MOTIF VATIONS

ACTIVE MOTIF®

Tools to Analyze Cellular Function

THE NEWSLETTER OF ACTIVE MOTIF — APRIL 2005 • VOLUME 6 • NUMBER 1

New: ELISA-based Method for Fast, Sensitive Quantification of Activated Ras GTPase

Active Motif is pleased to introduce the new Ras GTPase Chemi ELISA Kit. It is the first ELISA-based kit designed to detect and quantify activated Ras GTPase. As an ELISA, it offers a number of advantages over existing methods. The Ras GTPase Chemi ELISA Kit is much more sensitive than pull-down/Western methods, so you can detect even low levels of protein, and use less sample. In addition, ELISAs are more quantitative than Westerns, and they eliminate the need to run and develop gels. This means you'll get better results faster, and with less effort. And, as the assay uses a 96-well plate made up of 12-well strips, it is convenient and economical to run anywhere from 1 to 96 samples in a single experiment.

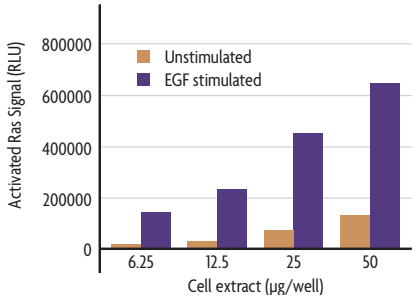

Figure 1: Quantification of activated Ras in stimulated HeLa cells. Increasing amounts of whole-cell extract from HeLa cells that had been stimulated with 50 ng/ml of EGF for 2 minutes were assayed using the Ras GTPase Chemi ELISA Kit.

continued on page 6

New: Measure Transcription Factor Binding at Any DNA Sequence You Choose

Active Motif's new TransAM™ Flexi Kits make it possible to efficiently study the binding of transcription factors at any DNA-binding site you choose. These kits are the latest version of Active Motif's original TransAM Kits, which have rapidly become the method of choice for studying transcription factor binding activity.

New version lets you choose your site

The original TransAM Kits are DNA-binding ELISAs that provide a fast, non-radioactive alternative to classical methods such as Electrophoretic Mobility Shift Assays (EMSAs). Each kit includes a 96-well plate that is coated with a double-stranded oligonucleotide, which contains a specific consensus-binding site. While this format makes it simple and convenient to measure transcription factor binding at this consensus site, it does not allow you to study binding of the transcription factor at alternative sites, such as those which contain mutations or that are putative, unproven binding sites. The new TransAM Flexi Kits give you the flexibility to immobilize any oligo or PCR product in the 96-well plate, while providing you with proven antibodies and all of the other components you'll need to quantitatively measure transcription factor binding. With TransAM Flexi Kits, you can now study variant transcription factor-binding sites, analyze native promoters, confirm chromatin immunoprecipitation (ChIP) results and determine isoform-binding affinity.

IN THIS ISSUE

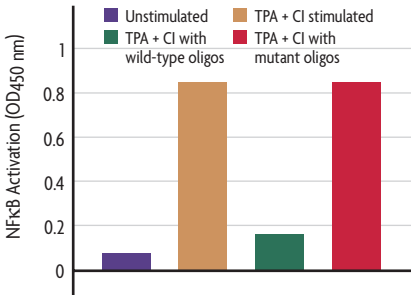

Figure 2: Specificity of TransAM Flexi assays. TransAM Flexi NFκB p65 assays are performed in the presence of wild-type and mutant competitor oligonucleotides using 5 µg/well of nuclear extract from Jurkat cells that were stimulated with TPA and calcium ionophore (CI).

continued on page 4

New: Simplify Chromatin Shearing Using the Enzymatic Shearing Kit for ChIP

Active Motif is pleased to announce the release of another innovative product that simplifies your chromatin immunoprecipitation (ChIP) experiments. The new Enzymatic Shearing Kit is your best bet for generating chromatin suitable for ChIP as well as other applications that study chromatin structure with little to no optimization required – nothing could be simpler.

