MOTIF VATIONS

ACTIVE MOTIF®

Tools to Analyze Cellular Function

THE NEWSLETTER OF ACTIVE MOTIF — November 2006 • volume 7 • number 4

New: Magnetic Beads Make Chromatin Immunoprecipitation Faster and Easier

Active Motif's new ChIP-IT™ Express Kits use protein G-coated magnetic beads instead of traditional agarose beads to make chromatin immunoprecipitation (ChIP) simpler and faster than ever before. These innovative beads have made it possible to simplify and streamline the protocol, optimize buffers and reduce or eliminate several time- and labor-intensive steps. The result is that ChIP-IT Express enables you to perform ChIP in a single day.

New technique for speed and success

The new components and protocol of ChIP-IT Express make it faster and easier than any other ChIP procedure. The kits include all buffers, reagents and components required to shear 5 samples of chromatin, using your choice of sonication or enzymatic digestion, then perform 25 ChIP reactions. We've even included a strong bar magnet. The protein G-coated magnetic beads, provided ready-to-use, have a high binding capacity for IgG. This enables them to be used in small volumes, which reduces non-specific binding. This improved specificity enables the number and length of washing steps to be reduced. It is not even necessary to block the beads or pre-clear the chromatin.

Optimized buffers eliminate steps

In addition to the magnetic beads and bar magnet, ChIP-IT Express Kits contain new buffers that eliminate the need for DNA purification, so you can simultaneously elute your DNA from the beads while reversing the cross-links. These simple protocol changes give you results quickly and easily while offering the freedom to simultaneously perform multiple ChIP assays: now you can even perform ChIP in PCR tubes with a multi-channel pipettor (Figure 1).

The power of magnetic beads

With ChIP-IT Express Kits, ChIP can be performed in the included Eppendorf tubes or in 8-well PCR strips. PCR strips make it convenient to process larger numbers of samples. The protein G-coated magnetic beads pellet in seconds, much faster than centrifuging agarose beads. An added advantage is that magnetic beads pellet on the tube side instead of the bottom, even coming completely out of the wash buffer. This makes it easy to remove buffers without disturbing the beads, which reduces sample loss and helps ensure sample-to-sample consistency.

INSIDE THIS ISSUE

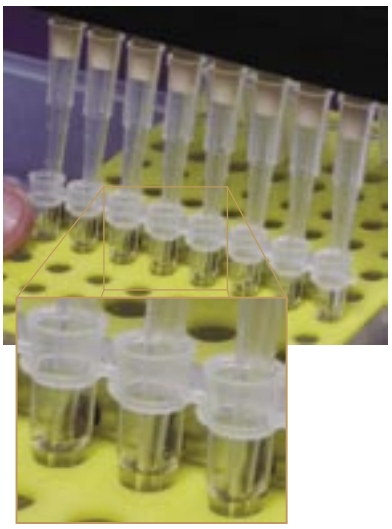

Figure 1: Multiple-sample ChIP using ChIP-IT Express. Washing magnetic beads is fast and easy because the pellet forms against the side of the tube in seconds. This makes it possible to ChIP multiple samples in 8-well PCR tubes using a multi-channel pipettor.

continued on page 6

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 (Figure 1). The plate is then read on a luminometer, which provides a sensitive, quantitative chemiluminescent readout of activated Ras (Figure 2).

"ELISAs are faster and more sensitive than other methods used to study GTPase activation, and eliminate the need for IP, gels and blots."

