MOTIF VATIONS

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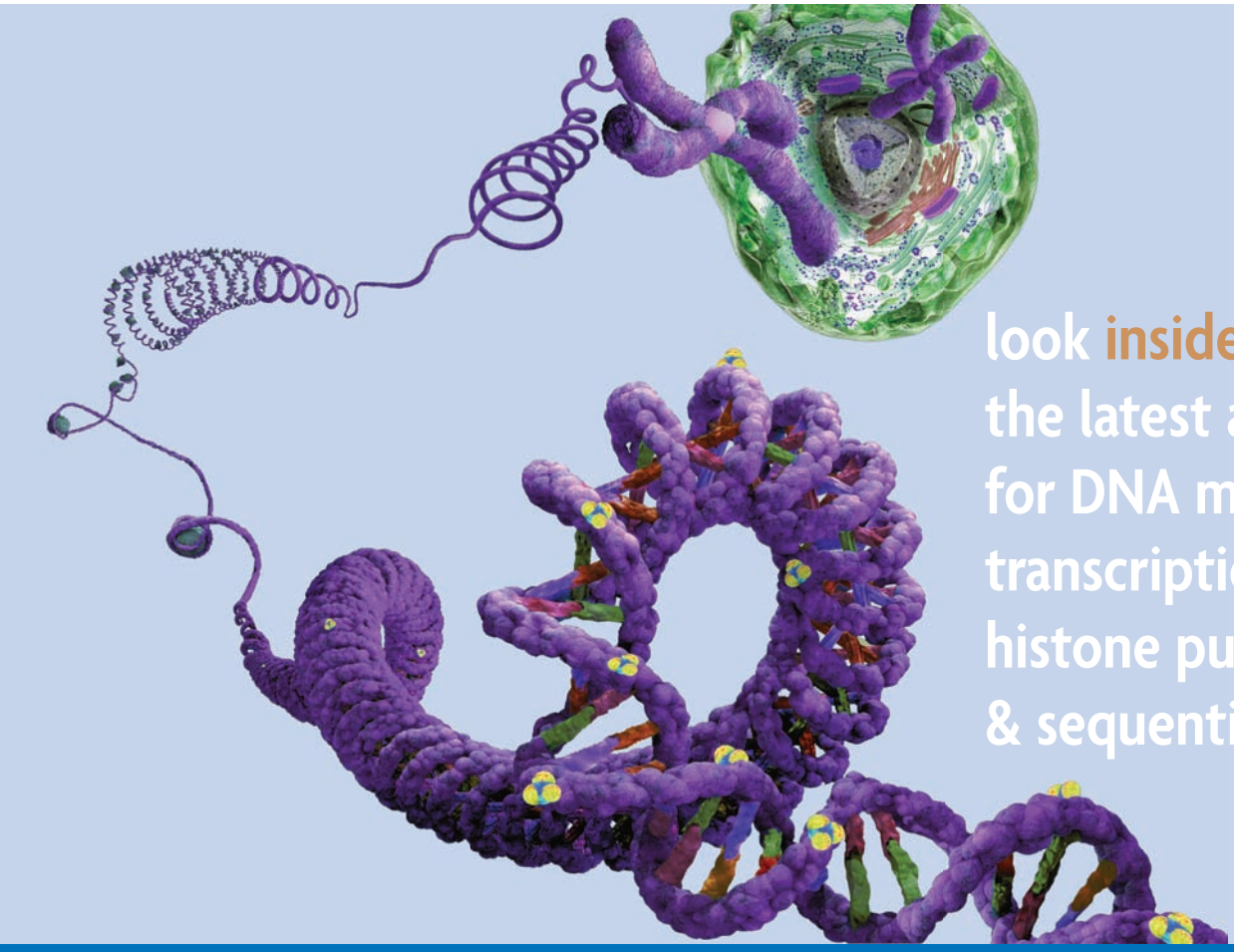
ACTIVE MOTIF®

Tools to Analyze Nuclear Function



look inside for the latest assays for DNA methylation, transcriptional regulation, histone purification & sequential ChIP

IN THIS ISSUE

Aberrant DNA Methylation and Pediatric Syndromes

Methylation of mammalian DNA has long been recognized to play a major role in a variety of different cellular functions. Dysregulation(s) in DNA methylation or in the DNA methylation machinery has been shown to lead to the development of severe pediatric syndromes.

Methylation of mammalian DNA has long been recognized to play a major role in different cellular functions, such as development or control of gene expression, and is often 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. Three families of DNMTs have been identified: DNMT1, DNMT2 and DNMT3. The DNMT3 family, containing two active methyltransferases, DNMT3A and DNMT3B, and one DNMT3-Like protein (DNMT3L), establishes the initial CpG methylation pattern *de novo* and shows the same propensity for methylating unmethylated duplex DNA as it does for hemi-methylated DNA. DNMT1 is considered a maintenance DNMT. Indeed, this enzyme shows a specificity for hemi-methylated DNA and is responsible for the establishment and regulation of tissue-specific patterns of DNA methylation in regulatory sequences (reviewed in 1).