Successful shearing every time

In order to perform a successful ChIP experiment, chromatin must first be sheared to 200-1000 bp fragments. Traditionally, shearing has been performed by subjecting the isolated chromatin to different pulses of sonication. Although sonication can be an effective method for shearing DNA, it can also be time consuming and difficult to optimize due to complications arising from emulsification and overheating during the sonication process. Also, because the quality of your sheared sample depends greatly upon the quality of your sonicator, it may be necessary to purchase an expensive, "high-end" sonicator to get reproducible shearing. Because of this, Active Motif has developed the Enzymatic Shearing Kit, which is a more robust and user-friendly method to shear chromatin for ChIP. The Enzymatic Shearing Kit uses a proprietary enzymatic shearing cocktail that quickly and easily shears DNA into 200-1000 bp fragments (Figure 1). And, because enzymatic shearing is dependent only on time and temperature, all of the problems associated with sonication are eliminated and ChIP results are improved.

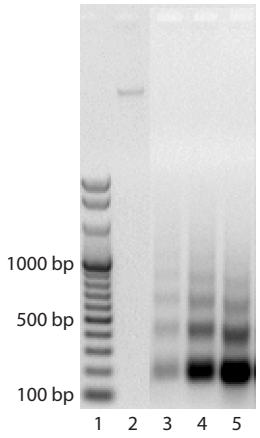

Figure 1: Analysis of DNA sheared using the Enzymatic Shearing Kit. HeLa cells were fixed for 10 minutes with 1% formaldehyde and then chromatin was prepared using the Enzymatic Shearing Kit protocol. Chromatin was sheared with the Enzymatic Shearing cocktail for 5, 10 & 15 minutes and the reaction was stopped. The sheared and unsheared chromatin samples were subjected to cross-link reversal, treated with Proteinase K, phenol/chloroform extracted and precipitated as described in the protocol. Samples were separated by electrophoresis through a 1% agarose gel to assess shearing results:

Lane 1: 100 to 1000 bp ladder.
Lane 2: Unsheared HeLa DNA.
Lane 3: HeLa DNA treated for 5 minutes.
Lane 4: HeLa DNA treated for 10 minutes.
Lane 5: HeLa DNA treated for 15 minutes.

How it works

Shearing chromatin with the Enzymatic Shearing Kit is simple to perform. First, cells are grown to confluency and then fixed using formaldehyde, which cross-links and therefore preserves protein/DNA interactions. A Glycine "Stop-Fix" buffer is then added to stop the fixation procedure and prevent over-crosslinking of the sample. Next, the cells are lysed and the nuclei are collected. A simple, 10-minute incubation with the enzymatic cocktail is then performed, the reaction is stopped and the chromatin is ready to use.

How can I get one

The Enzymatic Shearing Kit is sold separately and can be used in conjunction with Active Motif's ChIP-IT™ Kit buffers and controls. Order an Enzymatic Shearing Kit today and simplify your shearing.

Product	Format	Catalog No.
Enzymatic Shearing Kit	10 rxns	53005
ChIP-IT™ Kit	25 rxns	53001
ChIP-IT™ w/o controls	25 rxns	53004

ChIP Product Line

Complete ChIP-IT Kits

Active Motif's ChIP-IT Kit is the most effective product available for performing ChIP. The kit contains nearly everything you need to perform ChIP including a comprehensive protocol and optimized buffers, inhibitor cocktails, DNA purification columns as well as positive control primers and antibodies, all of which have been validated in actual ChIP experiments.

Pre-validated ChIP antibodies

Finding an antibody that has been validated in ChIP experiments can be difficult. As specialists in transcription factor research, Active Motif offers a large number of antibodies, which are actively being validated for use in ChIP. Below is a list of antibodies that have been positively tested in ChIP, which are ideal when used with our ChIP-IT w/o controls kit. Be sure to keep up to date on new ChIP-validated antibodies by visiting our website.

