MOTIF VATIONS

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ACTIVE MOTIF®

Tools to Analyze Cellular Function

Drop In & Learn About New Immunoprecipitation & Fluorescent Detection Products

IN THIS ISSUE

New: Simplify Co-Immunoprecipitation

Active Motif's new 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."

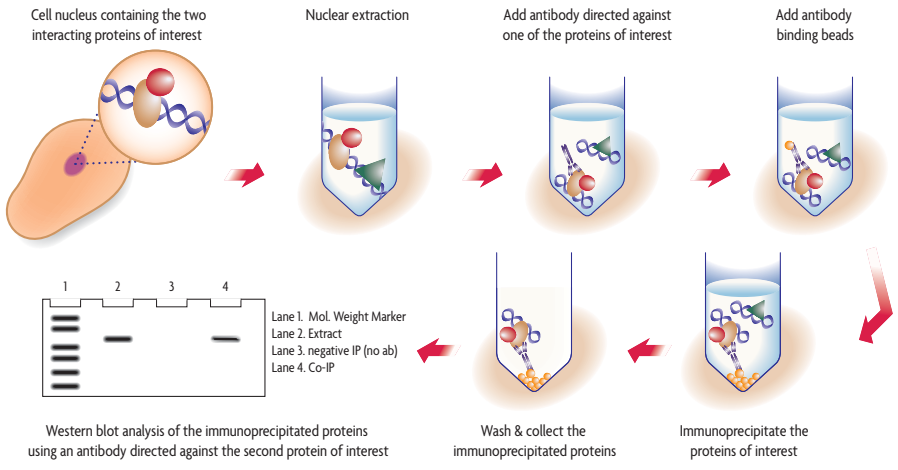

Figure 1: Flowchart of the Co-Immunoprecipitation process.

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

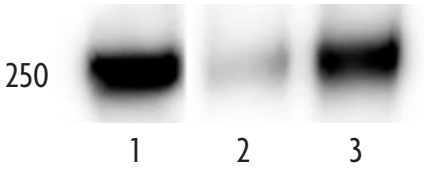

Figure 1: Western blot analysis of the IP'd p33 subunit of the RNA pol II complex.
HeLa cells were grown to confluence on 100 mm plates and nuclear extracts were prepared using the kit's extraction reagents. For IP experiments, the stringency of the IP High Buffer was increased by supplementing with 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 immunoprecipitations. To find out more about the new Nuclear Complex Co-IP Kit, visit us on the web at www.activemotif.com.

New: Effortless Quantification of Your Protein Samples

Active Motif's Fluorescent Protein Quantification Kit is a simple, sensitive alternative to traditional methods for determining protein levels. Offering high signal strength, unique spectral properties and robust conjugation, the kit offers limits of detection (60 ng/ml) superior to other fluorescent-based systems, as well as traditionally used methods, such as the Bradford assay.

Broad spectral shift for better results

A disadvantage of many methods used for protein quantification, such as the Bradford assay, is that the absorbance spectra of the free and conjugated forms of the dye partially overlap. This causes non-linear protein measurement because free dye is excited by the light used to excite bound dye. In contrast, the free versus conjugated absorbance maxima of the dye provided in the Fluorescent Protein Quantification Kit are separated by 108 nm. This means that when conjugated sample is excited at ~500 nm (for example, at 488 nm), free dye is not excited. In addition, the fluorescent intensity of free dye is 50-fold lower than conjugated dye; taken together, these features effectively eliminate background (Figure 1).

"The spectral properties and robust conjugation of the Fluorescent Protein Quantification Kit make measurement fast and accurate."

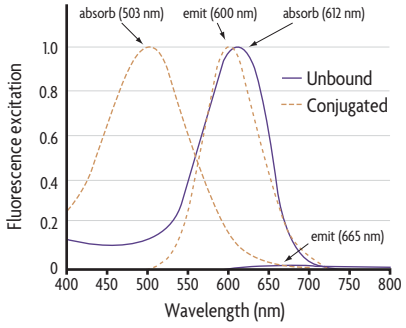

Figure 1: Absorption/emission spectra of free/bound dye.
Normalized absorption and emission spectra of free (solid lines) and conjugated dye (dotted lines) in phosphate buffer of pH 7.2.

