MOTIF VATIONS

ACTIVE MOTIF®

Innovative Tools for Cell & Molecular Biology

THE NEWSLETTER OF ACTIVE MOTIF — OCTOBER 2004 • VOLUME 5 • NUMBER 4

Profile Transcription Factor Phosphorylation Using New FACE™ NFκB p65 Profiler Kits

Active Motif's new FACE™ (Fast Activated Cell-based ELISA) NFκB p65 Profiler Kit makes it possible to rapidly profile the levels of three different phosphorylation sites of the transcription factor NFκB p65 in one simple experiment. Plus, the kit provides an antibody specific for the total form of the protein, enabling you to compare phosphorylated to native protein levels (Figure 1). Because FACE Kits are cell-based ELISAs, you grow, fix and assay your cells all in the same plate, eliminating the need to make extracts, run gels or perform membrane blotting. This makes FACE Kits faster, more sensitive and easier to use than other techniques, like Western blot, in-gel kinase and traditional Sandwich ELISAs.

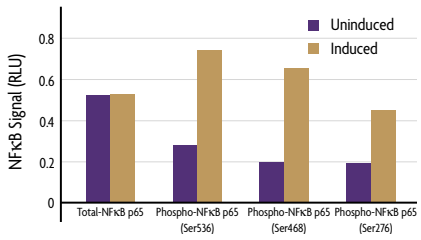

Figure 1: Monitoring phosphorylation of NFκB p65 at multiple sites. The FACE NFκB p65 Profiler Kit was used to assay levels of total and phosphorylated NFκB p65 in uninduced and TNF-α + Calyculin A induced HeLa cells. Data was plotted after correction for cell number (performed through use of the kit's Crystal Violet reagent).

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Nuclear Receptor Measurement Made Easy

Active Motif offers a variety of nuclear receptor analysis tools that make studying the activation of nuclear receptor proteins both faster and more accurate than using traditional technologies. Whether you're interested in DNA binding activity, activation state, protein level or agonist/antagonist effects, Active Motif has the tool for you.

Monitor tissue specific effects with NR Peptide ELISAs

Upon ligand-receptor binding, nuclear receptors undergo a conformational change, which can result in recruitment of co-activators (agonist) or co-repressors (antagonist). Depending on the nuclear receptor and the tissue location, a ligand

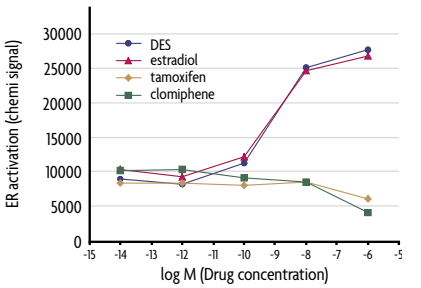

Figure 2: ERα agonism/antagonism dose-response curves. Nuclear extracts from the breast cancer cell line MCF-7 are incubated in wells of the NR Peptide ELISA ER plate in the presence of 100-fold serial dilutions (from 10^{-6} to 10^{-14} M) of the agonist compounds diethylstilbestrol (DES) and estradiol, and the antagonist compounds tamoxifen and clomiphene. Only ligand-activated ER protein can bind to the Capture Peptide immobilized in the plate. Bound ER is specifically detected with ERα antibody and quantified using a secondary antibody and Detection Solution.

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IN THIS ISSUE

Complete Kit & ChIP-validated Antibodies Improve Chromatin Immunoprecipitation

Chromatin immunoprecipitation (ChIP) is a powerful tool for analyzing genome regulation because it combines the specificity of immunoprecipitation with the sensitivity of PCR. However, ChIP is a complicated, multi-step procedure that can be technically challenging and yield results that are difficult to interpret. To overcome these problems, Active Motif developed its ChIP-IT™ Kit. ChIP-IT is a complete solution that contains all of the reagents required for DNA shearing, "pull down" and purification. Plus, the kit includes positive and negative control antibodies and PCR primers that help you interpret your results. With ChIP-IT, you get a complete package of high-quality, optimized reagents that make it possible to successfully obtain and validate your results. In addition, Active Motif offers a number of antibodies that have been tested and shown to work successfully with ChIP-IT (see table). To get the best results possible, while saving time and money, try ChIP-IT and our ChIP-validated antibodies.

Powerful technique

The ChIP technique is used because it can identify which DNA fragments are bound by a particular protein under specific conditions. A gene's promoter region typically contains multiple known or putative transcription factor binding sites that are involved in regulating the gene's expression. Determining if, when and where a specific transcription factor (or other protein) binds to the DNA is an important part of understanding the gene's regulation.

The ChIP process

In the ChIP process, cells are fixed with formaldehyde, which cross-links and therefore preserves the *in vivo* protein/DNA interactions. The DNA is then sonicated into small, uniform fragments and the protein/DNA complexes are immunoprecipitated using an antibody directed against the DNA-binding protein of interest. Following immunoprecipitation, the cross-links are reversed and the DNA is screened to determine which gene or groups of genes were bound by the protein (Figure 1).

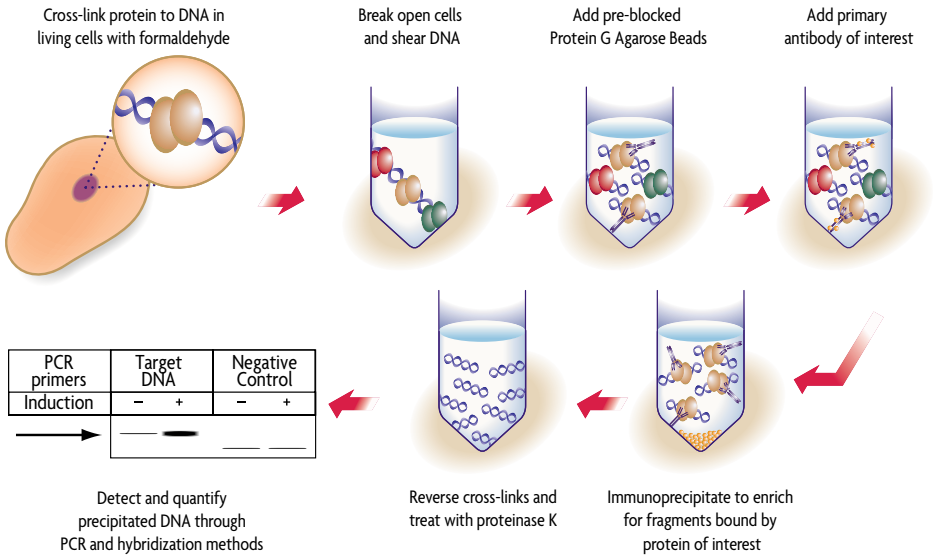

Figure 1: Schematic of chromatin immunoprecipitation. In ChIP, protein/DNA interactions are fixed, and the DNA is then sheared and precipitated using an antibody. After reversing the cross-links, the DNA is purified and then screened to determine which genes were bound by the protein of interest.