The complex series of events leading to a repressive chromatin state involve the coordinated regulation of DNA methyltransferases, other proteins called Methyl-CpG binding proteins (MBD proteins) and the Kaiso family proteins. The MBD family proteins include MeCP2, MBD1, MBD2, MBD3 and MBD4.[2, 3] In mammalian cells, DNA methylation is generally associated with gene silencing, either directly by inhibiting binding of transcription factors to their recognition sequences[4], or indirectly by preventing transcription factors from accessing their target sites through attachment of MBD proteins that "read" DNA methylation patterns.[1] These MBDs can recruit histone deacetylases and histone methyltransferases, thereby resulting in formation of a closed repressive chromatin structure. DNA methylation is involved in many cellular functions such as embryonic development, genetic imprinting, X chromosome inactivation and control of gene expression. Aberrant methylation patterns are associated with certain human tumors and developmental abnormalities. In this review, we will focus on the role of aberrant DNA methylation patterns in the development of pediatric syndromes.

Aberrant methylation patterns are associated with certain human tumors and developmental abnormalities.

Dysregulations of genomic imprinting

Diploid organisms possess two copies of each autosomal gene, one from each parent. Most autosomal genes are expressed simultaneously from both alleles. However, for a subset of genes, one allele is silenced in a parent-of-origin-dependent manner. This process is called imprinting. DNA methylation and histone modifications have been shown to play a major role in imprinting mechanisms.[5] Typical disorders associated with imprinted genes include Prader-Willi and Angelman syndromes, Beckwith-Wiedemann syndrome and multiple forms of neoplasia.[6-8]

The Prader-Willi syndrome (PWS) and the Angelman syndrome (AS) are two clinically distinct disorders, each caused by several genetic and epigenetic dysregulations within chromosome 15q11-q13, containing a number of imprinted genes.

Prader-Willi syndrome (PWS)

Individuals with Prader-Willi syndrome show neonatal hypotonia, failure to thrive, hyperphagia in early childhood leading to obesity, hypogonadotropism, short stature, behavior problems and moderate mental retardation.[9] One percent of PWS patients show a paternal copy of chromosome 15 that has a maternal pattern of imprinted gene expression and epigenetic modifications of 15q11-q13. In 85% of PWS patients who show imprinting defects, both copies of several genes (such as *SNRPN*, *NDN* or *MKRN3*) within chromosome 15 are methylated.[7]

Angelman syndrome (AS)

Angelman syndrome is a neurogenetic disorder characterized by severe mental retardation, seizures, abnormal gait, frequent smiling and laughter, microcephaly and ataxia.[10] Three to five percent of AS patients show an imprinting defect, with a maternally inherited chromosome 15 that has a paternal imprint and epigenetic modifications on the *UBE3A* gene, considered as the AS gene.[7, 11]

Beckwith-Wiedemann syndrome (BWS)

Beckwith-Wiedemann syndrome is a congenital overgrowth syndrome characterized by a number of developmental abnormalities as macroglossia, macrosomia or neonatal hypoglycemia.[7] Moreover, children with Beckwith-Wiedemann syndrome have an increased risk to develop embryonal tumors (Wilm's tumor, hepatoblastoma, *etc.*).[12] This syndrome is a multigenic disorder, implying

dysregulation of one or more imprinted genes of a major imprinted gene cluster on chromosome 11p15, involved in regulation of growth and the cell cycle. These genes include the paternally expressed genes *IGF2* and *KCNQ10T1* and the maternally expressed genes *H19*, *CDKN1C* and *KCNQ1B*.[13] It seems that there are several genotypes for the Beckwith-Wiedemann syndrome. In the majority of sporadic BWS cases, several groups have reported abnormal, biallelic expression of IGF2. This phenomenon is designated as loss of imprinting. Another subgroup of sporadic BWS cases shows biallelic expression of IGF2 and silencing of H19. These events are accompanied by a paternal methylation pattern on both chromosomes (reviewed in 6).

Dysregulations of tumor suppressor function and retinoblastoma

Some genes such as p53 or Rb1 have a tumor suppressor function. Dysregulation of these genes causes development of many cancers. Retinoblastoma is a childhood malignant tumor of the retina that can be caused by genetic and epigenetic alterations. Genetic alterations include mutation of one allele of the tumor suppressor gene Rb1, combined with chromosomal deletion or loss of the other allele.[14] Epigenetic alterations include the hypermethylation of the Rb1 gene leading to the inactivation of its tumor suppressor function[15] and to the development of retinoblastoma.

Dysregulations of DNA methylation machinery: DNMT and MBD proteins

Rett syndrome

Rett syndrome is an X-linked neurodevelopmental disorder, affecting female children of 17-18 months. Rett syndrome is characterized by an apparently normal development of the child prior to the onset of the symptoms. This period is followed by a skill regression in early childhood (speech, hand use) and patients develop severe dementia, autism and many other hallmark characteristics.[16, 17] Rett syndrome causative mutations occur mainly (in 85% of the cases) in the gene coding for the CpG-methyl binding protein, MeCP2.[18] Rett patients with no *MeCP2* mutation may show mutation in MeCP2-related genes, such as *CDKL5*, whose product interacts with MeCP2. MeCP2 recruits Histone deacetylases (HDACs) 1 and 2.[19] Mutations in the *MeCP2* gene result in hyperacetylation of histone H3 in mice and of histone H4 in cell cultures derived from Rett patients.[20, 21] Several studies have provided insights into the relationship between the genotype (type of mutation(s) and position of the mutation(s) in *MeCP2*) and the severity of Rett syndrome phenotypes.[22]

Typical disorders associated with imprinted genes include Prader-Willi, Angelman and Beckwith-Wiedemann syndromes and multiple neoplasias.