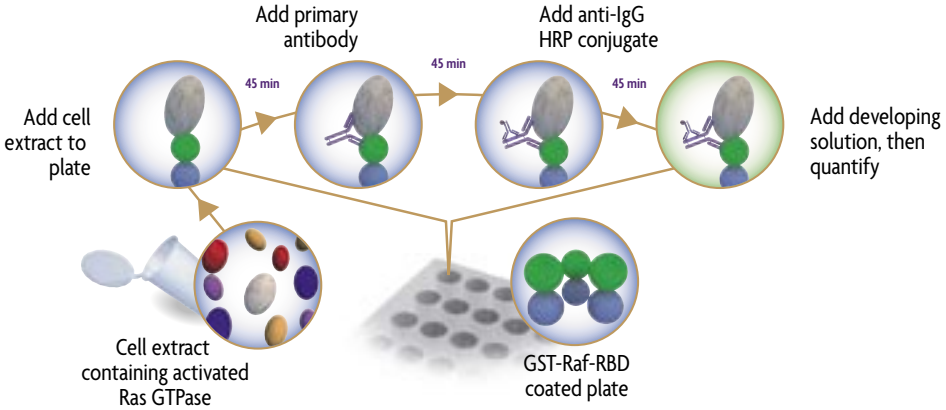

Figure 1: Flowchart of the Ras GTPase Chemi Kit.
Cell extract is added to a glutathione-coated plate that contains immobilized GST-Raf-RBD protein. Activated Ras in the extract binds to the Raf-RBD protein. Addition of primary & secondary antibodies and developing solution followed by reading on a luminometer enables sensitive quantification of activated Ras.

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

Ras 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
- **Versatile** – assay activated extracts from cells or tissue samples, or study recombinant Ras protein

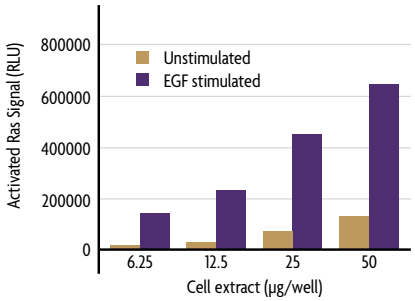

Figure 2: Quantification of activated Ras.
Increasing amounts of whole-cell extract from HeLa cells that had been stimulated with 5 ng/ml EGF for 2 minutes were assayed using the Ras GTPase Chemi ELISA Kit.

Try the quantitative, sensitive assay

The Ras GTPase Chemi ELISA Kit makes it fast and easy to detect and quantify activated Ras GTPase. The kit is ideal for the study of novel signaling pathways that activate Ras, as well as for determining if a particular malignancy is related to inappropriately activated, oncogenic Ras. Please give us a call, return the enclosed reply card or visit our website at www.activemotif.com/gtpase for complete information, including downloadable manuals. To get to the best Ras activation assay available, try the Ras GTPase Chemi ELISA Kit.

Simplify Co-Immunoprecipitation

Active Motif's Nuclear Complex Co-IP Kit simplifies co-immunoprecipitation studies of nuclear protein complexes by providing you with optimized reagents for both nuclear extract preparation and immunoprecipitation.

The Nuclear Complex Co-IP's extraction process provides a simple and effective method to obtain and maintain protein complexes contained in nuclear compartments of the cell, specifically those previously bound to DNA, while the versatile Co-IP reagents offers you the flexibility to vary the stringency of the Co-IP buffer compositions. This improves your results and enables you to study tightly bound or weak protein complexes with ease.

Co-Immunoprecipitation (Co-IP) is often used to find and 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. The complex is then detected by Western blot using a second antibody targeted against one of the bound, interacting proteins. However, traditional methods for performing Co-IP are not optimal for studying complexes of DNA-binding proteins as these complexes, being fragile, are frequently disrupted during the extraction process. In addition, many protein complexes are altered by the salt and detergent composition of the buffers used in the immunoprecipitation process, which can complicate their analysis. To overcome these problems, the Nuclear Complex Co-IP Kit extraction reagents were designed to help maintain nuclear protein complexes.

"The Nuclear Complex Co-IP Kit simplifies co-immunoprecipitation of DNA-binding proteins from cell and tissue samples by providing both extraction and immunoprecipitation components that maintain nuclear protein complexes."

