MOTIF VATIONS

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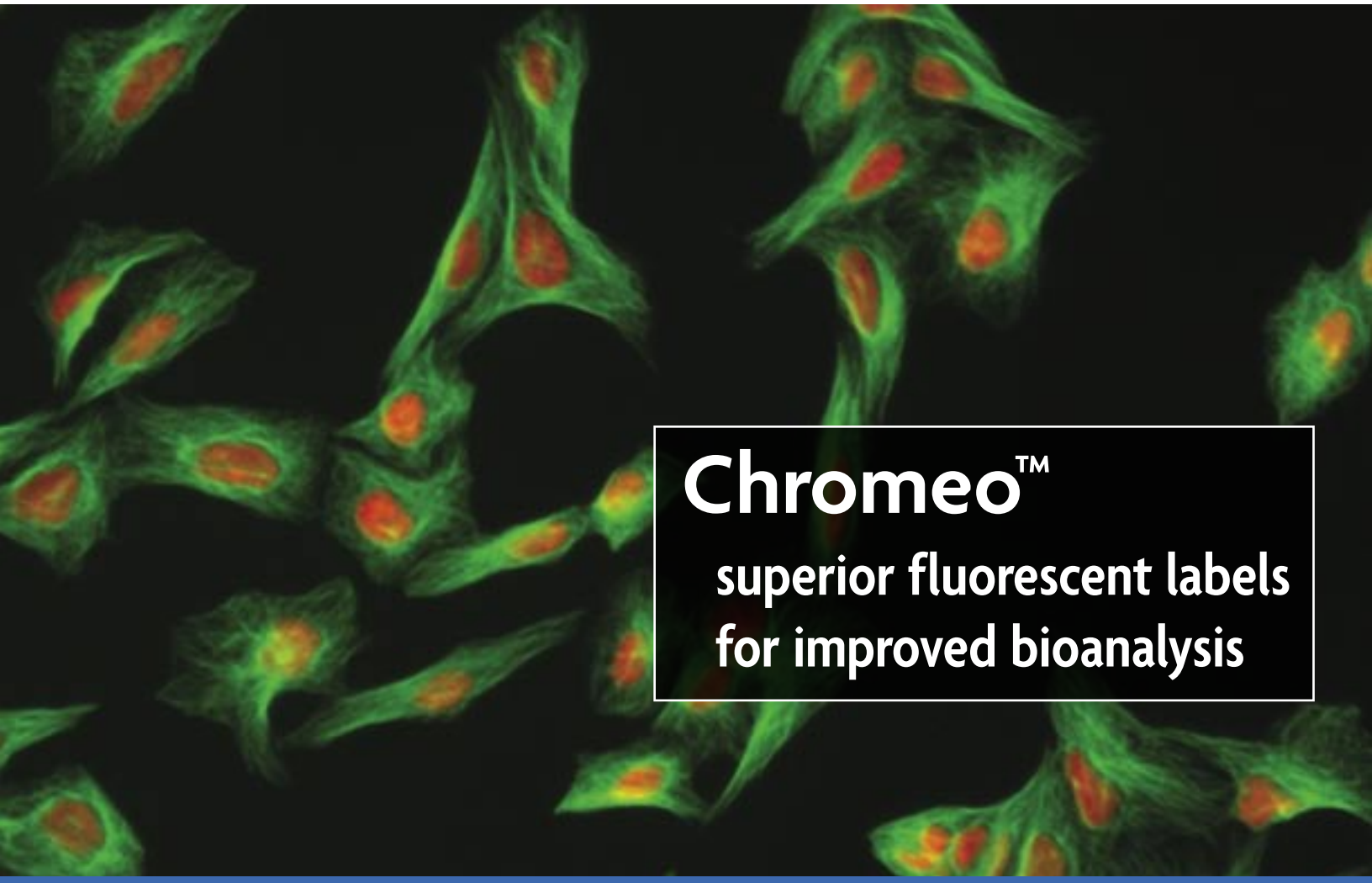
ACTIVE MOTIF®

Tools to Analyze Nuclear Function



Chromeo™

superior fluorescent labels for improved bioanalysis

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NEW: Mitotic Assay Kits – Quickly Test Treatment Effects on Cell Division

Active Motif's Mitotic Assay Kits make it simple for you to determine the percentage of cells undergoing mitosis within a specific population. The kits are available in fluorescent, chemiluminescent and colorimetric formats, enabling you to choose whichever assay best suits your research needs.

Cell division is a complex, tightly regulated process that is marked by mitosis. The Mitotic Assay Kits offer you a simple and accurate method to determine the percentage of cells undergoing mitosis within a specified population. Because mitosis is the most defining stage of cell division, accurate measurement of cells undergoing mitosis is a valuable method to characterize the effects of test compounds on cellular progression.

Specific mitosis marker

Two significant mitotic events include microtubule spindle formation and chromosome condensation. Histone H3 is phosphorylated on serine 28 during mitotic chromatin condensation before nuclear division occurs, which makes phosphorylation of histone H3 (Ser 28) a reliable marker for cells undergoing mitosis. The Mitotic Assay Kits use a highly specific phosphorylated histone H3 (Ser 28) monoclonal antibody as the mitosis marker.

What's in the kits?

Along with the mitosis marker antibody, all kits also include paclitaxel to treat cells for a high-mitotic index reference population. All kits include a method for normalizing cell number, and all the necessary buffers for a simple assay.

Fluorescent kit is ideal for HTS

The fluorescent Mitotic Index Assay Kit uses a bright fluorescent Chromeo™ 488-labeled secondary antibody to detect the mitosis marker primary antibody. Cell number is measured using the propidium iodide included in the kit. This kit is ideal for use with high-content screening fluorescent scanning technologies, like the Blueshift IsoCyte™ (Figure 1).

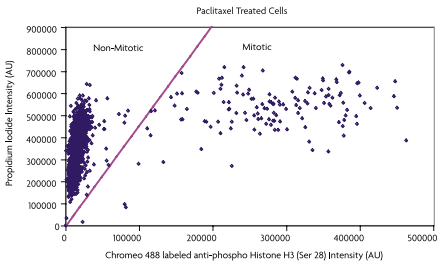

Figure 1: Mitotic Index results on an IsoCyte™ scanner. Single cell data analysis of HeLa cells treated with 1 µM paclitaxel for 6 hours.