The immunodeficiency, centromeric region instability and facial anomalies syndrome (ICF)

ICF is an immunodeficiency, autosomal and recessive disease. This syndrome is characterized by an instability of the pericentrometric regions of chromosomes 1, 9 and 16, and by a severe immunodeficiency. The patients present with an immunoglobulin deficiency in the presence of normal B- and T-cell counts. Other variable clinical signs are chronic respiratory and gastrointestinal infections, and facial anomalies (macroglossia, flat nasal bridge) (reviewed in 23). Mutations in the gene coding for the enzyme *DNMT3B* were found to be responsible for clinical presentations of ICF. Sixty percent of the patients have mutations in both alleles of *DNMT3B* within the coding sequence.[24] ICF patients who don't show mutations in *DNMT3B* display an additional hypomethylation in centromeric satellite alpha DNA.[25, 26] Some studies established that the loss of methyltransferase activity, and not other functions of DNMT3B protein, is responsible for the disease. Indeed, mutations in ICF patients are frequently found in the C-terminal portion of the protein, which contains the catalytic domain.[24] All patients, including those without mutations in DNMT3B, exhibit hypomethylation of the DNA, targeted to specific sequences, such as satellite DNA of the juxtacentromeric heterochromatin at chromosome 1, 9 or 16. These are sites of whole-arm deletions, decondensation, multiradial chromosome junctions and chromosome breaks, leading to aberrant rearrangements in mitogen-stimulated lymphocytes.[27]

All these studies show the importance of epigenetics in general, and of DNA methylation in the development of an organism and in various cell functions.

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NEW: MethylCollector™ Ultra for Enhanced Methylation Enrichment

The new MethylCollector™ Ultra Kit* is an enhanced version of our MethylCollector™ Kit*. It 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 alternative methyl-binding protein or antibody immunoprecipitation (MeDIP) methods, and in less than half the time. This makes MethylCollector Ultra suitable for use with smaller samples, as the new format requires only 1 ng of fragmented DNA. The kit also includes positive control human, male genomic DNA and methylation-specific PCR primers to ensure your success.

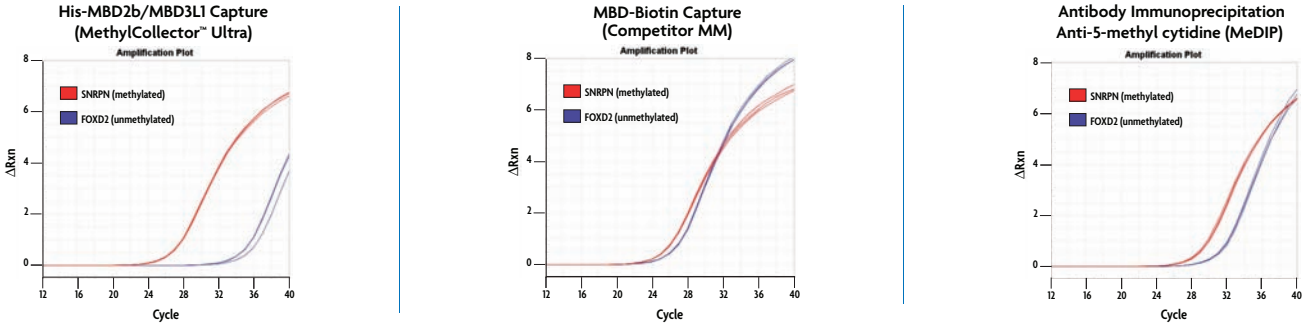


Figure 1: Real time PCR analysis reveals the specificity of MethylCollector Ultra versus competing technologies. 100 ng of human, male genomic DNA was digested with *Mse* I and tested in MethylCollector Ultra, competitor MM's kit and using an anti-5-methyl cytidine antibody immunoprecipitation method. Eluted DNA was analyzed using PCR primers for both methylated, SNRPN (red), and unmethylated, FOXD2 (blue) promoters. Only MethylCollector Ultra showed specific enrichment for CpG-methylated DNA as demonstrated by from the clear separation in amplification cycles. A comparison of 500 ng and 1 µg of starting material for each method revealed similar amplification profiles (data not shown).

MethylCollector Ultra advantages

- **Improved efficiency** – high-affinity binding provides greater enrichment than other MBD capture or 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 a variety of downstream applications such as endpoint or real time PCR, bisulfite conversion, or amplification and labeling for microarray analysis

MethylCollector™ Ultra method	MeDIP method
Input material: 1 ng - 1 µg	Input material: 1 µg
–	Denature DNA for 3 minutes at 95°C
–	Pre-block beads
Combine DNA with MBD2b/MBD3L1 protein mix and nickel-coated magnetic beads for 1 hour at 4°C	Combine DNA, antibody and magnetic beads, incubate 5 hours to overnight
Wash beads 4 times using the included magnet	Wash beads 4 times
Elute methylated DNA at 42°C for 30 minutes	Elute methylated DNA at 55°C for 15 minutes
Add Proteinase K Stop Solution	Incubate at 100°C for 15 minutes
DNA clean up	DNA clean up
Total time required: < 3 hours	**Total time required:** > 7 hours

Table 1: Comparison of MethylCollector Ultra process with MeDIP.