ChIP-validated antibodies available

AML1/Runx1	C/EBPβ	HDAC4	p53
AP-2	E2F-1	HDAC5	Pax-5
ATF-6	E2F-6	HDAC6	Sp1
c-Jun	GATA-1	IRF-3	
C/EBPα	HDAC3	JunB	

ChIP-IT advantages

- Complete solution for ChIP – all critical reagents and controls are supplied
- Direct measurement of transcription factor/DNA interactions or histone modifications
- Compatible with genome-wide profiling or selective PCR-based screening
- No need to optimize your own reagents and protocol

Ensure your success

ChIP-IT simplifies all aspects of chromatin immunoprecipitation by providing nearly everything you'll need to prepare chromatin, optimize shearing conditions, perform ChIP reactions and analyze ChIP results by PCR. All buffers (excluding formaldehyde) required for cell fixation, nuclei purification and chromatin shearing are included. Not only does this help ensure your results, but you won't have to spend hours preparing and optimizing your own buffers and protocols. Most importantly, the ChIP-IT Kit provides positive and negative control antibodies and PCR primer sets. These help to improve result interpretation and also make it easy for you to validate that your own antibodies and primer sets function in ChIP. No other kit or "home grown" method provides such a complete, convenient solution. For worry-free ChIP, use our ChIP-IT Kit and ChIP-validated antibodies.

Product	Format	Catalog No.
ChIP-IT™ Kit	25 rxns	53001

Sensitive, Specific Activation Assays for GATA-4, GR, Oct-4 and Many More

Active Motif's TransAM™ Kits are DNA-binding ELISAs* that facilitate the study of transcription factor activation in mammalian tissue and cell culture extracts. The method is faster and more sensitive than other commonly used methods, while eliminating the use of radioactivity. Over 30 different TransAM Kits are available for studying both individual transcription factors as well as entire transcription factor families. We are pleased this month to announce the introduction of 3 new TransAM Kits for assaying GATA-4, GR and Oct-4.

Simple, specific assay

Transcription factors bind to their specific DNA target(s) after they've been activated, commonly by phosphorylation. TransAM Kits take advantage of this property by supplying a 96-well plate in which multiple copies of a double-stranded oligonucleotide are immobilized. When sample is added, the activated transcription factor binds to the oligonucleotide at its consensus-binding sequence. Primary antibody directed against the bound form of the transcription factor is then added, followed by an HRP-conjugated secondary antibody (Figure 1). The colorimetric change is measured with a spectrophotometer, which provides a sensitive, quantitative measurement of the activated transcription factor (Figure 2). Each kit also contains wild-type and mutated competitor oligonucleotides that can be used to verify the specificity of the assay (Figure 3, page 6).

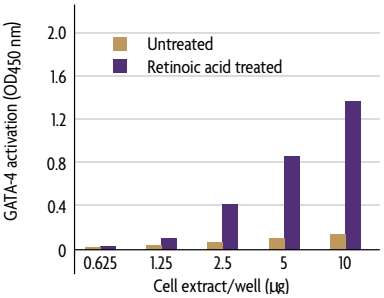

Figure 2: Quantitative measurement of GATA-4.
F9 cells are treated with retinoic acid and increasing amounts of nuclear extract from untreated and treated cells are assayed using the TransAM GATA-4 Kit.

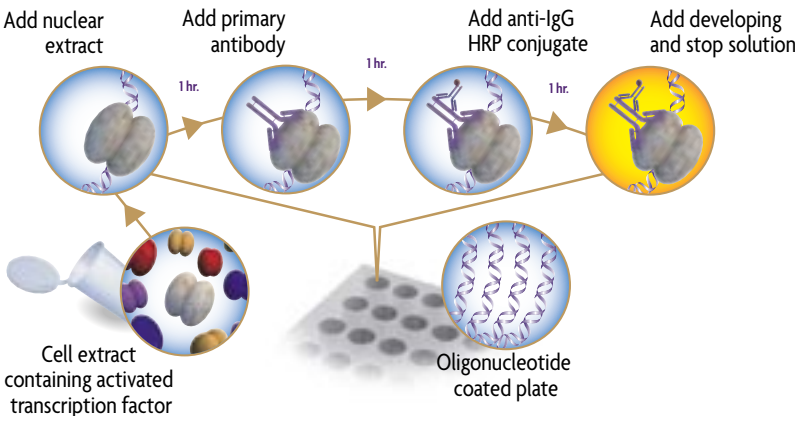

Figure 1: Flowchart of the TransAM procedure.

Improved method

TransAM assays are a marked improvement over other techniques used to study transcription factor activation. Unlike gelshift, Western blotting and reporter gene methods, TransAM does not use inefficient cloning and cell transfections, time-consuming gel exposures or radioactive probes. Inconsistencies due to variable reporter plasmid construction and the need to construct stable cell lines are also eliminated. TransAM assays are complete in less than 5 hours, are more sensitive and provide quantitative results. Moreover, TransAM can be used to study stimulated tissue samples.

TransAM assay formats

TransAM Kits are offered in a variety of formats – individual kits assay single transcription factors, while Family Kits make it possible to profile multiple members of a transcription factor family. And, TransAM Chemi Kits utilize a luminometer to provide the maximum sensitivity possible.

TransAM advantages

- Non-radioactive, colorimetric method provides quantifiable results
- Results in less than 5 hours
- 10-fold greater sensitivity than gelshift
- Simultaneous profiling of multiple family members
- Ability to assay cell and tissue samples

Complete solution for activation assays

TransAM Kits improve transcription factor activation research because they are faster, more sensitive and easier to use than existing methods. The new GATA-4, GR and Oct-4 Kits join an already extensive TransAM product line, including assays for NFκB p50 and p65, HIF-1, STAT3, c-Jun, as well as our popular Family Kits. Please visit www.activemotif.com/transam to learn which assays are available, and to download TransAM manuals and a list of publications that cite the use of TransAM. For a better method for studying transcription factor activation, try TransAM today.

* Technology covered under AAT-filed patents licensed to Active Motif.