Fast, simple conjugation

Using the Fluorescent Protein Quantification Kit is fast and easy. The kit provides fluorescent dye, dilution buffer and a protein standard. Simply resuspend the dye and add it to the wells of a microplate, then add a serial dilution of the standard protein to produce a Standard Curve, along with your sample. On conjugation, the dye changes from blue to red (Figure 2). After a 30 minute, room temperature incubation, simply read the fluorescence to quantify your samples.

Figure 2: Color shift caused by dye conjugation.
The dye is blue in its free state. After a 30 minute, room temperature conjugation, the dye's color changes to red.

Contaminating substances

Unlike many protein determination methods, which are influenced by the presence of contaminants such as detergents and salts, the Fluorescent Protein Quantification Kit has been shown to be resistant to the effects of many contaminating agents (Figure 3). However, to achieve the best results the protein standards should be assayed under the same conditions as your samples. For example, if your samples are prepared in an unusual buffer, then the standards used for the Standard Curve should be prepared in the same buffer.

Consistent performance

To ensure accurate determination of protein levels, it is important that the protein detection reagent perform consistently between samples. To demonstrate this, the Fluorescent Protein Quantification Kit was used to prepare Standard Curves using several different proteins. The fluorescence intensity obtained with the kit showed little variation between samples across a range of concentrations (download a kit manual for data). This means that you can have confidence that your results will not be influenced by protein-specific effects.

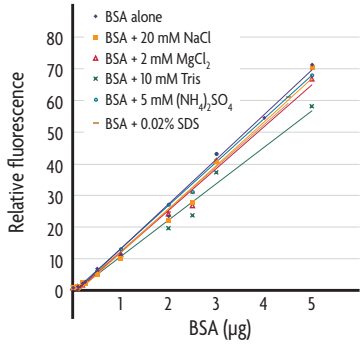

Figure 3: Standard Curves of BSA.
Increasing amounts of BSA protein were quantified using the Fluorescent Protein Quantification Kit in the presence of a variety of contaminants.

A simple way to improve your results

The large difference in the absorption maxima of the free dye compared to the conjugated dye and the ease of conjugation make the new Fluorescent Protein Quantification Kit an ideal tool for protein quantification. To improve the results of your downstream assays, use the Fluorescent Protein Quantification Kit.

Product	Format	Catalog No.
Fluorescent Protein Quantification Kit	1000 rxns	15001

New: Capillary Electrophoresis Made Easy

Active Motif is pleased to offer a new selection of novel dyes for capillary electrophoresis (CE). CE Dyes are a significant improvement over existing methods for analyzing proteins using CE. Their ease of use, spectral shift upon conjugation, high quantum yield and ability to maintain the natural ionic character of labeled protein make them ideal for use in CE.

Derivatization of proteins with fluorescent labels prior to CE is commonly used to overcome the lack of sensitivity when analyzing proteins via UV absorbance or natural fluorescence. In general, the proteins are labeled by attaching dye molecules to the primary amines of lysine residues. Lysine is used primarily because it is a relatively abundant amino acid, and also because lysine residues are often located at the surface of the protein. However, as dye labeling is non-uniform, and because proteins usually contain many lysine residues, inconsistent labeling of multiple lysine residues within a single protein is common. If the dye used affects the overall protein charge, then a single protein will give rise to a series of products, each with a different charge. This can cause problems during separation and other steps.

One charge – for clear separation

Like other dyes, Active Motif's CE Dyes utilize highly abundant and accessible lysine residues for their attachment chemistry. However, CE Dyes overcome the limitation of other dye systems by maintaining the positive charge of the amine group following dye conjugation (Figure 1). This means that proteins labeled with CE Dyes will not display band broadening or require adjusted ionic character calculations to be performed. The result is that CE Dyes provide a highly sensitive labeling system that maintains the natural charge properties of every protein within your sample.