Product	Format	Catalog No.
MethylCollector™ Ultra	30 rxns	55005
MethylCollector™	25 rxns	55002
Fully Methylated Jurkat DNA	10 µg	55003

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

REFERENCE
1. Rauch, T. and Pfeifer, G. (2005) *Lab. Investigation* 85: 1172-1180.

NEW: UnMethylCollector™ Positively Identifies Unmethylated CpG Regions

Active Motif is pleased to announce the launch of UnMethylCollector™*, the first commercially available kit for the specific isolation and enrichment of unmethylated CpG dinucleotides. UnMethylCollector utilizes the specificity of the CXXC binding domain towards unmethylated CpGs to capture and enrich for DNA fragments that lack methylation. This enables researchers to identify hypomethylated promoters and to study the effects of compounds that inhibit methylation. Instead of relying on negative data from methyl-specific binding techniques to identify unmethylated promoters, UnMethylCollector offers a specific, reliable technique that provides positive identification of unmethylated CpG regions.

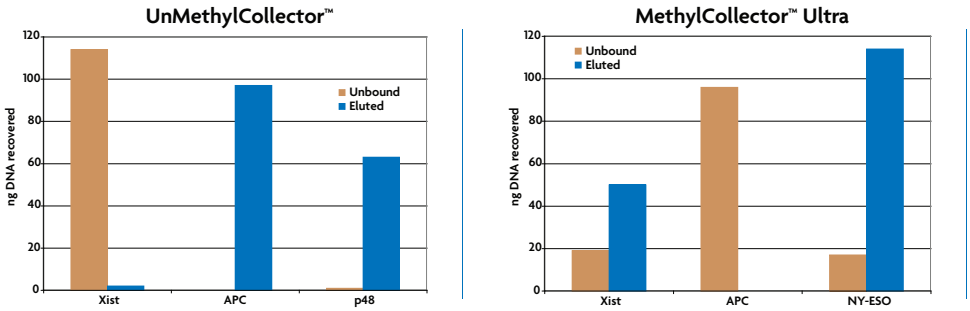


Figure 1: Direct comparison of UnMethylCollector and MethylCollector™ Ultra illustrates specificity. Both UnMethylCollector and MethylCollector™ Ultra were run according to the protocols using either 200 ng or 100 ng respectively of the provided *Mse* I digested human, male genomic DNA. Real time PCR 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).

APC – contains 29 CpGs and is normally unmethylated
p48 – contains 22 CpGs and is normally unmethylated
Xist – contains 8 CpGs and is methylated in males
NY-ESO – contains 6 CpGs and is normally methylated

UnMethylCollector advantages

- **Sensitivity** – detects unmethylated CpGs from 10 ng - 1 µg of DNA fragmented by sonication or enzymatic digestion
- **Faster procedure** – magnetic protocol can be completed in less than 3 hours
- **Specificity** – optimized reagents ensure successful capture from as few as one unmethylated CpG
- **Controls ensure success** – includes positive control DNA and PCR primers suitable for use in either endpoint or real time PCR analysis
- **Versatility** – eluted DNA is suitable for use in various downstream applications such as PCR, sequencing, or amplification and labeling for microarray analysis

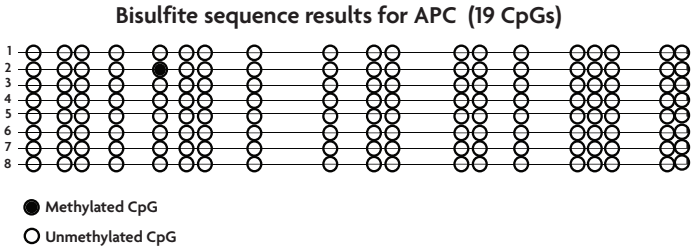


Figure 2: MethylDetector™ confirms the specificity of UnMethylCollector for unmethylated CpG dinucleotides. UnMethylCollector was used to enrich for unmethylated DNA fragments. This DNA was bisulfite treated using Active Motif's MethylDetector™ Kit. Bisulfite conversion changes unmethylated cytosines into uracils, while methylated cytosines remain unchanged. The converted DNA was then amplified by PCR and the gel extracted PCR product was cloned, with eight colonies being selected for sequencing of the unmethylated APC promoter region. The presence of a CpG site in the sequencing data indicates that the original CpG was methylated, thereby not converted. A C:T mismatch indicates that the original CpG was unmethylated, and therefore converted by the bisulfite treatment. Only one clone contained a single methylated CpG of the 19 CpG dinucleotides within the sequenced APC promoter region. This validates that UnMethylCollector specifically binds and enriches for unmethylated DNA fragments.

Product	Format	Catalog No.
UnMethylCollector™	30 rxns	55004
MethylDetector™	50 rxns	55001

* Patent pending.

Identify Protein Co-localization *In Vivo* Using Sequential Chromatin IP

Performing sequential chromatin IP (also called Re-ChIP) was technically challenging and difficult, until now. Active Motif's Re-ChIP-IT™ Kit makes it easy to perform sequential ChIP, so you can localize two different proteins or histone modifications to the same genomic locus.