Antibody	Cat. No.
AML-1/Runx1 pAb	39000
AP-2 pAb	39304
c-Jun pAb	39309
C/EBPα pAb	39306
DNMT1 mAb	39204
DNMT3A mAb	39206
DNMT3B mAb	39207
E2F-1 pAb	39313
E2F-6 mAb	39509
GATA-1 pAb	39025
HBP-1 mAb	39511
HDAC3 pAb	40968
HDAC4 pAb	40969
HDAC5 pAb	40970
HDAC6 pAb	40971
IRF-3 pAb	39033
JunB pAb	39326
JunD pAb	39328
p53 pAb	39334
Pax-5 pAb	39336
PPARγ pAb	39338
RNA pol II mAb	39097
Sp1 pAb	39058
TRF2 pAb	39223

Chariot™ Delivers Functionally Active Proteins, Peptides and Antibodies into Live Cells

Chariot™ is Active Motif's patented* protein delivery reagent that efficiently transports biologically active proteins, peptides and antibodies directly into cultured mammalian cells in less than two hours. The cells can be assayed immediately after delivery to determine the effects of the introduced material, without the need for fixing. This makes Chariot ideal for a variety of functional studies in living cells.

? How does Chariot work?

Chariot is a peptide that forms a non-covalent complex when combined with a purified protein, peptide or antibody. When added to cells, the complex is rapidly internalized. After delivery, the complex dissociates in a process called "decaging", leaving the macromolecule biologically active and free to proceed to its cellular target. One advantage of the complex is that it stabilizes the macromolecule, protecting it from degradation during internalization. It also eliminates the need to make fusion proteins or perform chemical coupling. Chariot delivery is also serum independent, which gives you the flexibility to culture your cells in whichever type of media you prefer.

? Does Chariot work with primary cells or even *in vivo*?

Chariot effectively delivers into primary cells, something that most other delivery systems cannot do. Most notable is the use of Chariot on hard-to-transfect primary neurons (Jurney, W. *et al.* (2002) *Journal of Neuroscience* 22: 6019-602) without any signs of cytotoxicity or rejection. Chariot has also been shown to deliver proteins *in vivo* (Aoshiba, K *et al.* (2003) *Am J Respiratory Cell & Molecular Biology*. 28: 555-562).

? What about protein functionality?

The ability of Chariot to deliver biologically active protein is shown using a 119 kDa subunit of β-galactosidase. β-galactosidase is composed of four subunits that must assemble to form functional protein. HeLa cells turn blue when X-gal is added after delivery of the Chariot-galactosidase complex, demonstrating successful delivery of functional β-galactosidase (Figure 1).

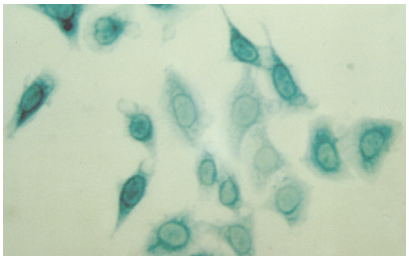

Figure 1: Chariot delivery of β-galactosidase.
One µg of a 119 kDa subunit of β-galactosidase was complexed with Chariot for 30 minutes and delivered into HeLa cells. Cells were fixed and stained with X-gal 2 hours post-delivery.

? What are researchers using Chariot for?

We have an ever growing list of citations from researchers who have used Chariot in their published research. Chariot is becoming the number one choice for protein, peptide and antibody delivery into both primary and transformed cell lines. Peluso *et al* transfected a peptide and an antibody into granulosa cells to study the PKCd-Dependent Pathway (*Endocrinology*. (2001) Vol. 142(10): 4203-4211).

Another recent publication shows the usage of Chariot in primary T cells to study the effects of a novel protein on T cell activation (Gorska, M. *et al.* (2004) *J Exp. Med*. 199(3): 369-379). And Ikari *et al.* used Chariot to study up-regulation of sodium-dependent glucose transporter by interaction with Heat Shock protein 70 in epithelial cells (*JBC*. (2002) Vol. 277, No. 5. 33338-33343).

Log on to www.activemotif.com/chariot to download the protocol as well as a current list of publications that cite Chariot.

? Is there any size limitation on the proteins that Chariot can deliver?

There is one limitation; peptides below 12 amino acids are too small for Chariot to efficiently deliver. However, we have not found a maximum size limitation. The key to remember is that the larger the protein or antibody, the longer it takes to get in; but it will get in.

? Does Chariot delivery use the endosomal pathway?