Product	Format	Catalog No.
TransAM™ GATA-4	1 x 96-well plate 5 x 96-well plates	46496 46996
TransAM™ GR	1 x 96-well plate 5 x 96-well plates	45496 45996
TransAM™ Oct-4	1 x 96-well plate 5 x 96-well plates	42496 42996

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NFκB phosphorylation

NFκB p65 plays a pivotal role in the regulation of inflammatory and immune responses. During phosphorylation and degradation of IκBs, NFκB p65 is phosphorylated on multiple residues, each triggered by different stimuli but essential for maintaining NFκB transcriptional activation (Figure 2). Despite the widespread interest in the study of NFκB, there is a lack of assays available that are suitable for monitoring NFκB phosphorylation in living cells. That's why Active Motif has developed the new FACE Profiler Kits. The FACE NFκB p65 Profiler Kit contains antibodies specific for three phosphorylated sites: Serine 276, Serine 468 and Serine 536, which enables you to screen these important sites of NFκB p65 regulation within a single assay.

The FACE Method

In the FACE method, cells are cultured in 96-well plates and stimulated to induce the pathway of interest (Figure 3). Following stimulation, the cells are fixed, which preserves protein modifications, including phosphorylation. Each well is then incubated with a primary antibody specific for the activated protein of interest. Subsequent incubation with secondary HRP-conjugated antibody and developing solution provides an easily quantified, colorimetric or chemiluminescent readout. The signals can then be normalized for cell number using the provided Crystal Violet solution and plotted (Figure 1, page 1). The entire assay is complete in about two hours of actual hands on time – nothing could be simpler.

Two types of detection

For added convenience, all of our FACE Kits are available in both colorimetric and chemiluminescent formats. The colorimetric kits detect phosphorylated and total protein levels using an HRP-

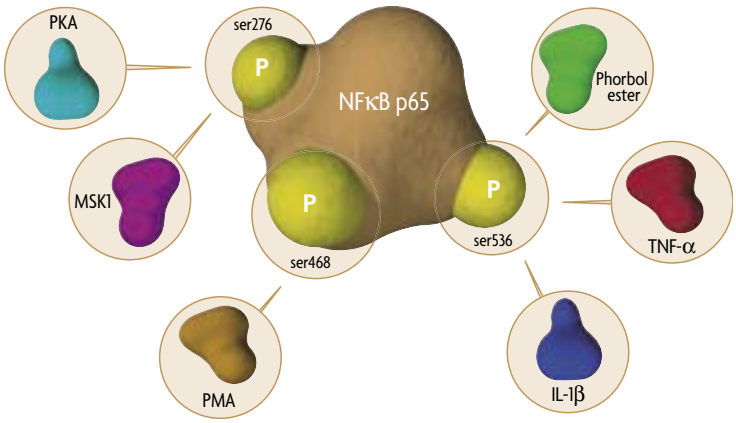

Figure 2: Multiple phosphorylation sites on NFκB p65.
Transcriptional regulation of NFκB p65 is dependent on the phosphorylation of multiple residues and is controlled by a variety of stimuli.

colorimetric signal at a wavelength of 450 nm and a standard ELISA-plate reader. If you require maximum sensitivity, take advantage of the FACE Chemi Kits. These kits use chemiluminescent detection on a luminometer to accurately monitor even the slightest changes in protein phosphorylation.

The FACE Family

FACE Kits provide you with a simple, efficient, quantitative method to monitor proteins activated by phosphorylation. No other phospho-specific assay available offers you such a quick and convenient solution. Currently, Active Motif has developed a number of FACE Kits to study MAPKs, transcription factors and receptors (see table below). These kits are available for studying single phosphorylation sites as well as multiple sites as in the new FACE Profiler Kits. As we are constantly adding to the FACE product line, be sure to give us a call or check our website at www.activemotif.com/face to find out about new additions to this innovative product line. To get to the best phospho-specific assay available, order a FACE Kit.

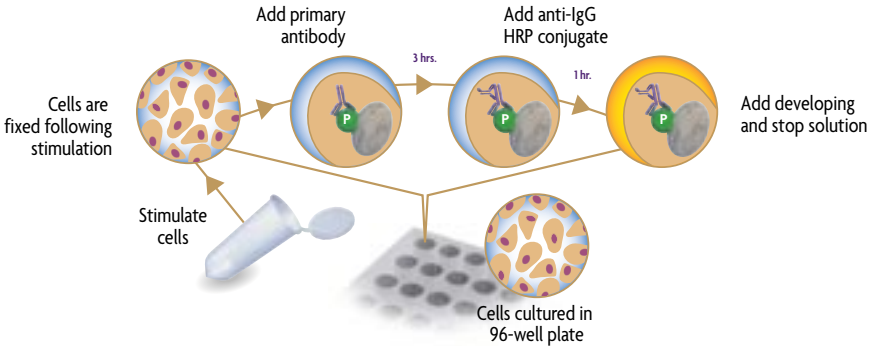

Figure 3: Flowchart of the FACE process.
Cells are grown, stimulated and fixed in the same 96-well plate. Addition of primary and secondary antibodies detects phosphorylated or total protein.

FACE Kits available

FACE™ AKT	FACE™ c-Jun	FACE™ ErbB-2	FACE™ FKHR (FOXO1)	FACE™ JNK	FACE™ p38
FACE™ ATF-2	FACE™ c-Src	FACE™ ERK1/2	FACE™ GSK3β	FACE™ MEK1/2	FACE™ PI3 Kinase
FACE™ Bad	FACE™ EGFR	FACE™ FAK	FACE™ JAK1	FACE™ NFκB p65 Profiler	

Accurate Monitoring of Apoptosis Using Mitochondrial Cytochrome c Levels

The FunctionELISA™ Cytochrome c Kit makes measuring cytoplasmic and mitochondrial cytochrome c levels both fast and accurate. Unlike other techniques used for studying proteins, such as Western blotting, FunctionELISA Cytochrome c is a sandwich ELISA-based approach that provides quantitative results in a few hours.

Sensitivity counts
Though it can be induced by a wide variety of events, once the cycle that leads to apoptosis begins, cytochrome c is released from the mitochondria into the cytoplasm within one hour. Therefore, assaying the cytochrome c levels in mitochondrial and cytosolic fractions is an extremely useful way to monitor apoptosis. FunctionELISA Cytochrome c simplifies this task by providing everything you need to perform sensitive, quantitative measurement of your samples and generate standard curves (Figure 1).

Convenient format saves time
For maximum convenience, the Capture Antibody in FunctionELISA Kits is supplied already immobilized to the wells of the 96-well assay plate. This makes it easy to process multiple samples quickly. Plus, it saves you the time and inconvenience of performing an overnight incubation to attach the antibody yourself.

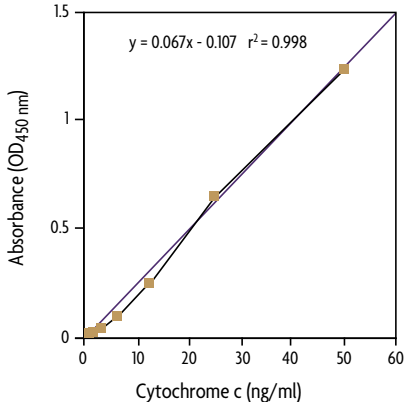

Figure 1: Cytochrome c Standard Curve.
Increasing amounts of recombinant human cytochrome c (0.78-50 ng) were assayed using the FunctionELISA Cytochrome c Kit.