Extend the utility of ChIP

When performing ChIP experiments, it is often useful to prove that two different proteins or histone modifications are present at the same site in the genome. Or, you may want to determine if a protein coincides with a specific histone modification at the same regulatory element. Re-ChIP is a relatively new technique in which sequential chromatin immunoprecipitations are performed using two different antibodies, enabling you to assay for the simultaneous presence of two proteins or distinct histone modifications at the same genomic region of interest (Figure 1).

How does Re-ChIP-IT work?

The Re-ChIP-IT Kit takes advantage of the same advances introduced in Active Motif's popular ChIP-IT Express Kit. Each method uses protein G-coated magnetic beads that have less background than standard agarose beads, so pre-clearing and blocking steps are not needed. Magnetic pull-down occurs in just seconds, and the low background has eliminated the need for DNA purification. With Re-ChIP-IT, the 1st ChIP is performed as in regular ChIP. Chromatin that has been immunoprecipitated is removed from the magnetic beads with a special buffer that prevents the first antibody from participating in the 2nd IP reaction. After desalting, a 2nd ChIP is performed using an antibody different from that used in the first ChIP. The cross-links of these sequentially immunoprecipitated protein-DNA complexes are then reversed and the DNA is analyzed by PCR, similar to conventional ChIP samples (Figure 2).

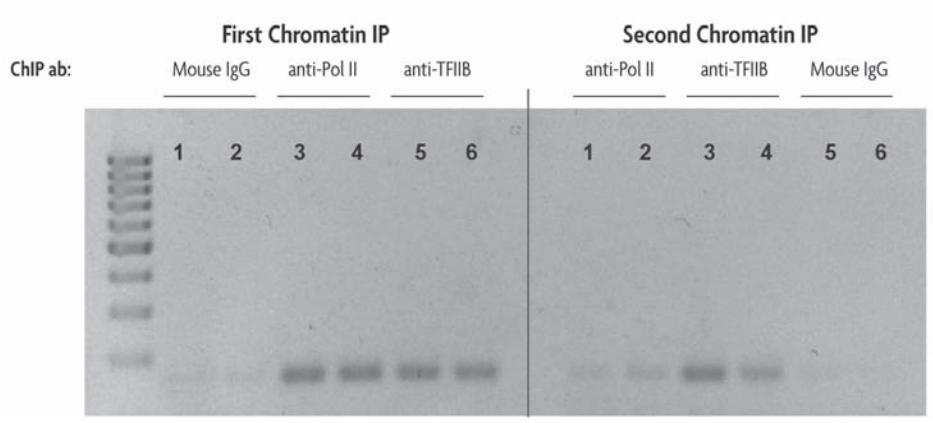


Figure 1: Sequential chromatin immunoprecipitation using Re-ChIP-IT.
The lane numbers are the same in each panel to indicate that the DNA is from the same chromatin sample. The left panel shows the results of PCR performed on an aliquot of DNA removed from the experiment after the first ChIP step; the right panel represents PCR results on DNA from chromatin samples after both ChIP steps. For example, chromatin samples subjected to first ChIP using Mouse IgG as a negative control (lanes 1 and 2 in the left panel) were then subjected to a second ChIP with an RNA Pol II antibody (lanes 1 and 2 in the right panel). Chromatin samples in which Mouse IgG was used as either the first antibody (lanes 1 and 2) or second antibody (lanes 5 and 6) show little amplification of GAPDH DNA in either the left (first ChIP) or right panel (first and second ChIP). Chromatin samples in which the first antibody used was anti-RNA Pol II and the second antibody was anti-TFIIB (lanes 3 and 4) show good amplification of GAPDH DNA after the second ChIP (right panel) indicating co-localization of RNA Pol II and TFIIB at the same region of the GAPDH promoter.

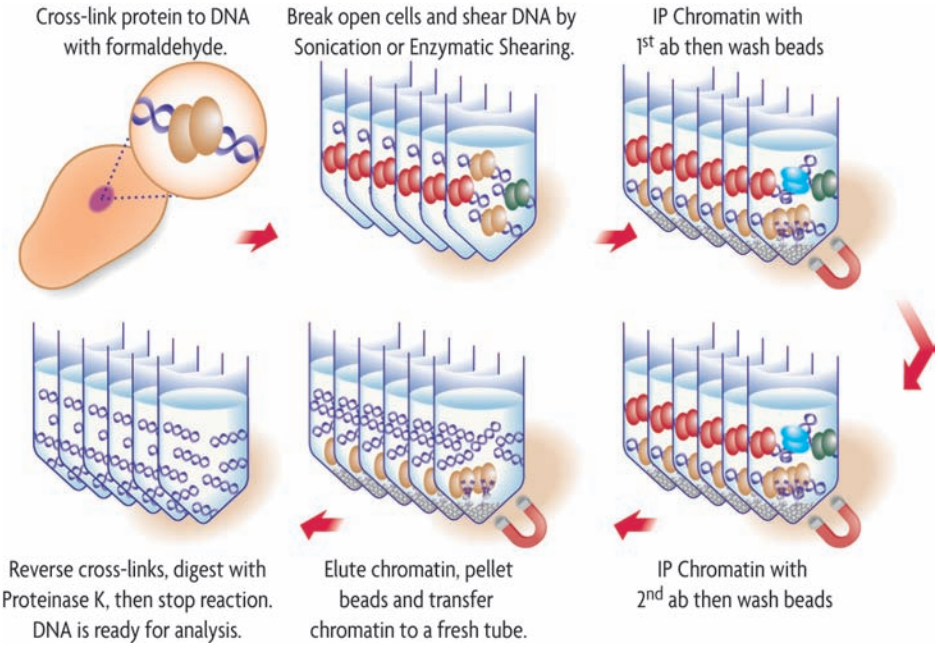


Figure 2: Schematic representation of the Re-ChIP-IT procedure.