High specificity and low background

The superior fluorescence detection with the Chromeo 488 Goat anti-Rat IgG secondary is highly specific with extremely low background, so your results are always accurate (Figure 3).

Chemiluminescent and colorimetric

The chemiluminescent and colorimetric kits work similarly to the fluorescent kit format, but use an HRP-conjugated secondary antibody for detection. Development is then performed with either the colorimetric or chemiluminescent developing reagents. Paclitaxel is included to create a high-mitotic reference population, and cell count can be normalized by crystal violet staining (Figure 2).

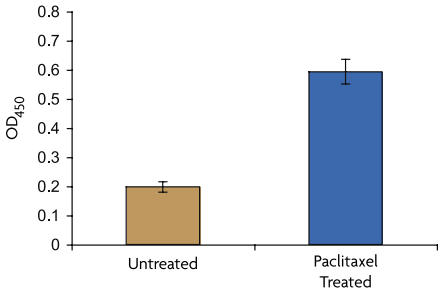

Figure 2: Mitotic Assay results with colorimetric kit. Fold induction of mitosis marker in HeLa cells treated with 1 µM paclitaxel for 6 hours.

Start screening today!

Screening the effects of compounds on cell division is easy with your choice of fluorescent, chemiluminescent or colorimetric Mitotic Assay Kits. For more information, please give us a call.

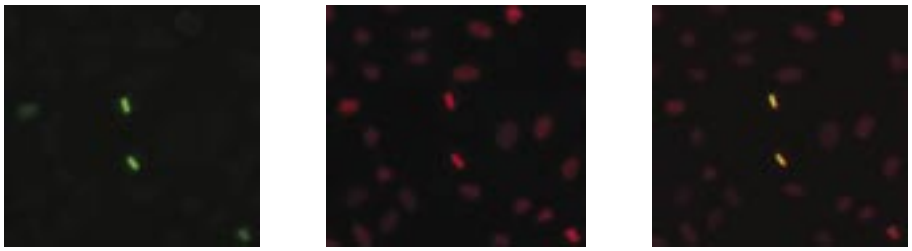

Figure 3: Mitotic Index results with fluorescent microscopy. Phospho-Histone H3 (Ser 28) detected with Chromeo 488 Goat anti-Rat IgG is shown in the first image. Nuclear staining of all cells with propidium iodide is shown in the center image. The merged image on the right show cells stained with both green 488 signal and red propidium iodide signals appearing yellow.

Product	Format	Catalog No.
Mitotic Index Assay Kit (Fluorescent)	5 x 96 rxns	18020
Mitotic Assay Kit (Color)	2 x 96 rxns	18021
Mitotic Assay Kit (Chemi)	2 x 96 rxns	18022

Monitoring Phosphorylation of Biomarkers is Simple and Effective Using In-cell Detection

Active Motif's Fast Activated Cell-based ELISA (FACE™) Kits are a simple, sensitive, cell-based method for detecting protein phosphorylation directly in the cell, without making cell extracts or the trouble of running gels.

FACE advantages

- **Cell-based** – no extraction, gels, or blotting needed
- **Accurate** – Fixation prevents additional protein modifications
- **Fast** – 5 hour protocol (only 2 hours of hands-on time)
- **Grow cells in 96-well plates** – save on reagents
- **Economical** – Total and phospho-specific antibody provided for 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 less than 2 hours of hands-on time. Cells are grown in 96-well cell culture plates and treated with a chemical of interest to change the phosphorylation status of the protein of interest. After the desired incubation time the cells are rapidly fixed with formaldehyde, which preserves the induced protein modifications. Each well is then incubated with a primary antibody specific for the phosphorylation site or the total protein. Subsequent incubation with a secondary labeled antibody provides a colorimetric or chemiluminescent read-out that is quantitative and reproducible (Figure 1). The number of cells in each well can be determined easily with the provided Crystal Violet.

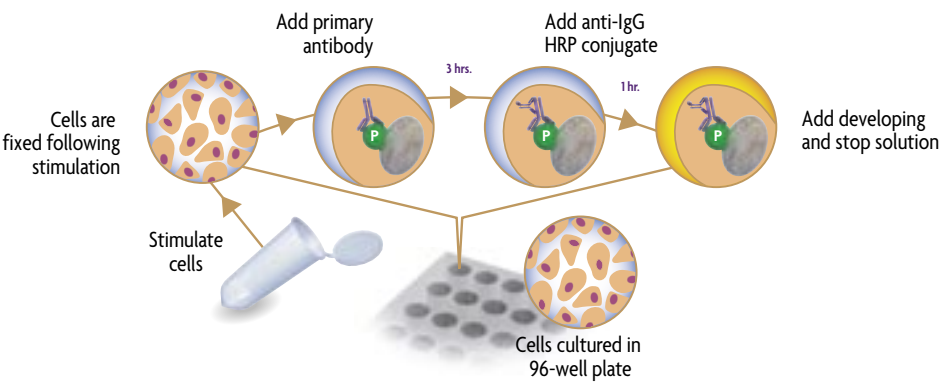

Figure 1: Flow chart of the FACE process.
Cells are grown, stimulated and fixed in the same 96-well plate. Addition of primary and secondary antibodies detects phosphorylated protein.