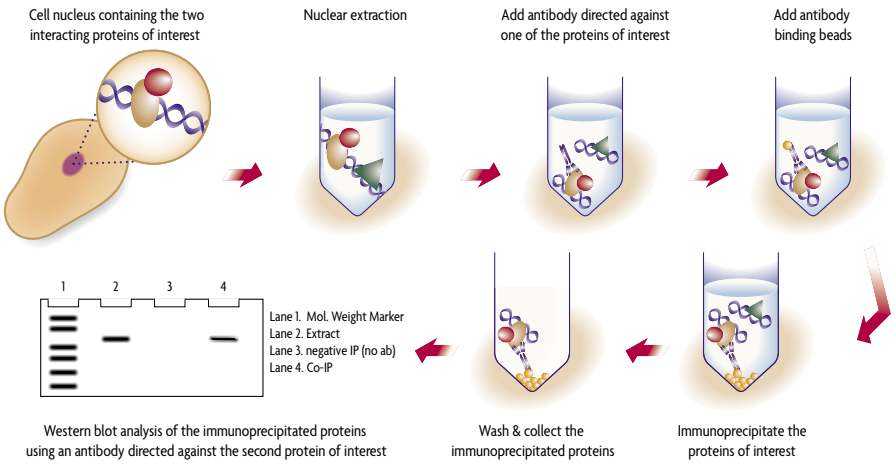

Figure 1: Flow chart of the Co-Immunoprecipitation process.

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

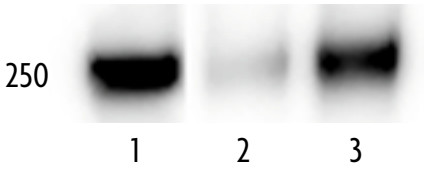

Figure 1: Western blot analysis of the IP'd p33 subunit of the RNA pol II complex.
HeLa cells were grown to confluence on 100 mm plates and nuclear extracts were prepared using the kit's extraction reagents. For IP experiments, the stringency of the IP High Buffer was increased by supplementing with 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

The Co-IP Kit also 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 it reduces background. However, as unstable protein complexes may not withstand high stringencies, this convenient kit format enables stringency to be modified as required for each particular protein complex.

Advantages

- Simple and efficient
- Optimized extraction procedure preserves nuclear protein complexes
- Flexible IP reagents to detect protein/protein interactions of varying strength

Order one today

The Nuclear Complex Co-IP Kit offers you a simple and flexible alternative to performing traditional immunoprecipita-tions. To find out more about the Nuclear Complex Co-IP Kit, visit us on the web at www.activemotif.com.

Investigate SUMOylation with Simple, Efficient SUMOlink™

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

Post-translational modification by SUMO (small ubiquitin-like modifier) can alter protein function in many different cellular processes including disrupting protein-protein interactions in signaling pathways, influencing intracellular protein localization and playing a regulatory role in transcription factor activity.

SUMOlink Kits enable you to easily perform and detect *in vitro* SUMO conjugation of your target protein alongside provided controls. In the kit, all required reagents are provided along with positive control p53 protein and antibody.

"The positive control protein and antibody supplied in SUMOlink Kits help ensure the accuracy of your *in vitro* SUMOylation assays."

The SUMOlink method

In easy-to-use SUMOlink Kits, 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. With the kit's p53 antibody and either SUMO-1 or SUMO-2/3 antibodies, you can easily determine the extent to which your target protein has been SUMOylated (Figure 1).

SUMOlink applications

- Investigate SUMOylation effects on transcription factor activity
- Study the role of SUMOylation in cellular processes
- Identify novel SUMOylation targets

SUMOlink advantages

- Simple and efficient method
- Complete kit with validated reagents and positive control protein
- SUMOylate proteins or cell extracts
- Antibodies cross-react with human, mouse and rat samples

SUMO-1 and SUMO-2/3 kits available

In vertebrates, the SUMOylation enzyme cascade can result in 3 distinct types of SUMO modification: SUMO-1, -2 or -3. Active Motif provides kits to detect and investigate SUMOylation of your protein of interest by each of these SUMO proteins. The kits contain E1 activating and E2 conjugating enzymes along with wild-type and mutant SUMO-1 protein (SUMO-1 Kit) or SUMO-2 and -3 proteins (SUMO-2/3 Kit). Antibodies to detect SUMO-1 or SUMO-2/3 modifications as well as control p53 protein and antibody are included. For more information please give us a call or visit our website at www.activemotif.com.