Product	Format	Catalog No.
Re-ChIP-IT™	25 rxns	53016

Histone Purification Made Simple in Your Choice of Assay Format

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.

Histone proteins are important regulators of genome function, comprising the scaffold around which genomic DNA is organized. Working with histones previously required long and tedious purification methods. Active Motif has simplified the process with two complete histone purification systems in your choice of kit format: the original Histone Purification Kit, which can be used in either gravity flow or spin column format, and the Histone Purification Mini Kit, with a convenient mini spin column for histone purification (Table 1).

Both kits utilize proprietary purification columns and buffers to isolate very pure fractions of histones. Using the Histone Purification Mini Kit, core histones can be purified from cultured cells and tissue samples as a single population containing H2A, H2B, H3 and H4 (Figure 1) from as few as $8x10^5$ cells. While you can not purify the same amount of histones as with the original Histone Purification Kit, mini spin columns make it easier to isolate histones from multiple samples simultaneously. The original Histone Purification Kit, which has a higher capacity than the Mini Kit, can purify all four core histones using the spin-column format, or purify the histones as separate fractions of H2A/H2B dimers and H3/H4 tetramers using the same column in a gravity flow format (Figure 2). Both kits result in the preparation of extremely pure fractions of histones while keeping important post-translational modifications intact (*e.g.* acetylation, phosphorylation and methylation).

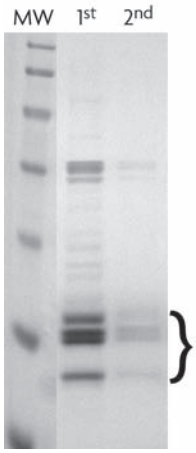


Figure 1: Histone Purification Mini Kit.
HeLa cell core histones (indicated by the bracket) were purified with the Histone Purification Mini Kit and run on an SDS-PAGE gel, which shows the first (1st) and second (2nd) elution fractions.

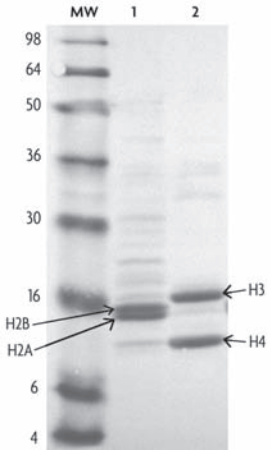


Figure 2: Histone Purification Kit.
The Histone Purification Kit was used in its gravity flow format to purify separate H2A/H2B (Lane 1) and H3/H4 (Lane 2) fractions from HeLa cells.

Better substrates make for better assays

Core histones isolated using the original Histone Purification Kit are highly pure and suitable substrates for downstream assays. Purified histones can be used with the Chromatin Assembly Kit to generate chromatin that very closely resembles native chromatin, ideal for use in functional assays (Figure 3).

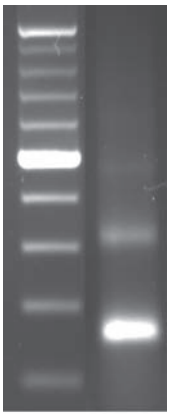


Figure 3: Chromatin assembled with purified histones.
Histones purified from HeLa cells were used with the Chromatin Assembly Kit. The ordered spacing of nucleosomes was confirmed by micrococcal nuclease digestion and agarose gel electrophoresis.

Kit	Format	Elution	Capacity
Original Histone Purification Kit	Gravity Flow	Separate H2A/H2B & H3/H4 fractions	0.5-2.5 mg
	Spin Column	H2A, H2B, H3 & H4 in a single fraction	0.5-2.5 mg
Histone Purification Mini Kit	Mini Spin Column	H2A, H2B, H3 & H4 in a single fraction	0.1-0.5 mg

Table 1: Comparison of the original Histone Purification Kit and the Histone Purification Mini Kit

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

TransAM™ Transcription Factor DNA-binding ELISAs

TransAM™ Kits offer a great alternative to traditional methods to study transcription factor activity. TransAM Kits are highly sensitive ELISA-based assays capable of detecting small changes in transcription factor activity without the need for gels or radioactivity. The standard TransAM Kits are 10-fold more sensitive than conventional gelshift assays, while TransAM Chemi Kits utilize chemiluminescent detection to improve sensitivity down to only 40 ng of extract.

TransAM advantages

- **Faster procedure** – ELISA-based assay can be completed in 5 hours, yet provide more quantitative results (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, HIF and our newest kits for Nrf2 and T-bet. For a complete product listing and additional details, please visit us at www.activemotif.com/transam.