No, Chariot delivery is independent of the endosomal pathway. Temperature studies performed at 37°C and at 4°C show nearly identical results. This demonstrates that Chariot is independent of the endosomal pathway, as it is shut down at 4°C. Because endosomal delivery can modify proteins, this is a further advantage of Chariot delivery.

? Do you guarantee that my protein will be delivered by Chariot?

Complex formation depends on the size and charge of the protein, and delivery of the complex is influenced by your specific cell line's membrane. Because of these variabilities, there are some proteins and/or cell lines that are not transfected efficiently by Chariot. However, the β-galactosidase protein is provided as a positive control with Chariot to make it easy for you to optimize delivery conditions for your cell line using our own gold standard.

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

* Technology covered under U.S. Patent No. 6,841,535, which is licensed to Active Motif. Purchase includes rights for research use. Other-use licenses available; contact technical services for details.

continued from page 1 — New: Measure Transcription Factor Binding at Any DNA Sequence You Choose

The TransAM Flexi method
In the TransAM Flexi method, a biotinylated oligo or PCR product, which contains the transcription factor-binding site of choice, is incubated with nuclear extracts that have been treated to activate the transcription factor. The samples are then transferred to a 96-well, streptavidin-coated plate, where the biotinylated oligonucleotide is captured. A primary antibody specific for the activated transcription factor is then added to the individual wells, followed by an HRP-conjugated antibody and developing reagent. The plate is then read on a spectrophotometer, which provides a quantitative, colorimetric readout of transcription factor activation (Figure 3).

Assay a variety of sequences for more accurate disease research
Historically, it has only been possible to measure the binding activity and affinity of a transcription factor to non-consensus sequences, such as putative or polymorphic sites, using either reporter gene constructs or radioactive electrophoretic mobility shift assays (EMSAs). Unfortunately, these approaches are time consuming, lack sensitivity and require significant validation before they can be used. The new TransAM Flexi Kits, however, make sequence-specific transcription factor studies more straightforward than ever before. This is because every TransAM Flexi Kit is supplied as a complete solution of validated antibodies, controls and preparation & reaction buffers, so all you need to do is decide how many different sequences you want to test.

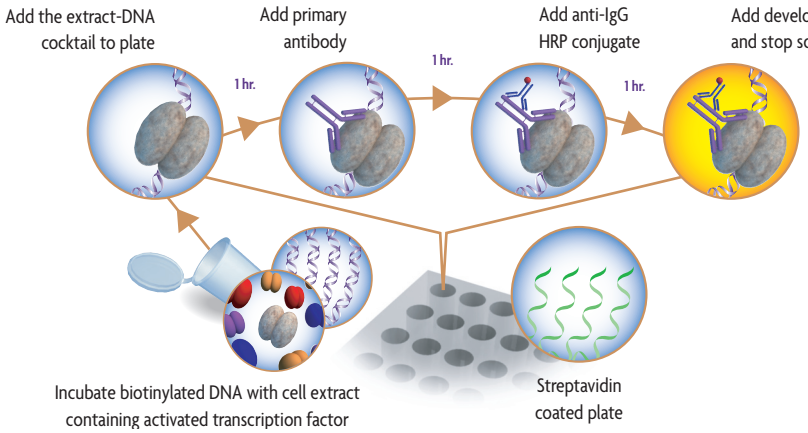

Figure 3: Flowchart of the TransAM Flexi procedure.

Proven antibodies to ensure your success
As in chromatin immunoprecipitation, transcription factor-binding assays require the use of highly specialized antibodies. Developing antibodies with the properties needed for use in TransAM is no easy task. They must be able to recognize the target protein when it is bound to DNA, and they must bind both specifically and with high affinity. To ensure that you get the highest quality results, every TransAM antibody has been extensively tested for sensitivity and specificity (Figure 2, page 1).

Confirm your ChIP results
ChIP is fast becoming an invaluable tool for the analysis of gene promoter regulation. However, there are currently no convenient tools that can be used to confirm if the transcription factor of interest is capable of binding to the promoter being studied. Because TransAM Flexi Kits make it possible to analyze the ability of a transcription factor to bind to any sequence, they offer a fast and convenient tool to confirm that your ChIP results are valid.