Non-toxic – for user-friendly labeling

A further disadvantage of commonly used dyes, such as *o*-pthaldialdehyde, naphthalene-2,3-dicarboxaldehyde and 3-(2-furoyl)quinoline-2-carboxaldehyde, is that derivatization must be performed in the presence of cyanide. Use of such a highly toxic compound is not only undesirable, but creates significant disposal problems for the unused material. In contrast, the CE Dye labeling process is simple to perform, non-toxic and requires only a 60 minute incubation at either room temperature or 50°C, depending on the CE Dye used.

The CE Dye method

Labeling samples with CE Dyes is fast and convenient. The protein sample of interest is dissolved in bicarbonate solution (pH unadjusted) and stock CE Dye is added, followed by incubation at either room temperature or 50°C, depending on the CE Dye used. Completion of the reaction can be observed by eye due to a change in sample color from blue to red. It's as simple as that!

Large Stokes Shift minimizes background

In addition to their non-toxic and charge properties, CE Dyes also undergo a significant change in spectral properties upon conjugation to amine groups. In their free state, CE Dyes have very weak fluorescence (quantum yield < 1%), with an absorbance maximum of 612 nm and an emission maximum of 665 nm. However, upon reaction with a primary amine the absorbance maximum shortwave-shifts by more than 100 nm to 503 nm and emits strongly with a quantum yield of more than 50% at 600 nm. This change in spectra and quantum yield means that the presence of unconjugated label does not affect downstream analysis and significantly reduces background effects.

Get better CE results starting now

To improve your CE results, give us a call to order CE Dyes today.

"Labeling proteins for capillary electrophoresis is easy with CE Dyes, while the large Stokes Shift and charge maintenance help improve your results."

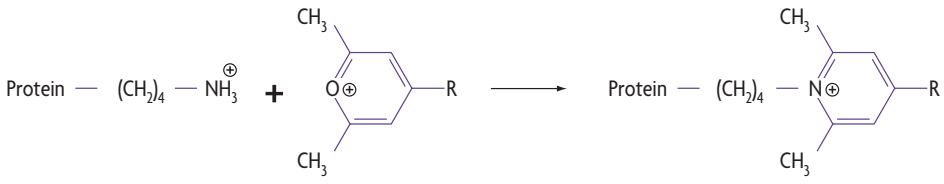

Figure 1: Labeling with CE Dyes maintains each protein's natural charge.
Chemical equation depicting the chemistry of CE Dye labeling, which demonstrates the preservation of the positive charge of the lysine residue of a protein. R stands for the respective chromogenic/fluorogenic group.

Product	Format	Catalog No.
CE Dye 503	1 kit	15101
CE Dye 540	1 kit	15102

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."

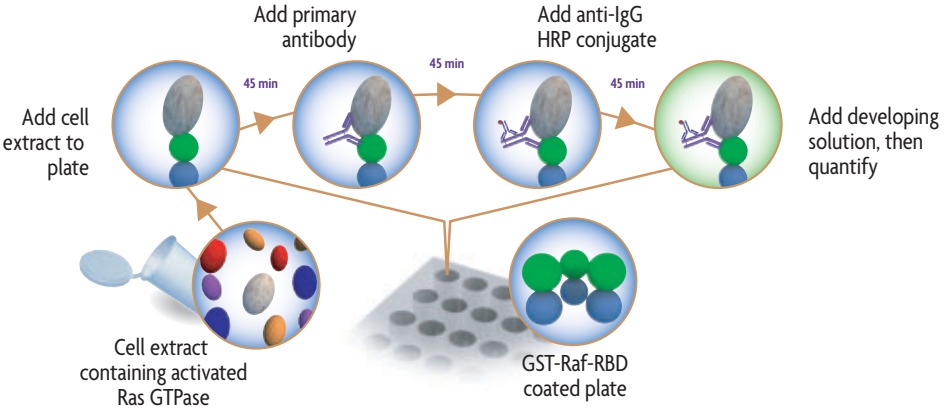

Figure 1: Flowchart of the Ras GTPase Chemi Kit.
Cell extract is added to a glutathione-coated plate that contains immobilized GST-Raf-RBD protein. Activated Ras in the extract binds to the Raf-RBD protein. Addition of primary & secondary antibodies and developing solution followed by reading on a luminometer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