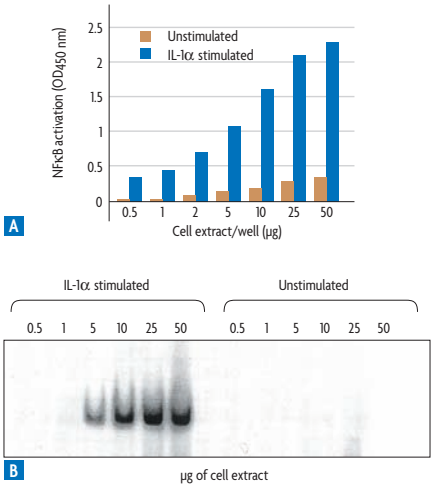


Figure 1: TransAM Kits are more sensitive than gelshift. Human fibroblast WI-38 cells were stimulated with IL-1α for 30 minutes. Increasing amounts of whole-cell extract are assayed using the TransAM NFκB p50 Kit (A) or gel retardation (B). The TransAM method is 10-fold more sensitive and provides quantitative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

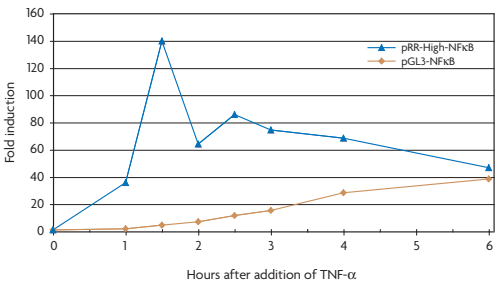
Improve Luciferase Assay Response with RapidReporter®

Active Motif's patented RapidReporter®* system includes a variety of vectors and assay reagents to study signal transduction pathways with greater sensitivity than standard reporter assays. The RapidReporter vectors contain a double destabilizing element that causes the luciferase mRNA and protein to degrade more quickly, enabling better detection of transient or minor effects on transcriptional activity.

RapidReporter vectors

RapidReporter® vectors are based on the extremely bright *Gaussia* luciferase gene. The vectors are offered in two different stringencies, pRR-High and pRR-Low, to allow even weak transcriptional effects to be detected.

In addition to the "empty" vectors that contain a multiple cloning site for insertion of promoters/enhancers of interest, Active Motif also sells several pre-made constructs for widely studied promoters such as NFκB, CREB (CRE) or STAT.



Optimized buffers for brighter signal

Active Motif's *Gaussia* Luciferase Assay Kits contain proprietary Lysis and Assay Buffers and a *Gaussia* Substrate, which are optimized for use with the RapidReporter vectors. For your convenience the RapidReporter *Gaussia* Luciferase Assay Kits are available in both 100 and 1000 reaction formats.

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

* RapidReporter is covered under U.S. Patent No. 7,157,272 and various other patents worldwide. It is sold under license granted by, and is a registered trademark of, GeneStream Pty Ltd.

Recombinant and Purified Proteins for Your Biochemical Assay Needs

Recombinant proteins are of particular interest in many aspects of research, from usage as substrates in biochemical assays to the reconstitution of active protein complexes. Active Motif offers a complete line of recombinant proteins to meet your research needs. These proteins are ideal for use in many biological applications with NFκB p50, NFκB p65, p53, c-Fos, c-Jun, c-Myc, CREB and Sp1 proteins validated for use as standards in TransAM™ DNA-binding ELISAs. We also offer a variety of active serine/threonine and tyrosine kinases, as well as Myelin Basic Protein (MBP), which is useful as a substrate for *in vitro* kinase assays, including those for MAPK, PKA, PKC cyclin-dependent and calmodulin-dependent protein kinases (Figure 1).

Active Motif Protein Categories

- Kinase and kinase substrates
- Transcription factors
- Histone modifying enzymes
- Nuclear receptors

A complete list of purified and recombinant proteins, as well as complete details on each protein, can be found at www.activemotif.com/proteins.

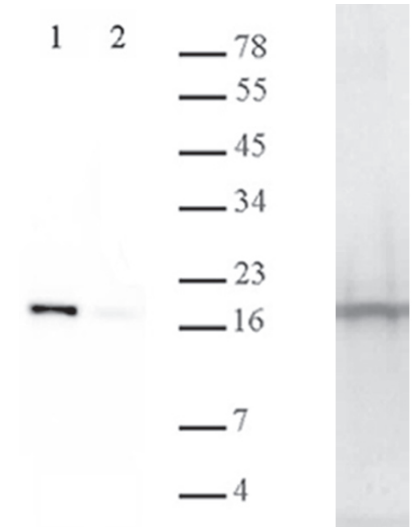


Figure 1: Use of MBP as a kinase assay substrate. Myelin Basic Protein was tested in an *in vitro* kinase assay by incubation with (Lane 1) or without MAPK2 (Lane 2), followed by Western blot detection using an MBP phospho Thr98 antibody. To the right of the Western blot is a lane from a Coomassie-stained SDS-PAGE gel containing 4 µg of purified MBP.

ChIP-validated Antibodies for Your Chromatin Experiments

Chromatin immunoprecipitation (chromatin IP or ChIP) is an extremely important technique, allowing the *in vivo* detection of sequences associated with chromatin proteins, and identification of regions of the genome associated with specific histone modifications. ChIP is also a very challenging technique, so only antibodies of the highest quality will do. For an antibody to work in ChIP, it must be of high titer and it must be very specific, with no detection of non-target proteins. Most importantly, it must be able to recognize the target protein in its native, chromatin-associated context after it has been fixed with formaldehyde. Active Motif specializes in the manufacture and of antibodies for use in ChIP.