TransAM Flexi advantages
- **Flexible assay** – enables analysis of binding at any DNA sequence
- **Reproducible** – proven antibodies and reagents ensure your results
- **Sensitive & quantitative** – ELISAs provide better results than Westerns
- **Fast** – have results in less than 5 hours
- **Versatile** – assay cell or tissue samples

Study your chosen sequences today
TransAM Flexi Kits make it fast and easy for you to study transcription factor binding at any DNA sequence you choose. Kits for NFκB p50 and p65 are now available, with more on the way. Try one today!

TransAM™ Product Line

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

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

New: A Novel, Cell-based Method for Monitoring STAT Phosphorylation

Fast Activated Cell-based ELISA (FACE™) Kits provide a simple, innovative alternative to classical methods for monitoring protein phosphorylation. With FACE, modification-specific analysis can be performed without time-consuming cell extractions, gel electrophoresis or membrane blotting.

The importance of STATs

The new FACE STAT Kits make it easy to study phosphorylation of STAT proteins. STATs (signal transducers and activators of transcription) are latent transcription factors that are activated by phosphorylation via tyrosine kinases. Because the disruption of STAT signaling blocks neoplastic transformation, inhibitors of STAT proteins have become important drug candidates for the treatment of cancer.

The FACE method

In the FACE method, cells are cultured in 96-well plates and stimulated to induce the pathway of interest. Following stimulation, the cells are rapidly fixed, which preserves activation-specific protein modifications. Each well is then incubated with a primary antibody specific for the protein modification of interest. Subsequent incubation with secondary HRP-conjugated antibody and developing solution provides a colorimetric or chemiluminescent readout that is quantitative and reproducible (Figure 1). FACE Kits also contain a primary antibody for the native non-modified protein, so you can monitor both native and activated protein levels in the same experiment.

FACE advantages

- **Cell-based ELISA** – no extracts, gels, blotting or radioactivity
- **Fast** – requires less than 2 hours of hands on time
- **Flexible** – high-throughput chemi and colorimetric formats
- **2 Antibodies** – compare phosphorylated and native protein levels in the same kit
- **Quantitative results** – ELISAs provide more meaningful data than Westerns (Figure 2)

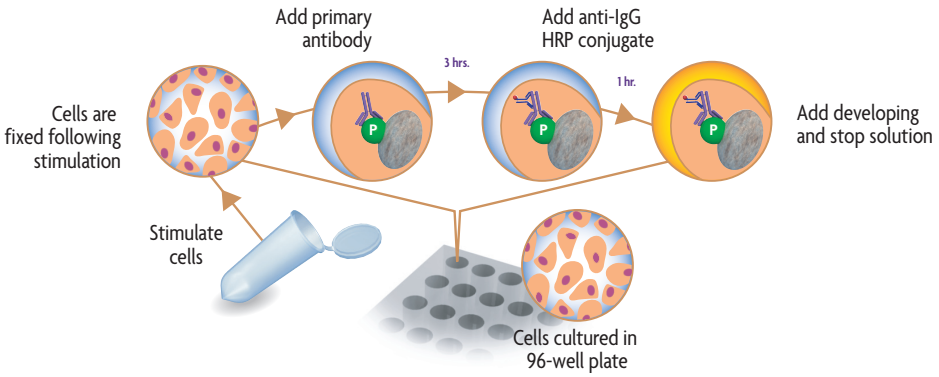

Figure 1: Flow Chart of the FACE Process.
Cells are grown, stimulated and fixed in the same 96-well plate. Addition of primary and secondary antibodies detects phosphorylated protein.

Reproducible, scaleable assay

Typically, phospho-specific analyses are performed on small sample numbers because the classical methods of study, such as Western blot and in-gel kinase assays, are both labor intensive and time-consuming. This forces you to run multiple experiments on different days and then to compare the results. However, because the variability of these methods is high and induction of phosphorylation is typically low, this can lead to statistically poor data.

Fortunately, FACE Kits make running more samples as simple as adding an extra tip to your multi-channel pipettor. And, because FACE Kits are highly reproducible, your results will be more statistically relevant.

Try FACE Kits today

Current FACE Kits available are listed below. For complete information, please give us a call, return the enclosed reply card or download the product manual and product citation list at www.activemotif.com/face.