Complete kit ensures reproducibility
FunctionELISA Cytochrome c contains all of the reagents required, including recombinant cytochrome c protein, to quickly quantify cytochrome c in your sample. For accurate quantification of cytochrome c, try the FunctionELISA Cytochrome c Kit.

Start your experiments out right
To add to the effectiveness of the FunctionELISA Cytochrome c, Active Motif recommends its Mitochondrial Fractionation Kit (see below). Use of this kit helps increase the accuracy of your results by ensuring that your mitochondrial and cytosolic fractions are properly segregated (Figure 2).

Product	Format	Catalog No.
FunctionELISA™ Cytochrome c	1 x 96 well-plate	48006
	5 x 96 well-plates	48506

Reproducible Isolation of Mitochondrial and Cytoplasmic Fractions

The Mitochondrial Fractionation Kit isolates highly enriched mitochondrial and cytosolic fractions from mammalian cell lines. This simplifies the study of protein translocation events that occur during apoptosis and in many other signal transduction pathways. The kit's high-quality reagents and optimized protocol eliminate cross-contamination and produce high yields of properly segregated mitochondrial and cytosolic fractions.

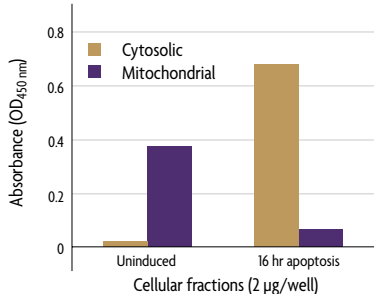

Figure 2: Monitoring cytosolic and mitochondrial cytochrome c.
HeLa cells were grown to 90-95% confluence and treated with 10 nM Actinomycin D to induce apoptosis. Cell samples were harvested prior to treatment as well as 16 hours post-treatment. The Mitochondrial Fractionation Kit was used to isolate cytosolic and mitochondrial fractions from each sample. Two µg of each fraction was assayed using the FunctionELISA Cytochrome c Kit to monitor changes in the mitochondrial and cytosolic cytochrome c levels.

Precise fractionation
To demonstrate its utility, Active Motif's Mitochondrial Fractionation Kit was used to isolate mitochondrial and cytosolic fractions from both uninduced and apoptotic HeLa cells. The FunctionELISA™ Cytochrome c Kit (see above) was used to determine the levels of cytochrome c in the mitochondrial and cytosolic fractions because the release of cytochrome c from mitochondria is a key indicator of apoptosis. As expected, nearly all cytochrome c in uninduced cells was found in the mitochondrial fraction, while nearly all the cytochrome c in the apoptotic sample was in the cytosol (Figure 2).

Upstream quality for downstream success
Using the Mitochondrial Fractionation Kit, with its optimized protocol and unique formulation of buffers, you'll be certain to get the specifically segregated fractions you need. For more consistent results, try the Mitochondrial Fractionation Kit.

Product	Format	Catalog No.
Mitochondrial Fractionation Kit	100 rxns	40015

continued from page 1 — Nuclear Receptor Measurement Made Easy

may have both agonist and antagonist properties, resulting in different biological outcomes. As such, studying the agonist/antagonist effects of target compounds is an important element of nuclear receptor-targeted drug discovery. Active Motif's NR Peptide ELISAs are specifically designed to capture ligand-activated nuclear receptor and can be used with both cellular extracts and recombinant proteins. Each NR Peptide ELISA Kit provides a 96-well plate that is coated with a Capture Peptide that includes the consensus-binding motif of the nuclear receptor's co-activator. Addition of sample results in binding of ligand-activated nuclear receptor to the Capture Peptide. Each well is then incubated with a primary antibody specific for the nuclear receptor of interest. Subsequent incubation with HRP-conjugated secondary antibody and developing solution provides an easily quantified readout (see Figure 2, page 1).

Perfect method for NR biomarker analysis
The ability to regulate nuclear receptor protein activity via addition of an inducing ligand makes nuclear receptors promising targets for development of improved therapeutic agents. However, one challenge of the drug discovery process is the identification of an appropriate biomarker for monitoring drug efficacy. Because the end point of nuclear receptor activation is DNA-binding, monitoring changes in DNA-binding activity of a target nuclear receptor can serve as an ideal biomarker. Classical methods such as radioactive electrophoretic mobility shift assays (EMSA) and time-consuming reporter gene assays are not well suited to this application. Active Motif's TransAM™ Kits provide an innovative alternative to these classical assays. TransAM Kits use a combination of DNA binding and antibody detection to give a specific, quantitative readout of DNA-binding activity from all sample types (Figure 3). To learn more about the TransAM method and the many different kits available for quantifying DNA-binding activity of various nuclear receptor and transcription factor proteins, please turn to page 3.

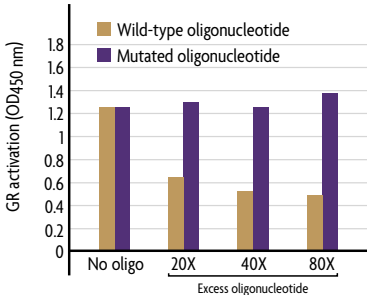

Figure 3: The TransAM method is specific.
Ten µg of nuclear extract from HeLa cells stimulated with 100 nM Dexamethasone are assayed using TransAM GR in the absence or presence of oligonucleotides containing either the wild-type or mutated consensus-binding site of GR. The inability of the mutated competitor oligonucleotides to reduce the signal demonstrates that the assay is specific for activated GR.

NR antibodies for all applications
Active Motif's extensive line of antibodies provide superior performance and reliable results. We offer over 200 highly characterized antibodies directed against transcription factors and nuclear receptors. Antibodies for GR, PPARα and γ, PXR, RAR-β, -β2 and -γ, RXR-α and -β, and VDR are all available, with more being added all the time. These mono- and polyclonal antibodies are suitable for a variety of applications including Western blotting, EMSA and chromatin immunoprecipitation. Check out our online search tool at www.activemotif.com to see if we have the antibody you need.

Extensive line of recombinant NR proteins
Active Motif's growing line of recombinant proteins includes an increasing number of recombinant nuclear receptor proteins. Our recombinant proteins are ideal for use as positive controls, in *in vitro* screening studies and in many other applications. For more details, see page 12 or go to our website at www.activemotif.com to download complete information.