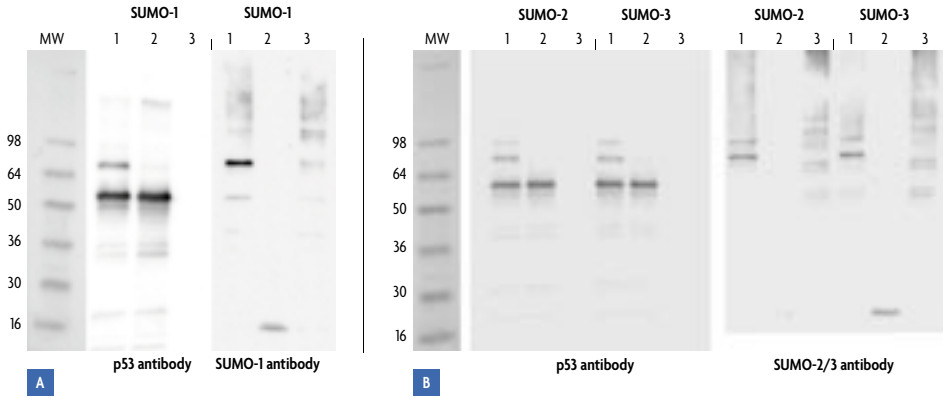

Figure 1: Specific protein labeling using SUMOlink.
Western blot analysis of *in vitro* SUMOylation of p53 protein by wild-type and mutated isoforms of SUMO-1, SUMO-2 and SUMO-3 proteins. A. SUMO-1 analysis: Western blots incubated with p53 antibody (1:5000 dilution) and SUMO-1 antibody (1 :4000 dilution); B. SUMO-2/3 analysis: Western blots incubated with p53 antibody (1:5000 dilution) and SUMO-2/3 antibody (1:4000 dilution).

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

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

New: Study Your Choice of Target in Any Cell Signaling Pathway

Measure downstream effects of signal transduction events with FACE™ Maker. This new, adaptable version of Active Motif's FACE (Fast Activated Cell-based ELISA) enables you to study any phosphorylated protein you choose in a simple, fast and sensitive cell-based assay. Using antibodies specific to your desired target protein, modification-state specific analysis is performed directly within the cell without the need for lysates, gels and time-consuming immunoblotting.

The FACE method

Cells are grown in a 96-well plate and stimulated to induce the pathway of interest. Following stimulation, the cells are fixed to preserve protein modifications, and each well is then incubated with a primary antibody specific to the target of interest. Original FACE Kits contain primary antibodies specific to particular targets, but FACE Maker Kits now give you the option to use your own choice of primary antibody. Subsequent incubation with secondary HRP-conjugated antibody and developing solution provides an easily quantified colorimetric or chemiluminescent readout (Figure 1). Results may be normalized for cell number using the included Crystal Violet solution. With FACE, a duplicate assay for the non-modified state of the protein can easily be run for comparison of constitutive levels versus the stimulation effect.

Prevent unwanted sample modifications

FACE eliminates the need to prepare cell lysates, which greatly reduces the possibility of introducing undesired alterations of the protein of interest. FACE Kits use a fixation step that "freezes" the cellular state and prevents further protein modifications. This enables detection of the exact protein state in the cells at your chosen timepoint(s), providing more accurate and reproducible results.

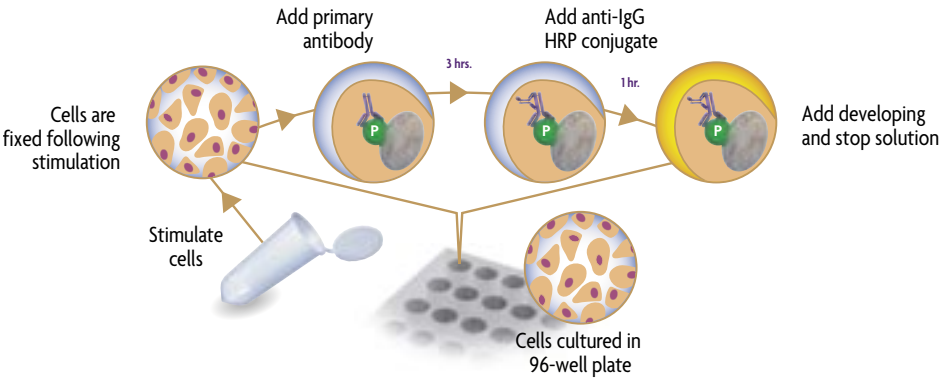

Figure 1: Flow chart of the FACE process.