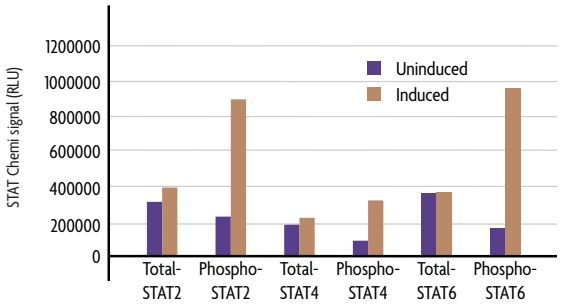

Figure 2: Measuring phosphorylated and total STAT.
NIH/3T3 cells were cultured in 96-well plates and serum-starved for 16 hours. Cells were then treated with 50 ng/ml PDGF for 5 minutes and fixed. Total and phospho STAT2, STAT4 and STAT6 were each assayed in triplicate using the phospho and total STAT antibodies included in the FACE STAT Kits. Data was plotted after correction for cell number (performed through use of Crystal Violet).

FACE™ Product Line

FACE™ AKT	FACE™ ATF-2	FACE™ Bad	FACE™ c-Jun (S63)
FACE™ c-Jun (S73)	FACE™ c-Src	FACE™ EGFR (Y992)	FACE™ EGFR (Y1173)
FACE™ ErbB-2 (Y877)	FACE™ ErbB-2 (Y1248)	FACE™ ERK1/2	FACE™ FAK
FACE™ FKHR (FOXO1)	FACE™ GSK3β	FACE™ JAK1	FACE™ JNK
FACE™ MEK1/2	FACE™ NFκB Profiler	FACE™ p38	FACE™ PI3 Kinase p85
FACE™ STAT2	FACE™ STAT4	FACE™ STAT6	

continued from page 1 — New: ELISA-based Method for Fast, Sensitive Quantification of Activated Ras GTPase

GTPases are important regulators
Small GTPases, also known as GTP-binding proteins, are a family of enzymes that serve as molecular switches in regulating a number of signal transduction pathways including cellular growth, apoptosis and differentiation. These proteins cycle between an inactive, GDP-bound state and an active, GTP-bound state. Activated GTPases exert their effects by activating a variety of downstream effector proteins such as Raf and PI3K. Activation of effector proteins in turn initiates phosphorylation cascades that modulate a number of different processes in the cell (Figure 2).

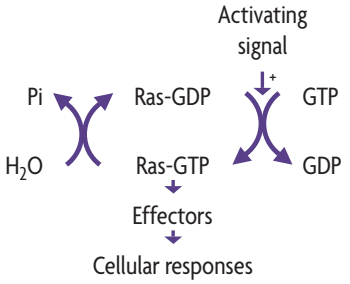

Figure 2: The Ras activation pathway.
Ras GTPase cycles between its inactive, GDP-bound form and its activated, GTP-bound form.

Aberrant Ras signaling causes many cancers
Ras GTPase is of particular interest because it is involved in many different pathways, and because aberrant regulation by Ras has been implicated in a number of disease states. Normally, GTPase-signaling cascades are only transiently activated because the intrinsic hydrolyzing activity of GTPases gradually converts GTP to GDP, leading to inactivation. However, a number of mutations in the *ras* gene have been identified that cause Ras to remain constitutively in its active, GTP-bound form. This results in continuous stimulation of cellular proliferation. Consequently, constitutively active, mutant forms of Ras GTPase are estimated to be present in ~30% of all human cancers.