Coming soon....Simple, fast measurement of nuclear receptor proteins
The new NR ELISAs offer a simple, rapid method to monitor changes in nuclear receptor proteins. NR ELISAs utilize a Sandwich ELISA-based method that is an improvement over other methods used to study proteins, such as Western blotting. Using NR ELISAs means that there is no need for gels, blotting or long, tedious incubations. The 96-well format is convenient and sensitive, with only a minimal amount of material required to give a quantitative readout of nuclear receptor levels. NR ELISAs are ideal for use on clinical material and will initially be available for ERα, PR and AR, with many more to be added over the upcoming months. Send in the enclosed reply card or give us a call, and we'll be happy to keep you updated of new NR ELISAs as they become available.

For a complete line of nuclear receptor analysis tools, there's no need to look further than Active Motif!

Product	Format	Catalog No.
NR ERα Peptide ELISA	1 x 96-well plate 5 x 96-well plates	49096 49596
NR ERα Peptide ELISA Chemi	1 x 96-well plate 5 x 96-well plates	49097 49597
TransAM™ ER	1 x 96-well plate 5 x 96-well plates	41396 41996
TransAM™ GR	1 x 96-well plate 5 x 96-well plates	45496 45996
TransAM™ PPARγ	1 x 96-well plate 5 x 96-well plates	40196 40696

Deliver Active Proteins Directly Into Mammalian Cells

The Chariot™ delivery reagent efficiently transports biologically active proteins, peptides and antibodies directly into cultured mammalian cells. Delivery is complete in less than two hours and provides efficiencies of 65-95%. After delivery, living cells can be assayed immediately to determine the effects of the introduced material. These features make Chariot the ideal tool for a variety of functional studies.

Targeted delivery

Chariot is a peptide that forms a non-covalent complex when incubated with your purified protein, peptide or antibody for 30 minutes at room temperature. Addition of the complex to cells results in its rapid internalization. Once inside the cell, the complex dissociates and Chariot is transported to the nucleus, while the delivered protein is biologically active and free to proceed to its cellular target (Figure 1).

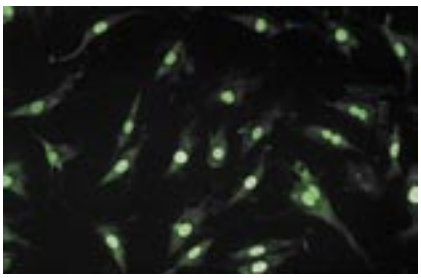

Figure 1: Targeted protein delivery.
50 ng of a 10 kDa nuclear protein labeled with Lucifer yellow at the C-terminus was complexed with Chariot and delivered into HS-68 cells. Unfixed cells were observed 90 minutes post-delivery.

Non-covalent delivery

Many protein delivery systems require that you begin by fusing a carrier protein to your macromolecule. However, this can change the protein folding characteristics of your protein and, ultimately, its function. Because Chariot forms a non-covalent bond with your protein, it does not affect the delivered protein's folding or function.

Deliver biologically active proteins

The ability of Chariot to deliver biologically active compound was demonstrated using $p27^{kip1}$, a 27 kDa cyclin-dependent kinase inhibitor that causes cell-cycle arrest in G_1 phase. Over 90% of cells receiving a Chariot-$p27^{kip1}$ complex were unable to progress beyond G_1 phase (Figures 2A & 2B), demonstrating the efficient delivery of active $p27^{kip1}$.

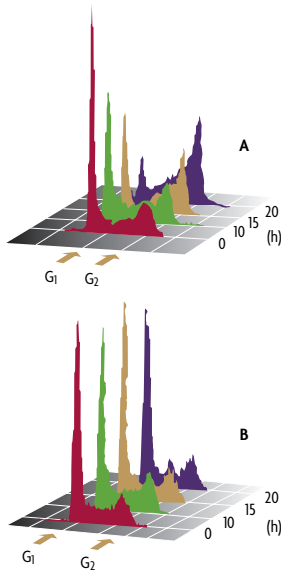

Figure 2: Chariot delivery of functional protein.
HS-68 cells arrested in G_0 phase by serum deprivation for 48 hours were released by addition of serum for 3 hours. Flow cytometry performed 0, 10, 15 and 20 hours after addition of Chariot alone and a Chariot-$p27^{kip1}$ complex indicate that cells receiving Chariot alone progressed into G_2 phase (A), while over 90% of the cells receiving the Chariot-$p27^{kip1}$ complex remained in G_1 phase (B). Data generously provided by Dr. Gilles Divita, Biophysics Dept., CNRS, Montepellier, France.

Deliver large proteins

Chariot can efficiently deliver a broad range of macromolecules directly into mammalian cells and the results can be assayed immediately. To demonstrate, Chariot was used to deliver functional β-galactosidase protein (a tetramer composed of four identical, 119 kDa subunits) into HeLa cells (Figure 3).

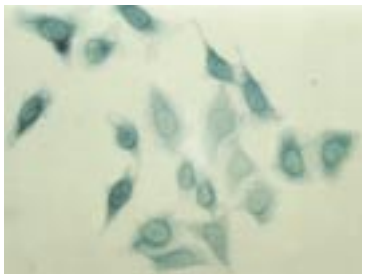

Figure 3: Staining of HeLa cells.
One µg of β-galactosidase protein, which is included with Chariot for use as a positive control, was complexed with Chariot for 30 minutes and delivered into HeLa cells. Cells were fixed and stained 2 hours post-delivery using Active Motif's β-Galactosidase Staining Kit.

Advantages

- Delivers active protein directly into living cells — no fixing needed
- Up to 95% efficiency in less than 2 hours
- Works in a variety of cell lines, as well as *in vivo*

Why use protein delivery?

Direct delivery of active protein makes it easy to perform studies not even possible using DNA transfection and expression. Successful Chariot delivery of proteins, peptides and antibodies has been shown in a wide range of cell lines, including hard-to-transfect neuronal, primary and plant cells. For a list of papers that cite the use of Chariot, simply return the enclosed reply card or download the list at www.activemotif.com/chariot.

Chariot delivers results

Chariot speeds and simplifies a variety of functional studies because it efficiently delivers biologically active proteins, peptides and antibodies directly into mammalian cells, even hard-to-transfect and non-dividing cells. To learn what your protein is *really* doing, study it using Chariot.

Product	Format	Catalog No.
Chariot™	25 rxns*	30025
	100 rxns	30100
β-Galactosidase Staining Kit	75 rxns	35001

* A rxn is defined as sufficient Chariot reagent to deliver protein to cells in a 35 mm plate.

Quantitative DNA Repair ELISAs for GTBP, Ku and RPA

Active Motif's DNA Repair Kits offer a better alternative to Western blots and gelshift assays for the study of DNA repair protein activity because their DNA-binding ELISA format eliminates radioactivity and provides quantitative results in less than five hours. The DNA Repair Kits are 10-fold more sensitive than traditional assays and can be used with both cell lines and tissues, providing you with even more flexibility.