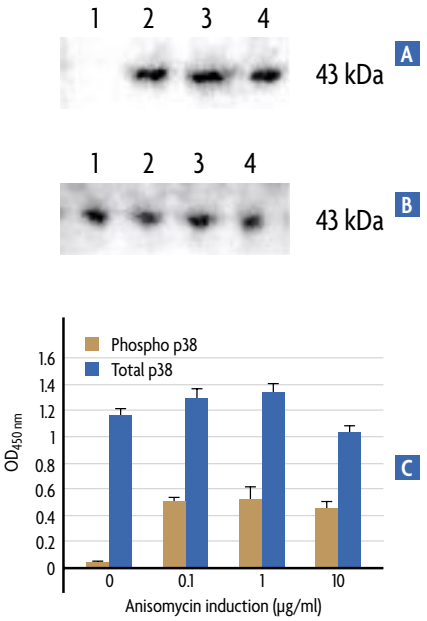

Figure 2: Phospho and total p38 MAPK assays.
Macrophage 4/4 cells were grown in 10 cm dishes to 80% confluency, serum-starved for 16 hours and stimulated with anisomycin for 15 minutes. Cell lysates were made and Western blots performed using phospho- (A) and total-p38 antibodies (B). For FACE, 4/4 cells 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 (T24)	FACE™ GSK3β (S9)	FACE™ HSP27 (S82)	FACE™ JAK1 (Y1022/Y1023)	FACE™ JNK (T183/Y185)
FACE™ Maker	FACE™ MEK1/2 (S217/S221)	FACE™ NFκB Profiler (S468 & S536)	FACE™ p38 (T180/Y182)	FACE™ PI3 Kinase p85	FACE™ STAT2 (Y689)
FACE™ STAT4 (Y693)	FACE™ STAT6 (Y641)				

Generate Chromatin *In Vitro* for Downstream Applications

Active Motif's Chromatin Assembly Kit enables you to generate chromatin *in vitro* from your linear or supercoiled DNA. It yields chromatin that closely mimics natural *in vivo* chromatin, so you can discover which histone modifications and associated proteins are crucial to regulation of your target.

A simple way to generate chromatin

Now you can investigate regulation of your gene of interest in its native form by assembling it into chromatin using Active Motif's *in vitro* Chromatin Assembly Kit. This kit includes all the recombinant proteins, core histones, buffers and ATP-utilizing factors needed to generate chromatin from your DNA sequence, and also to verify successful assembly. High-quality chromatin with more than six regularly spaced nucleosomes is made by adding the supplied components to 1 µg of your linear or supercoiled DNA, then incubating for 4 hours. A simple partial enzymatic digestion of the resulting chromatin confirms the ordered spacing of nucleosomes (Figure 1).

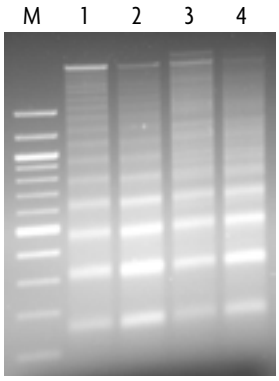

Figure 1: Enzymatic digestion of assembled chromatin. Chromatin assembled from 1 µg samples of circular DNA (Lanes 1 & 2) and linear DNA (Lanes 3 & 4) were digested for 2 and 4 minutes, respectively, deproteinated, phenol/chloroform extracted and run on an agarose gel. Each sample type resulted in regularly spaced nucleosomes.

Chromatin Assembly Kit advantages

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

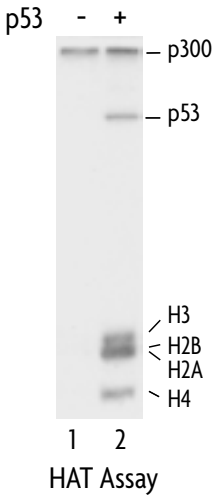

Figure 2: HAT assay using assembled chromatin. Chromatin was *in vitro* assembled with p300 and acetyl-CoA, in the presence (+) or absence (-) of p53 at 30°C for 30 minutes. The sample was then analyzed by 18% SDS-PAGE and visualized following fluorography.

Why bother to make chromatin?

When DNA sequences are assembled into chromatin by ATP-dependent factors, the resulting structure closely resembles the natural chromatin configuration. DNA that is in either a bare or unassembled state often cannot reveal the mechanism of transcriptional activation or repression with the associated factors and relevant histone modifications. However, properly assembled chromatin with regularly ordered nucleosomes is an excellent substrate for subsequent assays such as *in vitro* transcription assays, ChIP (Figure 3) and histone acetyltransferase (HAT) assays (Figure 2).

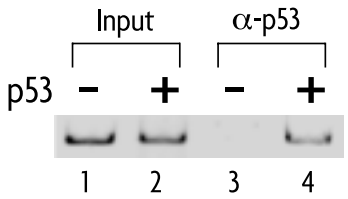

Figure 3: ChIP of *in vitro* assembled chromatin. Chromatin was *in vitro* assembled with p300 and acetyl-CoA in the presence (+) or absence (-) of p53 protein at 30°C for 30 minutes. ChIP was then performed with p53 antibody. Pull-down and Input DNAs were PCR amplified with primer pairs surrounding the p53-binding site. Pull down of chromatin by p53 antibody was dependent on the presence of p53 protein during *in vitro* assembly.

Complete kit ensures success

The kit includes recombinant h-NAP-1 chaperone protein, ACF assembly complex, HeLa core histones, and the buffers and enzymes necessary to generate assembled chromatin from your input DNA. Control Supercoiled DNA is also provided. To verify that your chromatin assembly reaction has been successful, reagents are provided to perform an easy partial enzymatic digestion to visualize the regular spacing of nucleosomes by agarose gel electrophoresis (Figure 1).

Try it today!

The Chromatin Assembly Kit makes it easy for you to assemble chromatin on your sequence of interest to investigate gene regulation in a context that closely resembles *in vivo* chromatin. For more information, please give us a call.

Product	Format	Catalog No.
Chromatin Assembly Kit	10 rxns	53500

NEW: Histone Acetyltransferase Assay Kit Makes It Easy to Screen Inhibitors and Test Activity

Now you can easily assay for histone acetyltransferase activity or screen potential HAT inhibitors with our simple and sensitive fluorescent histone acetyltransferase (HAT) assay kit.

An easy assay that works!

High background can make assaying for histone acetyltransferase activity or inhibition impossible. With Active Motif's new Histone Acetyltransferase Assay Kit, reagents and reaction buffers have been optimized to assay for activity and to screen inhibitor compounds.

Fluorescent simplicity

Assaying for histone acetyltransferase activity is easy with this 96-well plate format. Simply incubate your HAT with the histone substrate peptide and acetyl-CoA for 10-30 minutes, then develop!

What's in the kit?

Active recombinant p300 is provided as a control for use with your samples; it can also be used as a HAT to screen a full plate of inhibitor compounds. Histone H3 and histone H4 substrate peptides are provided to suit the needs of your purified HAT, and anacardic acid is provided as a potent control inhibitor for acetyltransferase activity (Figure 1).

