys20 pAb (Cat. No. 39180) was tested at a 1:1,000 dilution in Western blot using HeLa acid extract.

New: Chromatin-associated Enzymes from Active Motif

Changes in chromatin structure that result from the post-translational modification of amino acids within the tail of histones H2A, H2B, H3 and H4 are known to play an important role in regulation of gene expression. As a result, there is a growing scientific interest in gaining a better understanding of how proteins involved in chromatin modification function, and the role they may play in disease processes. One of the simplest methods for studying protein function and regulation is in a biochemical context. This is why Active Motif offers an ever-increasing line of functionally active proteins involved in many chromatin-associated processes. Active Motif has proteins available for the analysis of histone methylation, ubiquitination and acetylation as well as DNA repair and transcriptional regulation. To find out more, please visit our website at www.activemotif.com/proteins.



Purified wild-type and mutated histone methyltransferase proteins.
A variety of wild-type and mutated histone methyltransferase proteins were expressed in *E. coli*, purified, run on a gel then Coomassie Blue stained. The wild-type proteins are active and are for use in methyltransferase assays, while the mutated proteins have no activity, so are ideal negative controls.

Simple, Low Background Co-IP of Both Nuclear & Whole-cell Complexes

The Universal Magnetic Co-IP Kit includes protein G-coated magnetic beads that simplify the IP and wash steps while providing lower background, which improves your Co-IP results. 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).

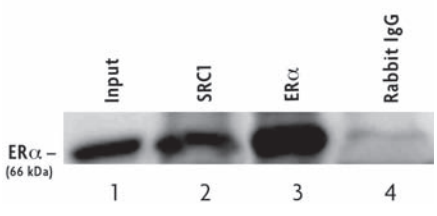


Figure 1: Nuclear Co-IP of SRC-1 and ERα.
The Universal Magnetic Co-IP Kit was used to make nuclear extract from MCF-7 cells induced 1 hour with 10 nM Estradiol. IP was performed on 300 µg samples using 2 µg of SRC-1 pAb, ERα pAb and rabbit IgG (as a negative control). Western blot was then performed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

Co-IP is often used to study cytoplasmic protein complexes. But, traditional methods are not optimal for studying DNA-binding proteins because nuclear complexes are very fragile, causing them to be disrupted during extraction. For this reason, in addition to containing components for preparing whole-cell extracts, 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 nuclear protein complexes from the DNA, so they are intact and ready for Co-IP.

Simpler procedure, lower background

The Universal Magnetic Co-IP Kit utilizes protein G-coated magnetic beads, which simplify Co-IP by enabling the IP and wash steps to be performed in seconds, rather than having to use centrifugation. Because these beads have very low non-specific binding, background is reduced even while using the kit's low-salt Co-IP/Wash Buffer, which is designed to help 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

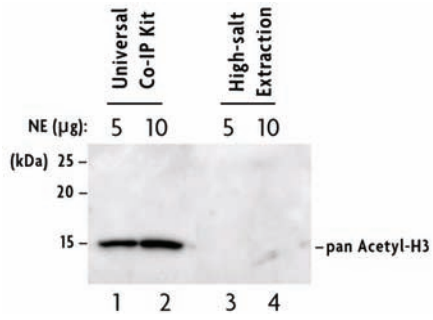


Figure 2: Detection of acetylated Histone H3.
HeLa nuclear extracts were made using the Universal Magnetic Co-IP Kit and a traditional high-salt extraction protocol, each supplemented with 1 µM trichostatin A, a deacetylase inhibitor. Five and ten µg samples of each extract were used in Western blot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

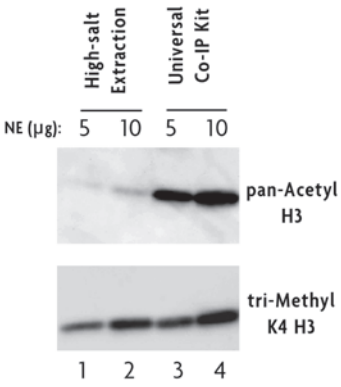


Figure 3: Preservation of acetylation and methylation.
Nuclear extracts were made from HeLa cells treated with 500 nM trichostatin A for 20 hours using either the Universal Magnetic Co-IP Kit (with its deacetylase inhibitor) or traditional high-salt extraction. Five and ten µg samples of these extracts 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 and protein modifications (Figures 2 & 3). 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/coip.