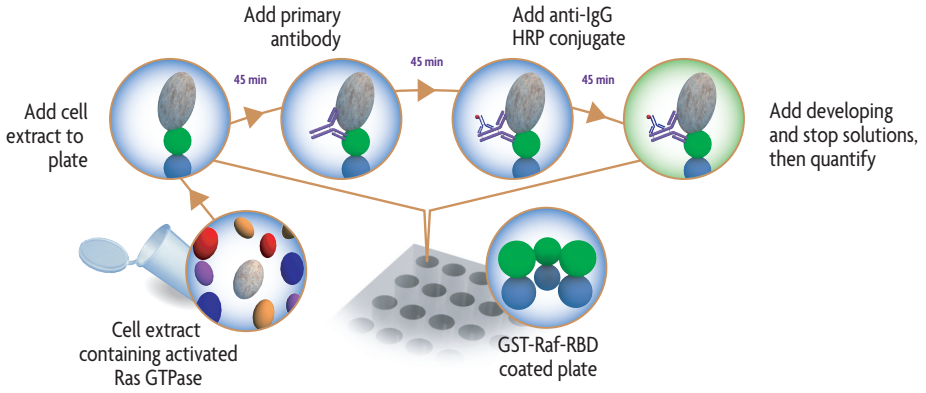

Figure 3: Flowchart of the Ras GTPase Chemi Kit.
Cell extract is added to a glutathione-coated plate that contains immobilized GST-Raf-RBD protein. Activated Ras in the extract binds to the Raf-RBD protein. Addition of primary & secondary antibodies and developing solution followed by reading on a luminometer enables sensitive quantification of activated Ras.

Ras GTPase Chemi ELISA advantages

- **More sensitive** – assay uses only 25 µg of extract, which is 20-fold less than pull-down/Western methods
- **Better results** – quantitative readouts make it easier to compare results
- **Less effort** – no need to run gels or develop Western blots
- **Save time** – results in less than 5 hours
- **Versatile** – assay activated extracts from cells or tissue samples, or study recombinant Ras protein

The Ras GTPase Chemi ELISA method
Because activated Ras binds specifically to the Ras-binding domain (RBD) of Raf effector protein, Raf-RBD can be used as a probe to isolate Ras-GTP. Active Motif's new Ras GTPase Chemi ELISA Kit contains a Raf-RBD protein that is 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 the capture probe. 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 to the individual wells, followed by an HRP-conjugated secondary antibody and developing reagent (Figure 3). The plate is then read on a luminometer, which provides a sensitive and quantitative chemiluminescent readout of activated Ras (Figure 1, page 1).

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. Additional assays to detect other GTPase proteins are in development. Please give us a call, return the enclosed reply card or log on to www.activemotif.com/gtpase for complete information, downloadable manuals and to find out about new additions to this innovative product line. To get to the best Ras activation assay available for Ras, order the Ras GTPase Chemi ELISA Kit.

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

A Complete Solution for Studying Nuclear Receptor Protein Activity

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

Monitor ligand activation with NR Peptide
Studying the agonist/antagonist effects of potential drug targets 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 cell extracts and 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, followed by an HRP-conjugated secondary antibody and developing solution to provide an easily quantified readout. This enables you to quickly and quantitatively measure the agonist/antagonist effects of target compounds on the binding of ligand-activated nuclear receptors (Figure 1).

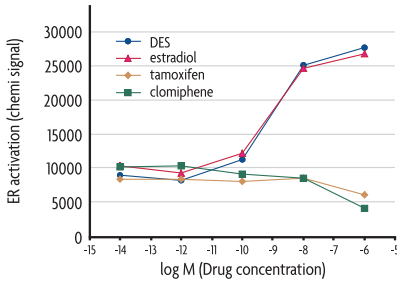

Figure 1: ERα agonism/antagonism dose-response curves. Nuclear extracts from the breast cancer cell line MCF-7 are incubated in wells of the NR Peptide ELISA ER plate in the presence of 100-fold serial dilutions (from 10^{-6} to 10^{-14} M) of the agonist compounds diethylstilbestrol (DES) and estradiol, 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.

Assess DNA-binding activity of NRs
Inappropriate nuclear receptor signaling is associated with numerous diseases including cancer, asthma and arthritis, which makes NRs promising drug targets. Because the end point of nuclear receptor activation is DNA binding, monitoring changes in the DNA-binding activity of a target nuclear receptor can serve as an ideal biomarker. Classical methods such as radioactive gelshifts 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. (See page 1 for more details.)