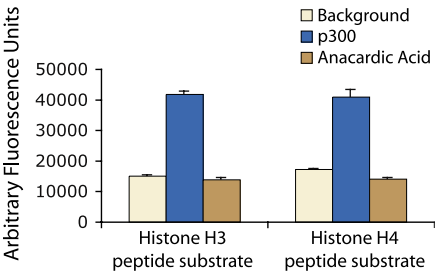

Figure 1: p300 activity.
p300 was assayed with 50 µM H3 or H4 peptide with 50 µM acetyl-CoA. Background is shown with p300 and acetyl-CoA. Inhibition of p300 activity was assayed with 15 µM anacardic acid.

How does it work?

The assay utilizes a thiol-reactive fluorescent dye that reacts with the Co-A-SH generated by the histone acetyltransferase activity to give a fluorescent readout. The fluorescent dye is excitable at 360-390 nm and emits at 450-470 nm. This simple reaction chemistry also makes it easy to generate standard curves for calculating enzymatic activity.

Determine specific activity

In addition to screening inhibitors, you can easily calculate the specific activity of your histone acetyltransferase. By preparing a standard curve with acetyl-CoA or β-mercaptoethanol, you can relate the fluorescence of your HAT to pmol/min/µg specific activity (Figure 2).

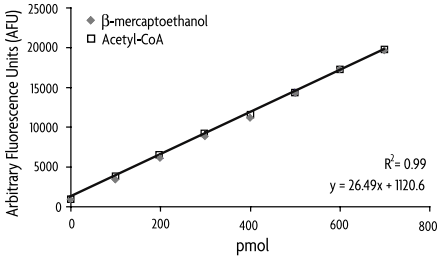

Figure 2: Standard curve to calculate activity.
Acetyl-CoA and β-mercaptoethanol were diluted from 0 to 14 µM and used to create a standard curve that can be used to calculate p300 activity.

Screen for inhibitors

Our assay buffers have been optimized to ensure there is no interference with HAT activity or the ability of compounds to inhibit activity (Figure 3). Depending on what you want to assay, you can use the p300 included in the kit to screen a full 96-well plate of inhibitors.

Fluorescent assay advantages

- Easy assay
- Determine activity of your HAT
- Screen compounds

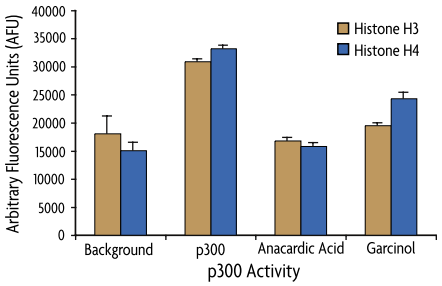

Figure 3: p300 activity and inhibition.
50 ng p300 were assayed per well with 50 µM acetyl-CoA and 50 µM histone H3 or H4 peptide substrates. Activity was inhibited with 15 µM anacardic acid and 25 µM garcinol.

Try it today!

Call or visit us at www.activemotif.com to find out more about the assay. The active p300 is also available separately, so you can use it for other applications. Be sure to visit our website and watch for additional active histone acetyltransferases and related assays.

Product	Format	Catalog No.
HAT Assay Kit (Fluorescent)	1 x 96 rxns	56100
Recombinant p300 protein, catalytic domain	5 µg	31205

TransAM™: The Most Published Alternative to EMSA

TransAM™ Kits are DNA-Binding ELISAs that are quickly becoming 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. 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), which allows 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 (Table 1). TransAM Kits are also available in either 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.

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?

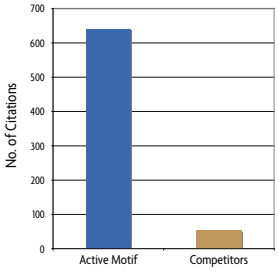

Figure 1: TransAM Kit citations.
Using HighWire Press, http://highwire.stanford.edu, a comparison was made by searching for citations of TransAM from Active Motif versus the tradenames of all competitor kits combined. TransAM is clearly the leader.

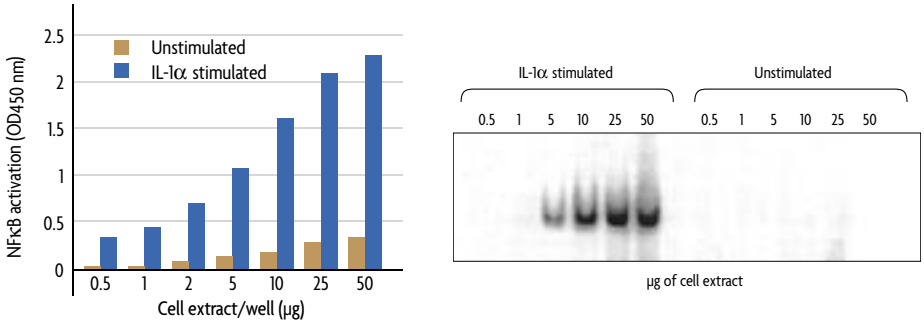

Figure 2: TransAM NFκB is more sensitive than gelshift.
Human fibroblast WI-38 cells are stimulated with IL-1α for 30 minutes. Increasing amounts of whole-cell extract are assayed using the TransAM NFκB p50 Kit (A) or gel retardation (B).

Table 1: TransAM™ Product Line

TransAM™ AP-1 Family	TransAM™ AML-1/Runx1	TransAM™ C/EBP α/β	TransAM™ HNF-1	TransAM™ Flexi NFκB p65
TransAM™ GATA Family	TransAM™ AML-3/Runx2	TransAM™ CREB & pCREB	TransAM™ MEF2	TransAM™ NFκB p65*
TransAM™ HNF Family	TransAM™ AP-1 c-Fos	TransAM™ Elk-1	TransAM™ MyoD	TransAM™ Oct-4
TransAM™ MAPK Family	TransAM™ AP-1 c-Jun	TransAM™ ER	TransAM™ NF-YA	TransAM™ p53
TransAM™ Flexi NFκB Family	TransAM™ AP-1 FosB	TransAM™ FKHR (FOXO1/4)	TransAM™ NFATc1	TransAM™ PPARγ
TransAM™ NFκB Family	TransAM™ AP-1 JunD	TransAM™ GATA-4	TransAM™ Flexi NFκB p50	TransAM™ Sp1 & Sp1/Sp3
TransAM™ STAT Family	TransAM™ ATF-2	TransAM™ GR	TransAM™ NFκB p50*	TransAM™ STAT3
	TransAM™ c-Myc	TransAM™ HIF-1	TransAM™ NFκB p52*	

*The TransAM NFκB p50, p52 & p65 Kits are offered in both colorimetric and chemiluminescent formats. TransAM Chemi Kits require the use of a luminometer.