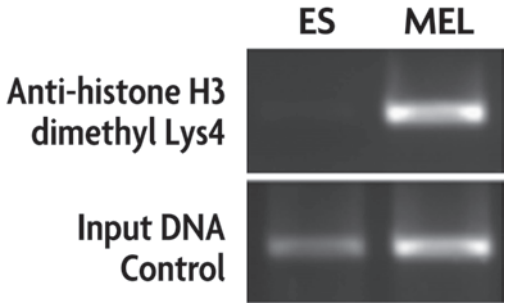


Figure 1: ChIP of methylated histone H3. ChIP was performed with Histone H3 dimethyl Lys4 pAb (Catalog No. 39141) using chromatin prepared from mouse embryonic stem cells (left lane) or mouse erythroleukemia cells (right lane). PCR primers specific for the beta-globin gene were used to amplify a 210 base pair region of the promoter following isolation of DNA.

To see our complete line of antibodies validated for use in chromatin IP, please visit www.activemotif.com/chipabs.

Faster, More Accurate Measurement of Nitric Oxide

The Nitric Oxide Quantitation Kit is faster and easier-to-use than existing methods for measuring the production of NO in biological samples. The kit employs an innovative cofactor technology that reduces the time and number of steps required, while increasing the dynamic range of NO measurement. The NO Quantitation Kit can be used with a wide variety of samples, including cell or tissue extract, urine, plasma, serum and saliva.

Active Motif's Nitric Oxide Quantitation Kit offers advantages in time, ease-of-use and accuracy. It employs an innovative cofactor technology that accelerates the conversion of nitrate to nitrite while simultaneously degrading NADPH. Thus, the time required for the reductase reaction is reduced to only 30 minutes, and sensitive colorimetric quantification can be performed without the need for LDH treatment. Moreover, the Active Motif method is linear over a broader dynamic range, which increases the range of sample concentrations that can be measured accurately (Figure 1). This saves time and money as fewer samples will need to be diluted and re-assayed to obtain accurate results. To make it easier to process large numbers of samples, the NO Quantitation Kit is provided in a convenient 96-well format that facilitates high-throughput automation. For a better method to measure NO, try the Nitric Oxide Quantitation Kit today.

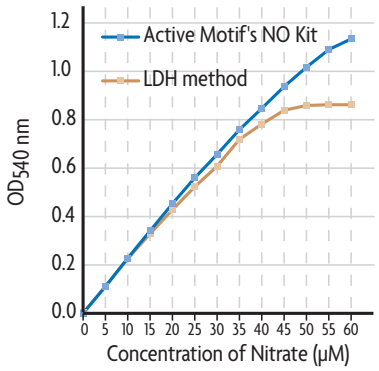


Figure 1: Dynamic range of nitrate standard curves. Nitrate standard curves produced using the Nitric Oxide Quantitation Kit and a conventional Three-Step LDH-method kit. The NO Kit provides linear, accurate measurement over a broader range of concentrations.

Product	Format	Catalog No.
Nitric Oxide Quantitation Kit	2 x 96-rxns	40020

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

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

Use only what you need

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

Don't pay for unused capacity

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

TAM1 genotype and contents

*mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lacZ*ΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G

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

Product	Format	Catalog No.
RapidTrans™ TAM1 Competent *E. coli*	1 x 96 rxns	11096
	5 x 96 rxns	115096

ELISA for 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 is more meaningful.

The Ras GTPase Chemi ELISA method

Because activated Ras binds specifically to the Ras-binding domain (RBD) of the Raf effector protein, Raf-RBD is used as a probe to isolate activated Ras. The Ras ELISA 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. A primary antibody specific for Ras is then added, followed by an HRP-conjugated secondary antibody and developing reagent. The plate is then read on a luminometer, which provides a sensitive, quantitative chemiluminescent 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
- **Save time** – results in < 5 hours

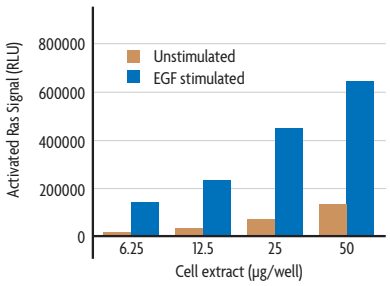


Figure 1: Quantification of activated Ras GTPase. Increasing amounts of whole-cell extract from HeLa cells either unstimulated or stimulated 2 minutes with 5 ng/ml EGF are assayed using the Ras GTPase ELISA Kit.

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

Investigate the Effects of SUMOylation with SUMOlink™

SUMOlink™ Kits are a simple, effective method for generating SUMOylated proteins *in vitro*. These fast, efficient and robust assays contain all necessary reagents for SUMOylation of target proteins, and include positive and negative controls that help to 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.

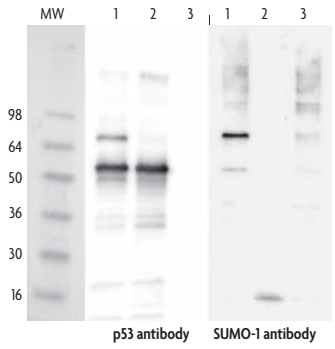


Figure 1: Enzymatic digestion of assembled chromatin. Western blot analysis of *in vitro* SUMOylation of p53 protein by wild-type and mutated isoforms of SUMO-1: The two Western blots were incubated with p53 antibody (1:5000 dilution) and SUMO-1 antibody (1 :4000 dilution). p53 is SUMOylated only by wild-type SUMO-1 protein. 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

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