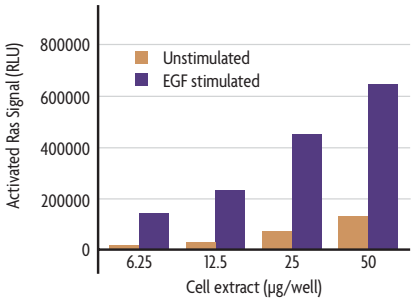

Figure 2: Quantification of activated Ras.
Increasing amounts of whole-cell extract from HeLa cells that had been stimulated with 5 ng/ml EGF for 2 minutes were assayed using the Ras GTPase Chemi ELISA Kit.

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. Additional assays to detect other GTPase proteins, such as RhoA, are currently 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 learn about new additions to this innovative product line. To get to the best Ras activation assay available, try the Ras GTPase Chemi ELISA Kit.

Use ChIP-IT™ for More Successful Chromatin Immunoprecipitation

Chromatin immunoprecipitation (ChIP) is a powerful, yet technically challenging tool used to study protein/DNA interactions. Active Motif's ChIP-IT™ Kits make your ChIP more successful by combining nearly everything you need in a single kit, including positive control antibodies and primers, DNA purification columns and a comprehensive protocol.

The ChIP method

In ChIP, intact cells are fixed using formaldehyde, which cross-links and preserves protein/DNA interactions. The DNA is then sheared into small, uniform fragments. The protein/DNA complexes are then immunoprecipitated using antibodies directed against the DNA-binding protein(s) of interest. Following immunoprecipitation, the cross-links are reversed and DNA fragments are purified and screened to determine which gene, or group of genes, was bound by the protein of interest (Figure 1).

ChIP-IT advantages

- Easy to use – all critical buffers, Protein G beads and DNA purification columns are provided
- No need to optimize reagents and protocol
- Your choice of enzymatic or sonication shearing
- Direct measurement of transcription factor/DNA interactions or histone modifications

Choose enzymatic or sonication shearing

The key to a successful ChIP experiment begins with correctly shearing the DNA into 200-1000 bp fragments. This can be achieved by using one of two methods: enzymatic digestion or sonication. Sonication shearing is an effective method for shearing DNA but can be difficult to optimize due to complications from overheating, emulsification and dependence on sonicator type. In contrast, enzymatic digestion can be used to quickly and easily digest DNA into fragments suitable for ChIP. And, as digestion is dependent only on time and temperature, very little optimization is required. For your convenience, ChIP-IT Kits are available with your choice of either sonication or enzymatic shearing.

Start your ChIP today

Active Motif's ChIP-IT Kits provide you with everything you need to make your ChIP experiments easier and more reproducible. For more information on ChIP-IT visit www.activemotif.com/chip.

"ChIP-IT makes performing ChIP with transcription factors more successful by combining all of the critical components you need in a single kit."