NEW: Improved ChIP-IT™ Express – Reduced Background Improves Results, Enables Less Starting Material

Active Motif has improved its ChIP-IT™ Express Kits by greatly reducing the background, which improves your results and enables you to use less starting material than ever before. In addition, the magnetic beads provided with ChIP-IT Express have made it possible to streamline the protocol so you can get results in half the normal time with much less sample manipulation. And, ChIP-IT Express makes it easy to perform ChIP on many samples at the same time.

ChIP-IT Express advantages

- No more need for pre-clearing, blocking or DNA purification steps
- Reduced background
- High throughput compatible
- Dramatically reduced hands-on time

ChIP-IT Express beats the competition

Not only does ChIP-IT Express save you time, but the simple method generates better results than traditional ChIP. To demonstrate, ChIP experiments were performed using ChIP-IT Express and a leading competitor's ChIP Kit (Figure 1).

The most efficient ChIP enrichment kit

ChIP is an enrichment technique, not a purification method. Thus, the less efficient your enrichment, the higher the sample background and the more material you will need to obtain an interpretable result. Conventional ChIP requires at least 2 million cells as starting material, which can be problematic with some cell lines. At the least, growing this many cells is labor intensive. Active Motif's improved ChIP-IT Express Kits, however, have been optimized to provide superior target gene enrichment, resulting in unmatched sensitivity. Using ChIP-IT Express, it is routine to perform ChIP on material from as few as 750,000 cells and the kits have even been shown to work with as few as 12,500 cells!

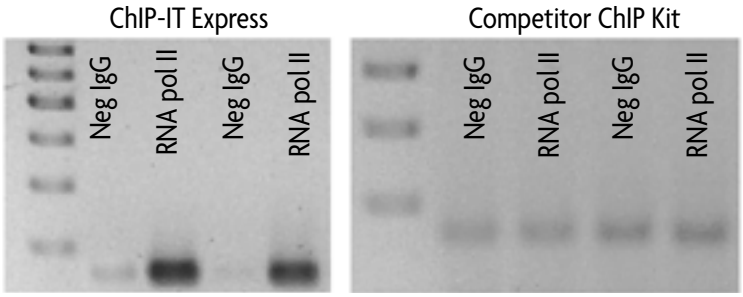

Figure 1: ChIP-IT Express is more efficient and has less background than the competition. ChIP-IT Express and standard ChIP (with Protein G agarose beads) were performed in duplicate with RNA pol II antibody and negative control IgG on HeLa cell DNA from 750,0000 cells. PCR was performed for 36 cycles using GAPDH positive control primers.

Product	Format	Catalog No.
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014
ChIP-IT™ Control Kit – Human	5 rxns	53010
ChIP-IT™ Control Kit – Mouse	5 rxns	53011
ChIP-IT™ Control Kit – Rat	5 rxns	53012
Ready-to-ChIP HeLa Chromatin	10 rxns	53015

The magnetic bead advantage

The ChIP-IT Express magnetic beads have much less background than standard agarose beads, and this means pre-clearing and blocking steps are no longer necessary. The magnetic pull-down occurs in just seconds, and re-formulated buffers allow steps to be combined and DNA purification to be eliminated. ChIP-IT Express is available in both sonication and enzymatic shearing formats.

Positive controls ensure success

Because interpreting ChIP data can be difficult, Active Motif has developed a complete set of controls to help you understand your results and troubleshoot your assays. To provide you with controls that are appropriate for your research, we removed the human-only controls from ChIP-IT Express Kits and now offer human, mouse and rat ChIP-IT Control Kits separately. These provide positive and negative control antibodies and species-specific primers, PCR buffer and a convenient 10X DNA loading dye so your PCR reactions are gel-ready. All reagents are quality control tested and validated to ensure your ChIP assay is working properly. In addition, we also offer convenient Ready-to-ChIP HeLa Chromatin, so you can be certain that the only variable in validating a new antibody for ChIP is the antibody itself.

Try the best ChIP kit today

For additional information on the new and improved ChIP-IT Express Kits, visit our website at www.activemotif.com.

NEW: Highly Specific Isolation of Methylated DNA

Active Motif's new MethylCollector Kit provides users with a fast and easy protocol for isolating CpG-methylated DNA from limited amounts of cell or tissue samples. Because it uses a recombinant Methyl-binding protein (MBD2b) to capture DNA, rather than traditional antibody-based immunoprecipitations, sensitivity is unmatched and the yield is greatly improved.

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. 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.

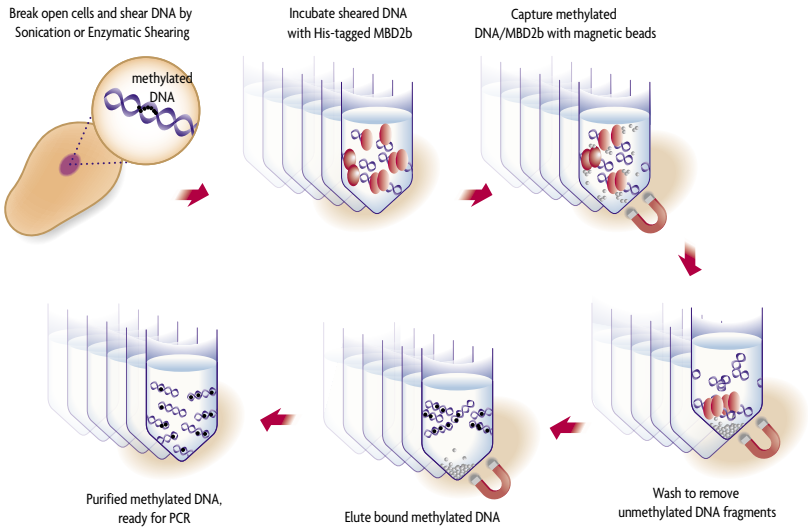

Figure 1: Flow chart of the MethylCollector process.
In a MethylCollector assay, genomic DNA of interest is sheared by either enzymatic digestion or sonication. The sheared DNA is then incubated with His-tagged recombinant MBD2b protein, which has a affinity for CpG-methylated DNA. These protein-DNA complexes are captured with nickel-coated magnetic beads and stringent washes are performed to remove DNA fragments that have little or no methylation. The methylated DNA is then eluted from the beads and PCR is performed on the resulting supernatant, using primers that are specific to amplify the locus of interest.