How it works

The DNA Repair Kits utilize the DNA-binding properties of DNA repair proteins to selectively capture activated protein. Each DNA Repair Kit includes a 96-well plate in which multiple copies of a specific oligonucleotide have been immobilized. When cellular extract is added, the repair protein of interest binds to the oligonucleotide on the plate. Each well is then incubated with a primary antibody that is specific for the repair protein being studied. Addition of a secondary HRP-conjugated antibody and developing solution provides an easily quantified colorimetric readout (Figure 1). This allows you to easily monitor the stimulation or deficiency of DNA repair proteins in a particular cell line, animal model or tumor biopsy.

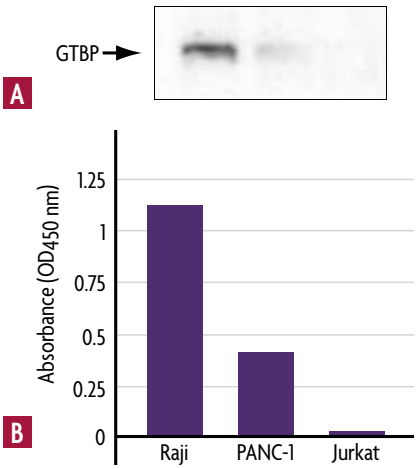

Figure 2: Measurement of GTBP activity in different cell lines using Western blot and the GTBP DNA Repair Kit.
Raji, PANC-1 and Jurkat nuclear extracts were assessed for GTBP activity using Western blot (A) and the GTBP DNA Repair Kit (B).

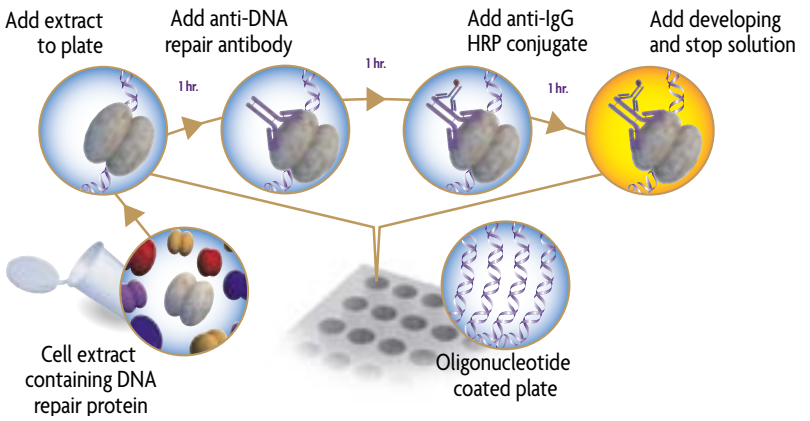

Figure 1: Flowchart of the DNA Repair Kit procedure.

More quantitative results

The ability to distinguish between small changes in DNA repair protein activity is important for understanding DNA damage and repair protein interactions. Assays like Western blots are sensitive yet yield more qualitative rather than quantitative results. Active Motif's DNA Repair Kits provide colorimetric readings that can be easily quantified, enabling the detection of even the slightest change in protein activity. To illustrate, GTBP activity was measured and compared using Active Motif's GTBP DNA Repair Kit and Western blot. The GTBP Kit results are clearly more quantitative than Western blot (Figure 2).

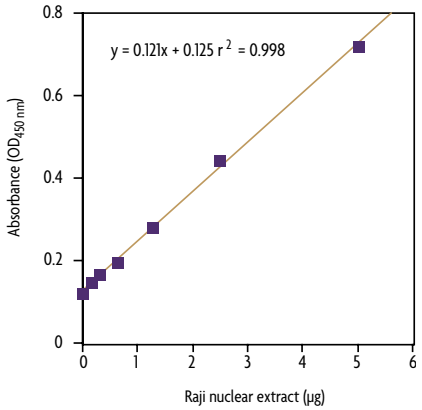

Figure 3: Monitoring Ku70 with the Ku70/86 DNA Repair Kit.
Different amounts of nuclear extract from Raji cells were tested for activity using the Ku70/86 DNA Repair Kit.

Improved accuracy for better results

In addition to their ease-of-use and increased sensitivity, DNA Repair Kits provide more accurate measurements because they use colorimetric detection, which is linear over a wider sample range (Figure 3). This saves you both time and money, as fewer samples will need to be reassayed to obtain accurate results. Get the most out of your experiments by using the DNA Repair Kits.

Try one today

Our DNA Repair Kits will simplify the way you study interactions between damaged DNA and repair proteins. These easy-to-use kits are flexible enough to use in a variety of functional studies, such as examining the specificity of repair proteins for a particular type of DNA damage. Don't waste time with inefficient blotting and radioactivity, try a DNA Repair Kit today.

Product	Format	Catalog No.
GTBP DNA Repair Kit	1 x 96 well-plate 5 x 96 well-plates	51096 51596
Ku70/86 DNA Repair Kit	1 x 96 well-plate 5 x 96 well-plates	51196 51696
RPA DNA Repair Kit	1 x 96 well-plate 5 x 96 well-plates	51296 51796

GeneDetective™ Generates Accurate Models for Exploring Alternatively Spliced Genes

GeneDetective™ provides the computational tools you need for constructing highly accurate gene models, which are necessary for exploring alternatively spliced genes. The system simplifies the development of gene models across entire genomes.

GeneDetective is on the case

GeneDetective elucidates intron/exon structure by comparing your cDNA, EST and protein sequences to genomic DNA. The system's algorithm pipeline reconstructs splice junctions in order to accurately align sequences to genomic regions. It identifies exons as small as 10 bases and can span introns of 100 Kb in order to deliver highly accurate gene models. GeneDetective has demonstrated greater sensitivity[1] than other current gene modeling tools such as GeneWise[2], and is considerably faster than software-only solutions.

GeneDetective advantages

- Easy visualization of intron/exon structure with a zoomable view (Figure 1)
- Rapid searches are processed by the ultra-fast DeCypher Engine™
- Utilizes splice site correction and multiple sequence alignment to identify correct exons
- Produces a predicted transcript for each gene model for further analysis
- Use via web browser or command line client for scripted batch searches
- Easily add additional software modules (BLAST, HMM, Smith-Waterman)

If you're focused on alternatively-spliced genes, GeneDetective's highly accurate gene models make it a valuable tool for your genome exploration studies. The turnkey, single-computer GeneDetective is an ideal solution for cost-effective gene modeling analyses. Give us a call or return the enclosed reply card to learn more.