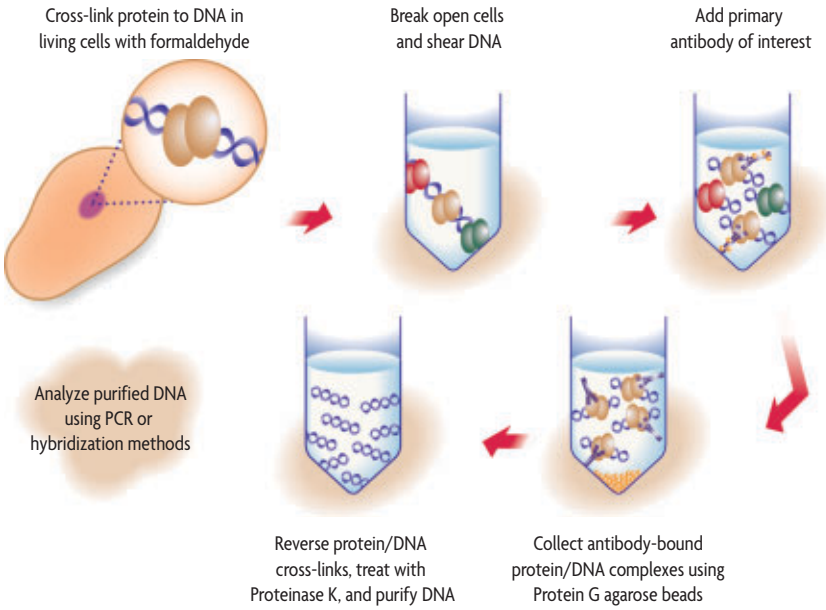

Figure 1: Flowchart of chromatin immunoprecipitation.
In ChIP, protein/DNA interactions are fixed, and the DNA is sheared and precipitated using an antibody. After cross-link reversal, the DNA is purified and screened to determine which genes were bound by the protein of interest.

Product	Format	Catalog No.
ChIP-IT™	25 rxns	53001
ChIP-IT™ w/o controls	25 rxns	53004
ChIP-IT™ Shearing Kit (included in 53001 & 53004)	10 rxns	53002
ChIP-IT™ Enzymatic	25 rxns	53006
ChIP-IT™ Enzymatic w/o controls	25 rxns	53007
Enzymatic Shearing Kit (included in 53006 & 53007)	10 rxns	53005

Sensitive, Specific Transcription Factor Analysis

Active Motif's TransAM™ Kits simplify the study of activated transcription factors by combining high sensitivity with convenience in a non-radioactive, ELISA-based assay.

Traditionally, transcription factor activity has been studied using either Electrophoretic Mobility Shift Assays (EMSA), immunoblotting or reporter gene assays. However, these methods are quite time-consuming and, at best, provide only semi-quantitative results. Moreover, use of these methods to determine the activity of multiple transcription factor family members would require the investment of significant amounts of both time and money; in many cases, it may not even be possible.

Simple, quantitative assay

In contrast, Active Motif's TransAM Kits make it straightforward to measure the activity of transcription factors. You can even study multiple members of a transcription factor family in a single experiment that is complete in less than 5 hours. In addition to being fast, the TransAM method is non-radioactive and provides quantitative results (Figure 1). Plus, TransAM Kits can be used on all sample types, including cell lines and tissues, giving you unsurpassed flexibility.

"TransAM Kits are sensitive, non-radioactive DNA-binding ELISAs that facilitate the study of transcription factor activation in cell and tissue samples."

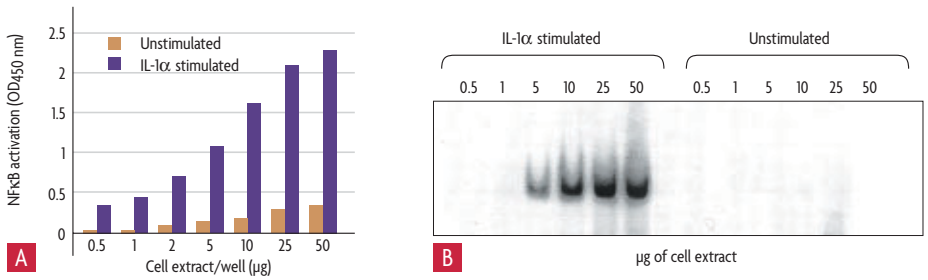

Figure 1: TransAM NFκB is more sensitive than gelshift, and provides more quantitative results.
Human fibroblast WI-38 cells are stimulated with IL-1α for 30 minutes. Increasing amounts of whole-cell extract are assayed using either the TransAM NFκB p50 Kit (A) or gel retardation (B).