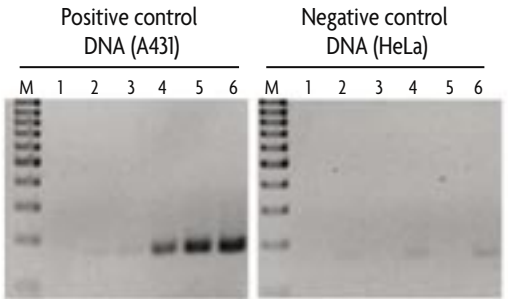

Figure 2: PCR analysis of isolated CpG-methylated DNA using MethylCollector.
Genomic DNA from A431 (positive control DNA) and HeLa (negative control DNA) cells were enzymatically digested by *Mse* I for 2 hours. Increasing amounts of fragmented DNA (from 5, 40 and 100 ng) were then incubated for 1 hour with 1 µg His-MBD2b recombinant protein in the presence of nickel-coated magnetic beads. After washing, CpG-methylated complexes were eluted. The isolated DNA was then analyzed by 36 cycles of PCR using the kit's control primers, which amplify a locus that is not methylated in HeLa but highly methylated in A431. PCR on MethylCollector-enriched A431 DNA generates robust signals that are proportionate to the amount of DNA starting material (left panel, lanes 4 to 6). No signal is observed with enriched HeLa DNA (right panel, lanes 4 to 6) or in those samples where His-MBD2b protein was omitted from the binding reaction (left and right panels, lanes 1 to 3). Taken together, these results indicate that MethylCollector specifically enriches for methylated DNA fragments, and that this enrichment is due to the presence of the kit's His-MBD2b protein.

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

Antibodies for Epigenetics Research

Active Motif offers a broad range of antibodies related to DNA methylation and repair. All are suitable for Western blotting and many are ChIP-validated. For additional information, including technical data sheets, please visit www.activemotif.com.

Product	Format	Catalog No.
CGBP rabbit pAb	200 µl	39203
DNMT1 mouse mAb	100 µg	39204
DNMT2 rabbit pAb	100 µg	39205
DNMT3A mouse mAb	100 µg	39206
DNMT3B mouse mAb	100 µg	39207
HDAC1 rabbit pAb	100 µg	40967
HDAC3 rabbit pAb	100 µg	40968
HDAC4 rabbit pAb	100 µg	40969
HDAC5 rabbit pAb	100 µg	40970
HDAC6 rabbit pAb	100 µg	40971
HDAC11 rabbit pAb	200 µl	39208
Histone H2A rabbit pAb	100 µg	39209
Histone H2B rabbit pAb	100 µg	39210
Histone H3 rabbit pAb	100 µg	39088

Product	Format	Catalog No.
Histone H4 rabbit pAb	100 µg	39212
INHAT-1/TAF-1α/TAF-1β rabbit pAb	200 µl	39202
INHAT-2/pp32 rabbit pAb	200 µl	39213
MBD1 mouse mAb	100 µg	39215
MBD3 rabbit pAb	100 µg	39216
MBD4 rabbit pAb	100 µg	39217
MeCP2 rabbit pAb	100 µg	39218
MLH-1 mouse mAb	100 µg	39219
MRE11 rabbit pAb	100 µg	39220
Rad17 (phosphorylated) rabbit pAb	100 µg	39222
TRF2 goat pAb	100 µg	39223
UBE2N rabbit pAb	100 µg	39224
UBE2V1α rabbit pAb	100 µg	39225
UBE2V2 rabbit pAb	100 µg	39226

LavaCell™ – a Bright Orange Fluorescent Cell Stain

LavaCell™ is a unique fluorescent compound that is an ideal stain for live or fixed cells in any cellular imaging application.

Perfect for cellular imaging

LavaCell is a small, neutral, non-toxic compound that readily diffuses into live or fixed cells and spontaneously reacts to produce bright orange fluorescence. This naturally fluorescent compound is excited by a variety of common lasers and can be multiplexed with a wide range of common blue, green and yellow-emitting probes (Figure 1).

Ideal fluorescent characteristics

Cells require no pre-treatment or permeabilization for staining as LavaCell is a neutral molecule that diffuses across cellular membranes. LavaCell then rapidly reacts with free amine groups on proteins to produce a bright fluorescent signal (Figure 2). LavaCell does not stain nucleic acids nor does it adversely affect cell growth. LavaCell can be excited with common laser sources at 405, 488 and 532 nm, and has a Stokes shift of over 100 nm for peak emission at 610 nm.

LavaCell staining advantages

- Excitable with common laser sources
- Long Stokes shift is great for multiplexing
- Non cytotoxic
- Fluorescent only when bound means no wash steps

Try LavaCell to visualize your cells

Call us for more information and to give it a try today!

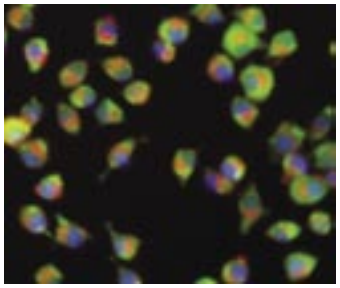

Figure 1: Live mouse macrophage cells were multiplex stained with LavaCell, calcein AM and Hoescht.