1. Roland Luethy, Ph.D. (2004) *Gene modeling using amino acid to genomic sequence alignments*, RECOMB poster. View at: www.timelogic.com/downloads/genedetective_poster.pdf

2. Birney *et al.* (2004) *Genome Res.* 14(5):988-995.

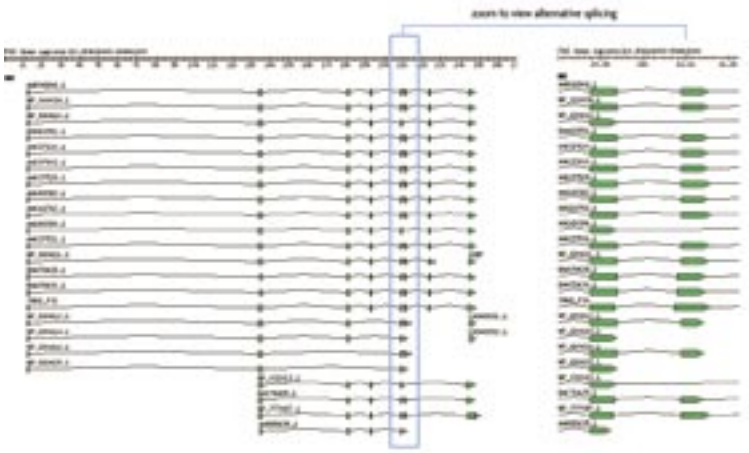

Figure 1: Intron/Exon structure visualization of the human Fatty Acid Synthase (FAS) gene. GeneDetective was used to align human ESTs with genomic regions. Gene models are valuable for identifying exon-specific primers for experimental validation of a gene. Exon-specific oligos can be used to construct microarrays for profiling the expression patterns of alternative splice forms and disease-related polymorphisms.

TimeLogic®
biocomputing solutions

The DeCypher Engine G4 speeds GeneDetective mapping comparisons

GeneDetective is a DeCypher application, so it has the computational horsepower for even large gene modeling projects. When deployed in a single 2-CPU server or workstation, the DeCypher Engine PCI card(s) easily handle the rigorous sequence comparison phase of the gene modeling pipeline. With GeneDetective, you don't need the complication of adding 30-60 computing nodes to undertake extensive annotation projects.

GeneDetective can be expanded with additional software modules for high-throughput BLAST, sensitive Smith-Waterman searches and Hidden Markov Model analysis methods for elucidating protein family (PFAM) and superfamily assignments. An additional DeCypher Engine can be installed in minutes to double your analysis throughput.

Software Module	DeCypher Engines
GeneDetective™ (software plus 1 DeCypher Engine*)	1
BLAST	—
Smith-Waterman	—
Hidden Markov Model (HMM)	—

* Start with GeneDetective software and one DeCypher Engine, then add additional software modules and/or DeCypher Engines as your needs evolve.
** Introductory GeneDetective pricing is valid until December 15, 2004.

Transcription Factor Antibodies for Westerns, EMSA & ChIP

Active Motif's extensive line of antibodies will provide you with superior performance and reliable results. We offer over 200 highly characterized antibodies directed against transcription factors, including members from the AML/Runx, AP-1, Cell Cycle Regulator, NFκB/Rel and STAT families. These mono- and polyclonal antibodies are suitable for a variety of applications including Western blotting, EMSA and chromatin immunoprecipitation.

Western blotting – specific and sensitive
Active Motif's antibodies are guaranteed to provide you with superior data in your Western blotting experiments. Every antibody has been fully characterized using appropriate cellular extracts, and the specificity of each antibody is verified by peptide competition (Figure 1). Each antibody is also accompanied by a detailed technical data sheet that provides specific recommendations on appropriate positive controls, dilutions and incubation conditions. Say goodbye to non-specific bands and endless optimization – get the results you need the first time.

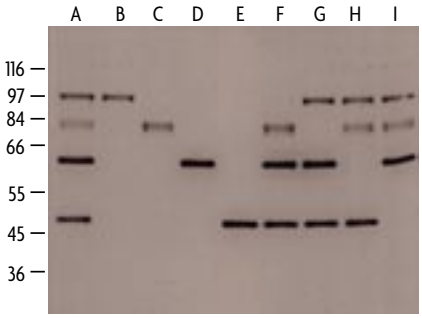

Figure 1: Western blot with Active Motif antibodies.
Using nuclear extract from Raji cells, antibodies for Sp1 (B), c-Rel (C), YY1 (D) and Pax-5 (E) were tested individually or as a mixture (A) to reveal their respective antigens. The mixed antibodies were simultaneously incubated with one peptide at a time for Sp1 (F), c-Rel (G), YY1 (H) and Pax-5 (I), demonstrating specific competition of each antibody by its respective peptide.

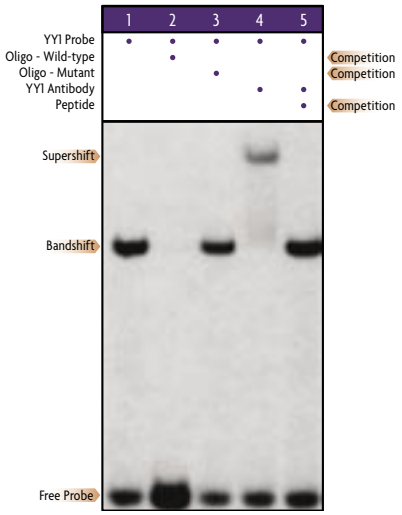

Figure 2: Highly specific YY1 mobility shift.
The Nushift YY1 Kit (Catalog No. 37043) detects YY1 using nuclear extracts prepared from K-562 cells. Specificity is determined by use of the wild-type and mutated competitor oligonucleotides and neutralizing peptide.

Nushift™ – for fail-safe supershifts
Locating commercially available antibodies that have been optimized for performing Supershifts in EMSA can be a lengthy and difficult process. Active Motif has removed the guesswork with its line of Nushift antibodies. These antibodies are specifically formulated for Supershift experiments and are available both individually and as complete Nushift Kits (Figure 2). The Nushift Kits come with everything needed to successfully perform Supershift experiments including the transcription factor antibody of interest, neutralizing peptides (if available), wild-type and mutated oligonucleotides, ready-to-label wild-type oligonucleotide probe, positive control nuclear extract, reaction buffers and G-25 purification columns. Don't waste your time and effort developing and optimizing "home grown" kits; purchase a Nushift Kit for error-free results every time.

Antibodies for ChIP
Chromatin immunoprecipitation is one of proteomics' powerful new tools for analyzing genome regulation (see page 2). However, one of the drawbacks of performing ChIP is finding antibodies that are proven to work in this technique. To help, we are testing most of our antibodies in ChIP to determine which ones function in this procedure. Please see the table on page 2 for a list of ChIP-validated antibodies. As this list will be growing, please be sure to check our website for up-to-date information.