Flexi or Original format

The Original TransAM Kits offer a fast, non-radioactive alternative to gelshift by providing a 96-well plate that is precoated with oligonucleotide that contains a consensus-binding site for the factor of interest. Activated transcription factor binds the oligo and is quantified on a spectrophotometer using the supplied antibodies and developing reagent. This format is convenient for measuring binding at the consensus site, but does not enable you to study alternative sites. For this reason, we developed TransAM Flexi Kits; they contain all of the optimized reagents provided in the Original kits, including antibodies, but give you the flexibility to immobilize any oligo in the 96-well plate.

TransAM advantages

- Non-radioactive, colorimetric method provides quantifiable results
- Results in less than 5 hours
- 10-fold greater sensitivity than gelshift (100-fold with Chemi Kits)
- Simultaneous profiling of multiple family members
- Assay both cell and tissue samples

Try sensitive, quantitative ELISAs

TransAM Kits make it simple to quantify activated transcription factors. Please give us a call or visit our website to learn more about the TransAM product line.

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

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

Fast, Efficient Analysis of Protein Phosphorylation

As interest in kinase activation and its effect on cellular regulation increases, so does the need for fast, efficient and robust assays to measure protein phosphorylation. To fill this need, Active Motif developed its Fast Activated Cell-based ELISA (FACE™) Kits.

FACE Kits provide a simple, sensitive, cell-based method for monitoring protein phosphorylation. They enable you to perform modification-specific analysis directly within the cell, without the need for time-consuming cell extractions, gel electrophoresis or membrane blotting, all of which saves you time and money.

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 (Figure 2).

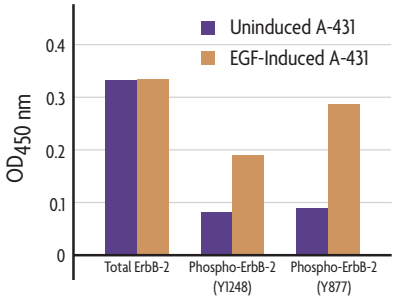

Figure 2: Monitoring total- and phospho-ErbB-2 using FACE.
The FACE ErbB-2 (Y1248) and ErbB-2 (Y877) Kits were used to assay the levels of total and phosphorylated ErbB-2 contained within uninduced or EGF-induced A-431 cells.

Specificity counts

To be certain that you detect only your protein of interest, all FACE antibodies are stringently tested for cross-reactivity. All phospho-specific antibodies are verified to detect only the activated form of the target protein. In addition, the phospho-specific and total antibodies are used in tandem to ensure the phospho-antibody doesn't interact with other phosphorylated proteins. This ensures that FACE Kits are highly specific, so you'll get the results you need.

FACE advantages

- Simple, quantitative method
- Cell-based assay eliminates extractions and gel electrophoresis
- Fixing cells preserves protein activation state
- Fast – requires less than 3 hours of hands on time
- Primary antibodies to compare phospho and native protein levels

Choose from a variety of kits

FACE Kits are available for a variety of different factors, listed below, and each assay is available in both colorimetric and chemiluminescent formats. To simplify the measurement of your phospho proteins, try a FACE Kit today.

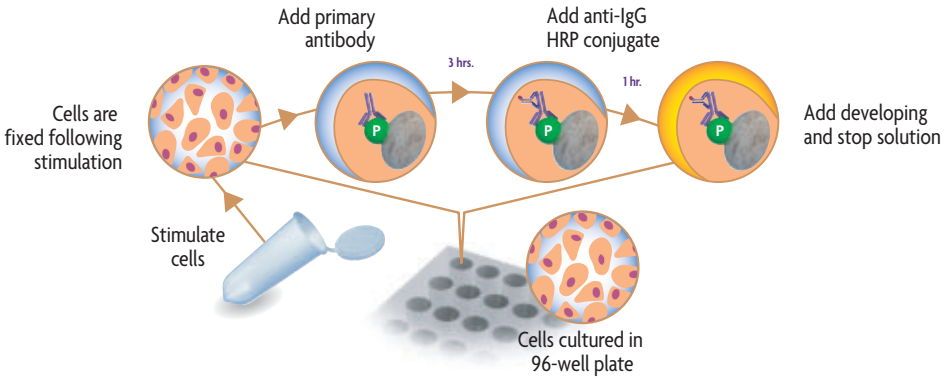

Figure 1: Flowchart of the FACE process.
Cells are grown, stimulated, fixed and assayed in a single 96-well plate. Addition of primary and secondary antibodies and developing solution enables quantitation of phosphorylated and total protein levels on a spectrophotometer or luminometer.