Product	Format	Catalog No.
FACE™ Maker	1 x 96 rxns	48000
	5 x 96 rxns	48500
FACE™ Maker Chemi	1 x 96 rxns	48050
	5 x 96 rxns	48550

Two types of detection

All FACE Kits are available in both colorimetric and chemiluminescent formats. The colorimetric kits detect your protein of interest using an HRP-colorimetric signal at a wavelength of 450 nm and can be read with a standard plate reader. The more sensitive FACE Chemi Kits use chemiluminescent detection that is measured by a luminometer, which accurately monitors the slightest changes in protein phosphorylation.

FACE advantages

- Simple, quantifiable method
- Fixing cells preserves protein activation state
- Cell-based assay eliminates lysate preparation and immunoblotting
- Fast – results after less than 3 hours of hands on time

What's in the kit

Active Motif already offers FACE Kits that have been optimized for 25 different targets. With new FACE Maker Kits, the possibilities are now endless because you decide which protein to target. FACE Maker Kits include 96-well plates for cell culture, optimized Reaction Buffers and Crystal Violet Cell Quantification Solution. You supply only your own phospho-specific and total antibodies and an appropriate HRP-conjugated secondary antibody. For the simplest way to study phospho-proteins, try FACE and the new FACE Maker Kits.

continued from page 1 — New: Magnetic Beads Make Chromatin Immunoprecipitation Faster and Easier

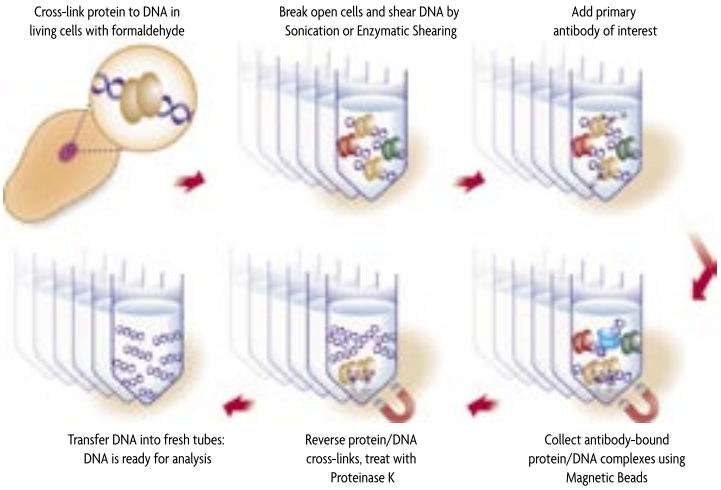

Figure 3: Flow chart of chromatin immunoprecipitation with ChIP-IT Express.

A powerful magnet

ChIP-IT Express kits include a strong bar magnet that can be used to turn pipette tip boxes into magnetic stands for immunoprecipitation and washing. P1000 tip boxes can be used with Eppendorf tubes (Figure 2), while P200 boxes work with PCR strips (Figure 1, page 1). Commercially available magnetic tube stands, such as those from Ambion and Promega, can also be used.

ChIP-IT Express advantages

- Dramatically reduced hands-on time
- Simplified wash steps improve consistency
- No pre-clearing, blocking or DNA purification
- High-throughput compatible

Improvements of ChIP-IT Express

ChIP-IT Express improves on traditional ChIP by reducing or eliminating several time-consuming steps (Figure 3). The magnetic beads have much lower background than traditional agarose beads, so pre-clearing and blocking are no longer necessary. This dramatically reduces the amount of hands-on time during the assay. Washing is easier because the spin steps have been replaced by rapid magnetic pull-down. Other optimizations include a specialized ChIP Elution Buffer that eliminates the need for DNA purification after the ChIP is complete. This saves time, minimizes manipulations and eliminates the DNA loss that can occur during purification.