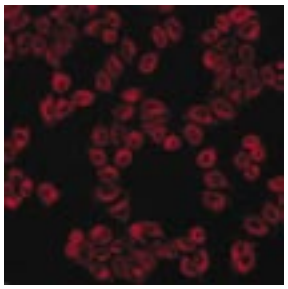

Figure 2: Live HeLa cells stained with LavaCell.

Product	Format	Catalog No.
LavaCell™	200 µg	15004

RapidReporter® – Double Destabilization Improves Response & Sensitivity

Active Motif's patented* RapidReporter® is the only luciferase reporter gene assay that utilizes both mRNA and protein destabilizing elements. This greatly reduces background, which increases both the magnitude of the response that can be measured after stimulation or repression, and the speed in which the assay can measure changes in transcription (both increases and decreases).

Stop living in the past

Standard luciferase assays are limited by the fact that basal activity of the cloned promoter results in accumulation of both the luciferase mRNA and protein. The slow clearance rate of these pre-existing molecules substantially delays and dilutes the measurable response. Thus, a large proportion of the luciferase measured is derived from transcription that took place before the test agent was even added. As a result, in standard assays transient or relatively minor effects are hidden and kinetics are inaccurate. Consequently, drugs or treatments that are disregarded because they have little or no detectable effect may often elicit a 10-fold or more change in transcription.[1]

Half measures will only get you so far

To solve the problem of long half-life proteins, some vectors include protein-destabilizing elements, which causes the luciferase to degrade more rapidly. Destabilizing the protein, however, only partly addresses the problem, as clearance rates are also dependent on the half-life of the mRNA. As long as the reporter mRNA is intact, it continues to produce new reporter protein.

Double destabilization is the key

To solve the problem, RapidReporter utilizes vectors that include both protein AND mRNA destabilizing elements. Such double destabilizing vectors have been shown to reduce luciferase half-life an additional 33% compared to vectors with only protein-destabilizing elements.[2]

Measure what's actually happening

The use of double destabilizing vectors makes RapidReporter more sensitive and responsive, so you can detect smaller changes and get more accurate real-time measurement than with non-destabilized reporter gene assays, or those that destabilize the protein only (Figure 1).

Your choice of stringency

RapidReporter vectors are offered in two different stringencies. pRR-High is highly destabilized, while pRR-Low has fewer elements, which is appropriate when studying weaker stimulation.

Pre-made vectors & complete kits

In addition to empty RapidReporter vectors, Active Motif offers vectors that contain widely studied promoters. All are available separately or in complete assay kits, which also include a positive control vector and buffers & substrate optimized for *Gaussia* luciferase. To learn more about the only reporter assay with double destabilization, please give us a call or visit www.activemotif.com.

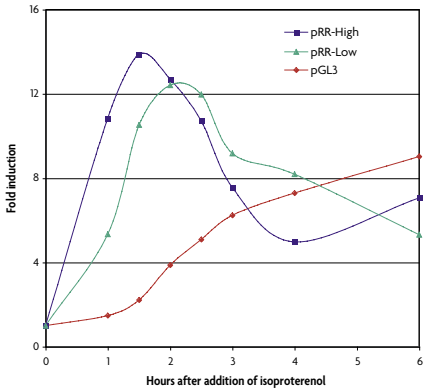

Figure 1: More accurate kinetics, higher fold induction. 293 cells transiently transfected with pRR-High-CRE, pRR-Low-CRE and a pGL3 vector containing CRE were plated onto 96-well plates. Twenty-four hours post-transfection, cells were stimulated with 4 µM isoproterenol, then measured for *Gaussia* (pRR vectors) and firefly luciferase activities (pGL3 vector).

Product	Format	Catalog No.
RapidReporter® Gaussia Luciferase Assay	100 rxns	33001
	1000 rxns	33002
RapidReporter® pRR-High vector	10 µg	33003
RapidReporter® pRR-High Assay	100 rxns	33004
RapidReporter® pRR-Low vector	10 µg	33005
RapidReporter® pRR-Low Assay	100 rxns	33006
RapidReporter® pRR-High-CRE vector	10 µg	33007
RapidReporter® pRR-High-CRE Assay	100 rxns	33008
RapidReporter® pRR-High-NFκB vector	10 µg	33009
RapidReporter® pRR-High-NFκB Assay	100 rxns	33010
RapidReporter® pRR-High-GR vector	10 µg	33011
RapidReporter® pRR-High-GR Assay	100 rxns	33012
RapidReporter® pRR-High-STAT3 vector	10 µg	33013
RapidReporter® pRR-High-STAT3 Assay	100 rxns	33014

* RapidReporter is covered under U.S. Patent No. 7,157,272 and various other patents worldwide and is sold under license granted by GeneStream Pty Ltd. Purchasers are subject to a Limited-use License; please contact Active Motif's Technical Services for details, or download a copy from our web site. RapidReporter is a registered trademark of GeneStream Pty Ltd.

REFERENCES

1. Voon, C. *et al.* (2004) *Nucleic Acids Research* 33(3): e27.
2. Almond, A. *et al.* (2004) *Promega Notes* 87: 18-22.

Simplified Co-Immunoprecipitation of Intact Nuclear Complexes

The Nuclear Complex Co-IP Kit simplifies co-immunoprecipitation of intact nuclear protein complexes because its reagents have been optimized for preparing nuclear extracts and immunoprecipitating DNA-bound proteins.

Co-Immunoprecipitation (Co-IP) is often used to study protein/protein interactions. In Co-IP, a first antibody is used to immunoprecipitate a target antigen, which also co-precipitates any bound, interacting proteins. These are then detected by Western blot using antibodies targeted against the interacting proteins. However, traditional Co-IP methods are not optimal for studying DNA-binding proteins because their complexes are very fragile, so are frequently disrupted during extraction. In addition, these complexes can be altered by the salt and detergent composition of the immunoprecipitation buffers, which can further complicate their analysis.

Perform Co-IP of DNA-bound proteins

To overcome these problems, the Nuclear Complex Co-IP Kit contains extraction reagents that were designed to help maintain nuclear protein complexes. The kit's extraction process provides a simple, effective method for isolating intact protein complexes contained in nuclear compartments of the cell, specifically those previously bound to DNA. After isolation of the intact complexes, the supplied Co-IP reagents were designed so that you can vary the stringency of the Co-IP buffers. This improves your results by making it easy for you to study any protein complex, whether its members are tightly or weakly bound.