Figure 3: Active Motif's antibody search engine.
Active Motif's online search engines enable you to find antibodies by Catalog No., keyword, isotype, species reactivity and application. You can also generate a list of cell extracts that can be used as a positive control with a particular antibody in Western blotting, gelshift or ChIP.

Find what you need on the web
Finding your antibody of interest and a suitable positive control is easy using our online search tools (Figure 3). You can also download technical data sheets that provide complete information on every antibody and cell extract. With such a large line of specialized antibodies and extracts to choose from, chances are we'll have what you're looking for. Go ahead and test drive our website today.

High-quality Nuclear Extracts

Active Motif offers over 100 different ready-to-use nuclear, whole-cell and cytoplasmic extracts from a variety of cell lines and tissue types (see table). Many are prepared from cells that have been treated to specifically induce activation of hard-to-detect transcription factors. Our extracts are ideal for use as positive controls in Western blot, EMSAs, ELISAs and more. They can also be used as a starting point for isolating and purifying proteins for use in other downstream applications.

Quality is the key
When used as a positive control, quality extracts are the key to ensuring that your applications are working correctly. All Active Motif extracts are tested for protein viability and functionality by various methods, such as Western blot, EMSA or ELISA.

Don't see the extract you need?
Active Motif's Nuclear Extract Kit makes it simple for you to isolate high yields of active nuclear, whole-cell and cytoplasmic extracts from your own mammalian cell and tissue samples. Its use eliminates the need to optimize your own reagents and protocols, so you get error-free results every time.

Nuclear Extract Kit advantages

- Complete kit contains all required reagents
- No need to optimize your procedure
- QC'd reagents ensure reproducibility

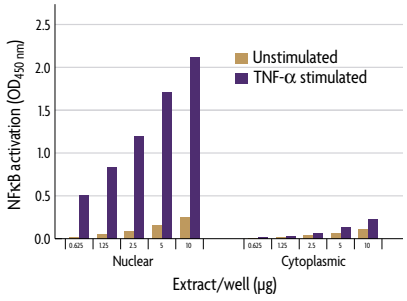

Figure 1: NFκB p50 activity in nuclear and cytoplasmic extracts. Increasing amounts of nuclear and cytoplasmic extracts isolated with the Nuclear Extract Kit are assayed using TransAM™ NFκB p50.

Nuclear, whole-cell & cytoplasmic extracts available			
293	HaCat	MCF-7	R2C
3T3	HeLa	MDA-MB-231	Raji
3T6	HeLa S3	MEL c88	Rat Tissue (various)
624 mel	Hep G2	MG-63	Saos-2
A-431	HL-60	MIA PaCa-2	Schneider's Drosophila L2
AtT-20/D16v-F2	HT-29	Mouse Brain	SK-N-BE (2)
B16	Human Tissue (various)	Nb2	SW48
C2C12	JEG-3	NCI-H441	T-47D
Caco-2	Jurkat	NCI-H446	THP-1
CCF-STTG1	K-562	NCI-H661	U-373 MG
CCRF-CEM	Kelly	NIH/3T3	U-87 MG
COS-7	KG-1	NIH:OVCAR-3	U-937
Daudi	L6E9	P19	WI-38
DU 145	LA-4	PANC-1	Y-1
F9	LNCaP	PC-3	Y-79
GH3	LoVo	PC-12	ZR-75-1

Results you can rely on
Accurate research often depends on the precise isolation of proteins from a specific cellular compartment. If your nuclear extract is contaminated with cytoplasmic proteins, your results may not be valid. The Nuclear Extract Kit helps ensure that proteins remain segregated in their particular fraction. To demonstrate the importance of using a quality extraction procedure, nuclear and cytoplasmic extracts were prepared from unstimulated and TNF-α-stimulated HeLa cells using our Nuclear Extract Kit and assayed for activated NFκB p50 using our TransAM™ NFκB p50 DNA-binding assay (Figure 1). As activated NFκB is not present in the cytoplasm, only nuclear extract from the stimulated cells should test positive. This is precisely what was seen, demonstrating the specificity of the kit.

Quality in means quality out
Extracts prepared using the Nuclear Extract Kit are ideal for use in any experiment that requires nuclear, whole-cell or cytoplasmic extract. Whether you choose to make your own extracts using our Nuclear Extract Kit or to purchase our ready-to-use extracts, the use of high-quality extracts will improve the accuracy of your downstream experiments. For complete details, visit our website at www.activemotif.com.

Product	Format	Catalog No.
Nuclear Extract Kit	100 rxns 400 rxns	40010 40410
All extracts	200 µg*	**

* Most human, rat and mouse tissue extracts are supplied as 120 µg aliquots.
** Call us or visit our website for complete information on all of our extracts, including catalog numbers and details on growth & stimulation conditions.

Recombinant Proteins for Your Research

Active Motif offers an ever-growing line of recombinant proteins that are ideal for use in many different biological applications, including *in vitro* transcription assays, as EMSA controls, in protein-protein interaction studies and as protein standards in ELISAs. The c-Fos, c-Jun, c-Myc, CREB, NFκB p50, NFκB p65, p53, and Sp1 proteins have been validated for use in making standard curves in our TransAM™ Transcription Factor ELISAs (see page 3). Complete information on the recombinant proteins, including detailed technical data sheets that specify protein length, the species it was produced in, the method used for purification, *etc.*, can be found at www.activemotif.com.

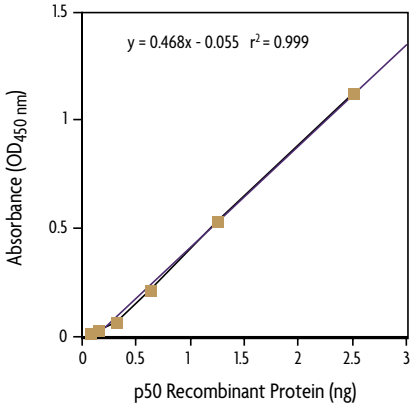

Figure 1: NFκB p50 Standard Curve.
A NFκB p50 standard curve was generated using the recombinant NFκB p50 protein and the TransAM™ NFκB p50 Kit.

Recombinant proteins available			
AKT1	ER	NFκB p65	RARβ
ATF-2	FXR	p53	RARγ
BRCA1	GR	p300	RXRα
c-Fos	IκBα	PPARα	RXRβ
c-Jun	JNK2α1	PPARβ(δ)	RXR-LBD
c-Myc	JNK2α2	PPARγ	Sp1
CREB	LXRα	pRB	STAT1
CTF1 (NF-1)	LXRβ	Rad51	TRα1
eIF2α	NFκB p50	RARα	TRβ1

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