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

In-cell Westerns Make it Easy to Monitor Activation by Phosphorylation

Fast Activated Cell-based ELISA (FACE™) Kits are a simple, sensitive, cell-based method for detecting protein phosphorylation directly in the cell. These convenient in-cell Westerns eliminate the need to make extracts and run gels.

FACE advantages

- **Cell-based** – no extraction, gels or blotting needed
- **Accurate** – fixation prevents additional protein modifications
- **Fast** – 5-hour protocol, with just 2 hours of hands-on time
- **Simple** – grow your cells, stimulate, then assay in the same 96-well plate
- **Complete** – kits include both total and phospho-specific antibodies so you can assay 2 x 96 wells
- **Semi-quantitative results** – normalize to total protein and cell number

The "in-cell" method

Fast Activated Cell-based ELISAs (FACE™) are easy to use and require just 2 hours of hands-on time. Cells are grown in 96-well cell culture plates and treated to induce phosphorylation of the protein of interest. The cells are rapidly fixed with formaldehyde to preserve the phosphorylation state, and then each well is incubated with a primary antibody specific for either the total protein or its phosphorylated form. Subsequent incubation with a labeled secondary provides a colorimetric or chemiluminescent readout that is quantitative and reproducible (Figures 1 & 2). Data is easily normalized to cell number using the provided Crystal Violet Dye.

A variety of kits to choose from

FACE Kits are available for over 20 different targets (Table 1) in both colorimetric and chemiluminescent formats. The Suspension Cell FACE module was designed to work 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. With FACE Maker Kits, you can use your own primary and secondary antibodies to detect any target or modification state of interest.

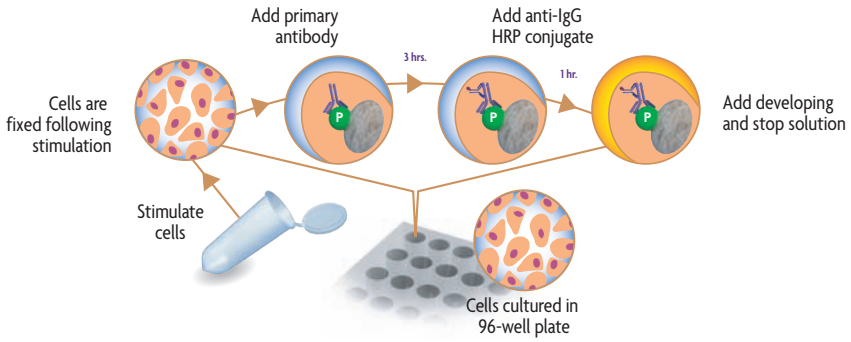


Figure 1: Flow chart of the FACE process.
Cells are grown, stimulated and fixed in the same 96-well plate. Addition of primary and secondary antibodies detects total protein as well as the phosphorylated form of the protein.

Simplify your phospho-assays today!

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

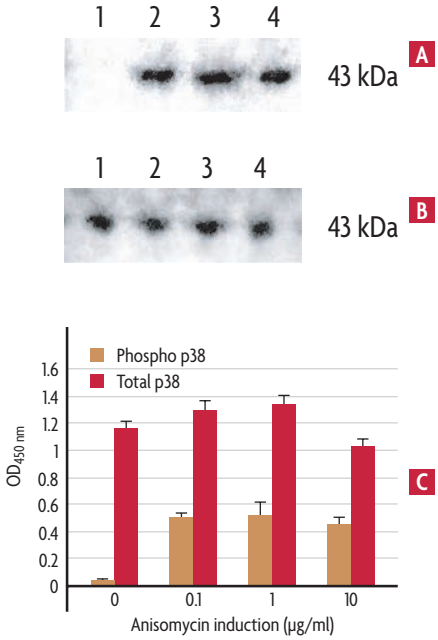


Figure 2: Phospho and total p38 MAPK assays.
Macrophage 4/4 cells were grown in 10 cm dishes to 80% confluency, serum-starved for 16 hours and stimulated with anisomycin for 15 minutes. Cell lysates were made and Western blots performed using phospho- (A) and total-p38 antibodies (B). For FACE, 4/4 cells 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.

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 (FOXO1) (T24)	FACE™ HSP27 (S82)	FACE™ JAK1 (Y1022/ Y1023)	FACE™ JNK (T183/Y185)	FACE™ MEK1/2 (S217/S221)
FACE™ NFκB Profiler (S468 & S536)	FACE™ p38 (T180/Y182)	FACE™ PI3 Kinase p85	FACE™ STAT2 (Y869)	FACE™ STAT4 (Y693)	FACE™ STAT6 (Y641)
FACE™ Maker	Suspension Cell FACE™				