"The Nuclear Complex Co-IP Kit improves co-immunoprecipitation of DNA-binding proteins by providing extraction and immunoprecipitation components that are optimized to maintain nuclear protein complexes."

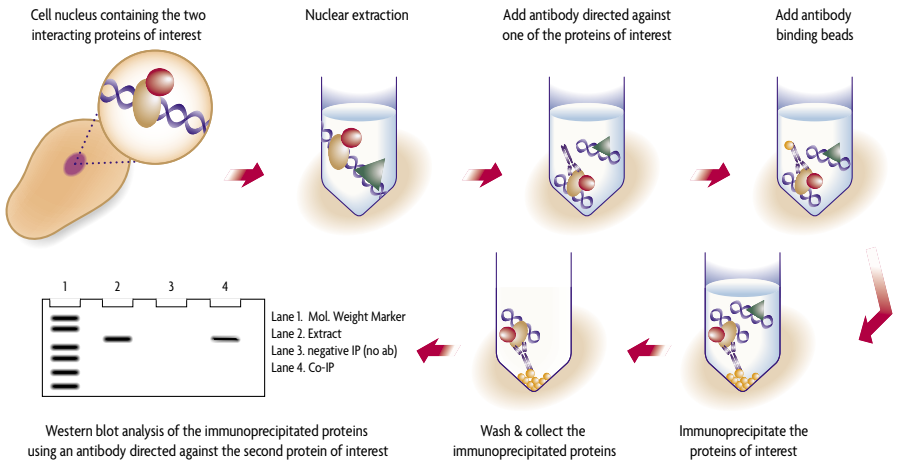

Figure 1: Flow chart of the Co-Immunoprecipitation process.

Product	Format	Catalog No.
Nuclear Complex Co-IP Kit	50 rxns	54001

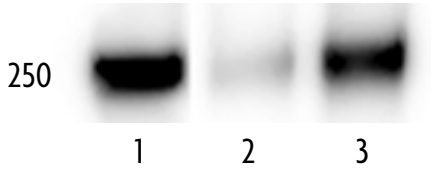

Figure 2: Analysis of p33 in the RNA pol II complex. HeLa cells were grown to confluence on 100 mm plates and nuclear extracts were prepared using the kit's extraction reagents. For IP experiments, the stringency of the IP High Buffer was increased by supplementing with NaCl and Detergent. 100 µg of nuclear extract was used per IP reaction and incubated with either 2 µg p33 antibody or no antibody. Following the IP, Western blot analysis was performed using RNA pol II mouse mAb at 0.1 µg/ml followed by anti-mouse HRP at 1:1000. Detection of the p33/RNA pol II complex by the RNA pol II antibody (lane 3) demonstrates that the Co-IP was successful in maintaining the protein complex. The input HeLa extract (lane 1) was run as a control for the Western blot using 0.1 µg/ml RNA pol II.

Lane 1 Western blot control
Lane 2 Negative Control (no antibody used in IP)
Lane 3 Co-IP: IP using p33/WB using RNA pol II

Easy to optimize for ideal stringency

With the Nuclear Complex Co-IP Kit, nuclear extracts are prepared using low-salt buffers and enzymatic shearing. The low-salt buffers keep the protein complexes intact, while digestion gently releases them from the DNA. Immunoprecipitation is then carried out to detect the bound proteins. The kit contains high- and low-stringency IP buffers, as well as salt and detergent. Addition of salt and detergent is ideal for robust protein/protein interactions because higher stringency reduces background. However, as unstable protein complexes may not withstand high stringencies, the kit makes it simple to optimize the stringency as required for each particular protein complex.

Find the complex members you missed

The Nuclear Complex Co-IP Kit offers you a simpler, more flexible alternative than traditional co-immunoprecipitation methods. To find out more about how it can help you, please give us a call or visit us on the web at www.activemotif.com.

NEW: Chromeo™ Fluorescent Secondary Antibodies

Now the superior fluorescence properties of the Chromeo™ Dyes are available as mouse and rabbit secondary antibody conjugates to provide you with flexible options for your assays.

New Chromeo 488 nm label

Chromeo Dyes have the superior fluorescent properties you need in an assay, with their bright fluorescent intensity, stability over a broad pH range, and limited photobleaching. Now, the Chromeo Dyes are available as 488, 494, 546 and 642 nm conjugates to high-quality mouse and rabbit secondary antibodies to suit your fluorescent assay needs.

On the cover:
U2OS cells were fixed and permeabilized by methanol. Tubulin was detected with rabbit primary and Chromeo 488 Goat anti-Rabbit IgG. Cells were also stained with propidium iodide.

Compatible with assay conditions

Chromeo fluorescent secondary antibodies work in all popular fixation conditions such as methanol, formaldehyde or formalin (Cover image and Figure 1). Please call or visit us online for more complete information.

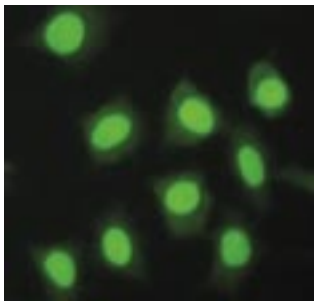

Figure 1: Lamin staining in fixed HeLa cells.
HeLa cells were fixed with formaldehyde and Chromeo 488 Goat anti-Mouse was used to detect a primary monoclonal mouse lamin A antibody.

Product	Format	Catalog No.
Chromeo™ 488 Goat anti-Mouse IgG	1 mg	15031
Chromeo™ 488 Goat anti-Rabbit IgG=	1 mg	15041
Chromeo™ 494 Goat anti-Rabbit IgG	1 mg	15042
Chromeo™ 546 Goat anti-Mouse IgG	1 mg	15033
Chromeo™ 546 Goat anti-Rabbit IgG	1 mg	15043
Chromeo™ 642 Goat anti-Mouse IgG	1 mg	15034
Chromeo™ 642 Goat anti-Rabbit IgG	1 mg	15044

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Tools to Analyze Nuclear Function