Secrets to success

A feature that made the original ChIP-IT Kits successful was the positive and negative controls, as these help troubleshooting and results interpretation. But, because many customers requested species-specific controls, we've removed these human-only controls from ChIP-IT Express Kits and are pleased to release new ChIP-IT Control Kits for human, rat or mouse samples. These kits provide positive and negative control antibodies and species-specific PCR primer sets, PCR buffer and a convenient 10X DNA loading dye that makes your PCR reactions gel-ready. The human control kit is now available; check our website for release of the rat and mouse kits.

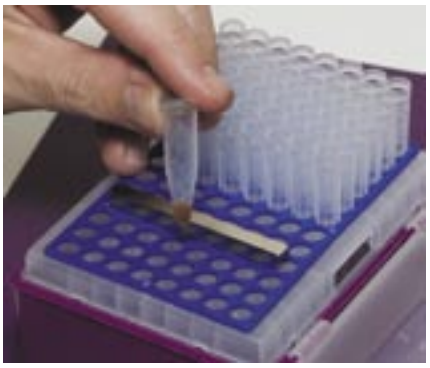

Figure 2: Use of a standard tip box for magnetic ChIP. ChIP-IT Express Kits include a strong bar magnet that can be used to convert tip boxes into a magnetic stand for washing the protein G-coated magnetic beads.

Are you Ready-to-ChIP?

For your convenience, Active Motif now offers Ready-to-ChIP Chromatin. Combined with our controls, this ensures that all components are confirmed to work in ChIP, so you can more easily validate your own antibodies and primer sets. Ready-to-ChIP Chromatin has been optimally sheared by sonication and validated in ChIP. It can be used in conjunction with all of the ChIP-IT Kits, so you can be certain the only variable in testing an antibody for ChIP is the antibody itself.

Get started today

Active Motif's ChIP-IT products provide you with everything you need to make your ChIP experiments easier and more reproducible. No ChIP is faster than ChIP-IT Express, and the validated ChIP-IT controls and reagents help ensure your success. For more complete information, please give us a call or visit us online at www.activemotif.com/chip.

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
Ready-to-ChIP HeLa Chromatin	10 rxns	53015

TransAM™: The Simple Way to Assay Transcription Factor Activation

Simplify the study of activated transcription factors with the combined convenience and increased sensitivity of Active Motif's non-radioactive, ELISA-based TransAM™ assays.

What is TransAM?

Active Motif's TransAM Kits are simple DNA-binding ELISA-based assays that facilitate the study of transcription factor activation in mammalian tissue and cell culture extracts. The TransAM method is fast, delivering results in less than 5 hours, and it is up to 100-fold more sensitive than classic EMSA, or gelshift, techniques.

How does TransAM work?

Commonly, transcription factors are activated by phosphorylation as a result of a cell-signaling cascade. Upon activation, transcription factors bind to their DNA targets to regulate gene expression. TransAM Kits operate on this principle by using a 96-well plate in which multiple copies of a double-stranded oligonucleotide that contains a specific target-binding sequence are immobilized. When nuclear or whole-cell extracts are added to the plate, the activated transcription factor of interest binds its consensus sequence on the plate-bound oligonucleotide. Next, a primary antibody specific to the transcription factor is added. This is detected by incubation with a secondary HRP-conjugated antibody and developing reagent. Colorimetric changes are measured by a spectrophotometer; with the sensitive chemiluminescent kits, results are read with a luminometer. The readout provides a sensitive measure of the activated transcription factor.

How is TransAM an improvement over other methods?

Two commonly used methods to study transcription factor activation are gelshift/EMSA and reporter gene assays. TransAM has a number of advantages over these procedures. With respect to gelshift/EMSA, TransAM assays are 10-fold more sensitive so you get more accurate results while using less sample. TransAM eliminates the use of radioactivity as well as the need to run, blot, expose and develop gels. This saves you a considerable amount of time; TransAM is complete in less than 5 hours, while gelshift takes days. In addition, data from TransAM is easier to interpret and compare than gelshift results (Figure 1). TransAM also overcomes the problems associated with the inconsistency of reporter gene assays. Because transfection is a variable process and gene expression is subject to many confounding factors, reporter assays must be repeated many times to obtain statistically reliable data. And, unlike reporter assays, TransAM can also be used with tissue samples.