Quantify total NR with Sandwich ELISAs
In order to fully examine the activation of a given nuclear receptor, it is important to be able to quantify the total levels of a given nuclear receptor within a sample. The new NR Sandwich ELISAs offer a simple, rapid method to quantify the total amount of nuclear receptor protein present in both cell and tissue samples. NR Sandwich Kits utilize the Sandwich ELISA-based method that is an improvement over other methods used to study proteins, such as Western blotting. Using NR Sandwich 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 quantitative readout of nuclear receptor levels (Figure 2).

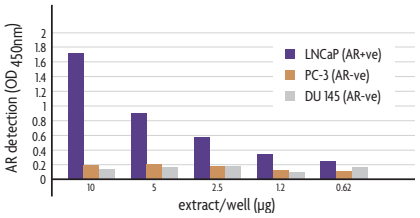

Figure 2: Monitoring expression levels of AR using NR Sandwich AR. Different amounts of nuclear extracts from three human prostate cancer cell lines, LNCaP, PC-3 and DU 145, were analyzed for levels of AR protein using the NR Sandwich AR Kit.

A collection of NR abs & proteins
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, including antibodies for GR, PPARα and γ, PXR, RAR-β, -β2 and -γ, RXR-α and -β, and VDR. In addition, Active Motif offers a number of recombinant nuclear receptor proteins that are ideal for use as positive controls, in *in vitro* screening studies and in many other applications.

Get the tools you need for NR research
To get complete information on all of Active Motif's tools for studying nuclear receptors, please give us a call, visit our website or send in the enclosed reply card.

Product	Format	Catalog No.
NR Peptide ERα	1 x 96-well plate 5 x 96-well plates	49096 49596
NR Peptide ERα Chemi	1 x 96-well plate 5 x 96-well plates	49097 49597
NR Sandwich AR	1 x 96-well plate 5 x 96-well plates	49196 49696
NR Sandwich ERα	1 x 96-well plate 5 x 96-well plates	49296 49796
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

New Antibodies for Chromatin and DNA Methylation

Active Motif has recently added a number of antibodies related to epigenetics and signal transduction to its already extensive line of antibodies. The tables below list these recent additions. Active Motif also offers numerous cell extracts that are ideal positive controls for use with its kits and antibodies. For complete information, please visit www.activemotif.com.

Product	Format	Application	React.	Cat. No.
APE pAb	100 µg	WB	H	39200
APEXL2 pAb	200 µl	WB	H	39201
INHAT-1/TAF-1α/TAF-1β pAb	200 µl	WB	H	39202
CGBP pAb	200 µl	WB	H	39203
DNMT1 mAb	100 µg	Ch, IHC, IP, WB	H, M	39204
DNMT2 pAb	100 µg	WB	H, M	39205
DNMT3A mAb	100 µg	Ch, IF, IHC, WB	H, M	39206
DNMT3B mAb	100 µg	Ch, IF, IP, WB	H, M	39207
HDAC11 pAb	200 µl	WB	H, M	39208
Histone H2A pAb	100 µg	WB	H	39209
Histone H2B pAb	100 µg	WB	H	39210
Histone H3 (Phosphorylated) mAb	100 µg	WB	H	39211
Histone H4 pAb	100 µg	WB	H	39212
INHAT-2/pp32 pAb	200 µl	WB	H	39213
LIG1 pAb	200 µl	WB	H	39214

Product	Format	Application	React.	Cat. No.
MBD1 mAb	100 µg	WB	H	39215
MBD3 mAb	100 µg	WB	H	39216
MBD4 pAb	100 µg	WB	H	39217
MeCP2 pAb	100 µg	WB	H	39218
MLH-1 mAb	100 µg	WB	H	39219
MRE11 pAb	100 µg	WB	H	39220
PMS2 mAb	100 µg	WB	H, M	39221
Rad17 (Phosphorylated) pAb	100 µg	IF, WB	H	39222
TRF2 pAb	100 µg	Ch, IP, WB	H, M	39223
UBE2N pAb	100 µg	IP, WB	H	39224
UBE2V1a pAb	100 µg	WB	H	39225
UBE2V2 pAb	100 µg	WB	H	39226

Application Key:
Ch = Chromatin Immunoprecipitation; IF = Immunofluorescence; IHC = Immunohistochemistry; IP = Immunoprecipitation; WB = Western blot

Reactivity (React.) Key:
H = Human; M = Mouse

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