What types of samples can be assayed with TransAM?

TransAM assays are typically used with nuclear extracts. Most commonly, extracts are made from cells in their normal state as well as after stimulation to activate the targeted transcription factor; the results are compared to determine the effect of the treatment on transcription activation. For some transcription factors, such as NFκB, whole-cell extract can be used.

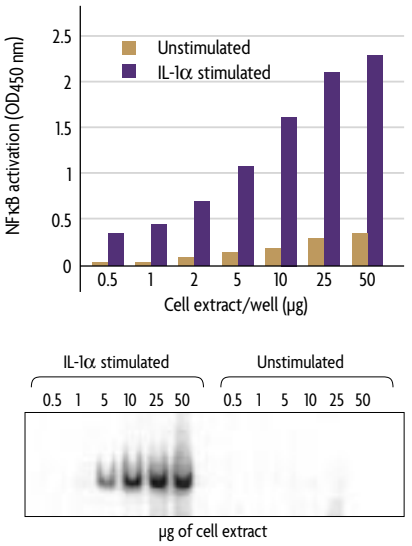

Figure 1: TransAM assays are more sensitive than gelshift and provide quantitative results.
Human fibroblast WI-38 cells are stimulated with IL-1α for 30 minutes. Increasing amounts of whole-cell extract are assayed using the TransAM NFκB p50 Kit (A) or gel retardation (B).

Which transcription factors can be studied with TransAM?

Active Motif has developed a TransAM Kits to study over 30 individual transcription factors, as well as kits designed to study transcription factor families. TransAM Family Kits contain multiple antibodies so that you can simultaneously profile the activation levels of different family members. TransAM Flexi Kits provide you with optimized reagents, but give you the flexibility to use your own oligonucleotide, enabling you to study alternative binding sites. For more information complete about the TransAM Kits, please give us a call or visit our website at www.activemotif.com/transam.

Simple Analysis of DNA Methylation

Active Motif's MethylDetector™ Bisulfite Modification Kit provides an efficient method for DNA methylation analysis by combining optimized reagents for performing DNA conversion with time-saving DNA purification columns, as well as positive control PCR primers to validate your results.

Proven controls ensure success

DNA methylation analysis frequently begins by treating DNA with bisulfite to convert unmethylated cytosines to uracils while leaving methylated cytosines unchanged. The bisulfite-treated DNA is then amplified by PCR and analyzed by sequencing or restriction digest. However, the conversion process can be technically challenging. To help, MethylDetector provides optimized conversion reagents that enable a streamlined protocol. Positive control PCR primers that are specific for bisulfite-converted DNA enable you to confirm that the conversion step was successful before beginning costly downstream sample analysis, saving you time, effort and materials (Figure 1).

MethylDetector advantages

- **Flexible** – works with high G/C content sequences and uncut DNA
- **Consistent results** – 99% conversion efficiency
- **Proven reagents** – optimized reagents, simple protocol and positive controls
- **High yield** – plenty of converted DNA for further analysis

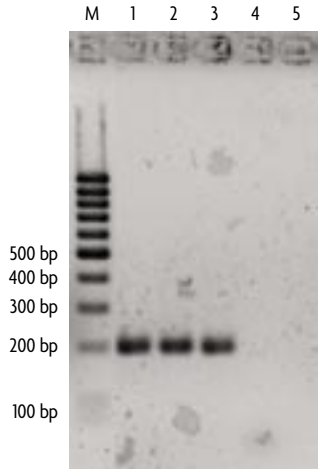

Figure 1: Agarose gel analysis of PCR products generated with MethylDetector.
Three different DNA conversions were performed (Lanes 1-3) and compared to an unconverted DNA control (Lane 5) and to a no DNA control (Lane 4). The presence of PCR product in only the converted samples demonstrates the conversion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

Product	Format	Catalog No.
MethylDetector™	50 rxns	55001

8 U.S.
Toll Free: 877 222 9543
Direct: 760 431 1263
Fax: 760 431 1351
Email: sales@activemotif.com

THE NEWSLETTER OF ACTIVE MOTIF — November 2006 • volume 7 • number 4

ACTIVE MOTIF®
Tools to Analyze Cellular Function