"FACE Kits make it possible to detect phosphorylated proteins in the cell, eliminating the need for cell extractions, gels and Western blotting."

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	

Deliver Functional Proteins Directly into Living Cells

Chariot™ is Active Motif's patented* protein delivery reagent that 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 an 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).

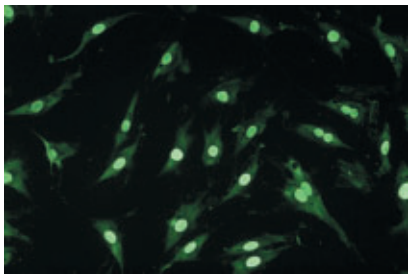

Figure 1: Targeted protein delivery.
50 ng of a 10 kDa nuclear protein that was labeled with Lucifer yellow at the C-terminus was complexed with Chariot and delivered into HS-68 cells. Unfixed cells were observed 90 minutes post-delivery.

Non-covalent delivery of native protein

Many protein delivery systems require that you begin by fusing a carrier protein to your macromolecule. In addition to being time-consuming, this can alter the 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 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 2).

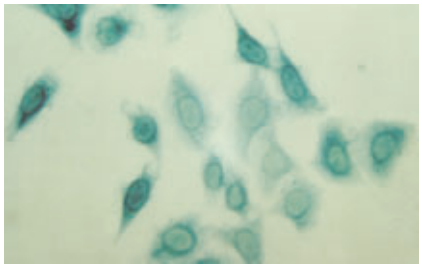

Figure 2: Chariot delivery of β-galactosidase.
One µg of a 119 kDa subunit of β-galactosidase was complexed with Chariot for 30 minutes and delivered into HeLa cells. Cells were fixed and stained with X-gal 2 hours post-delivery.

Advantages

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

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.

"The ability to deliver active proteins directly into living cells and then assay their effect makes feasible studies not even possible with other methods."

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 into 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

* Chariot is covered under U.S. Patent No. 6,841,535. Purchase includes the right to use for basic research purposes only. Other-use licenses are available; please contact Active Motif Technical Services for additional information.

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

Accurate Detection of Signal Pathway and Nuclear Receptor Proteins

FunctionELISA™ & NR Sandwich Kits offer a simple, rapid method to monitor changes in protein levels. They utilize the Sandwich ELISA technique to capture and quantify the amount of a specific protein in your sample.

The Sandwich ELISA method is faster and simpler to perform than other techniques used to study proteins, like Western blot. And, it enables you to make quantitative rather than just qualitative protein measurements.

Specific, quantitative assay

Sandwich ELISAs utilize two antibodies that recognize different epitopes on the protein to be measured. The first antibody, termed the Capture Antibody, binds the protein of interest from the cell lysate. A Detecting Antibody is then used to bind to the captured protein. In FunctionELISA Kits, accurate quantification can be made by comparing the sample to a standard curve made using recombinant protein included in each kit (Figure 1).

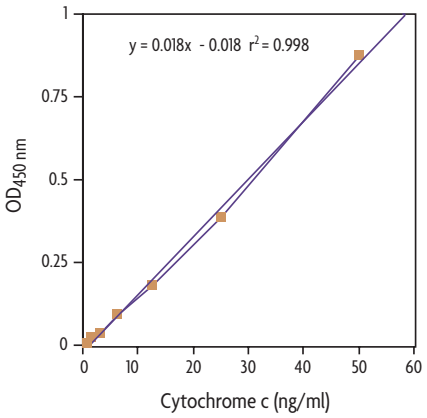

Figure 1: Cytochrome c Standard Curve.
A Standard Curve was generated by assaying increasing amounts of recombinant human cytochrome c with the FunctionELISA Cytochrome c Kit.

> "Sandwich ELISA Kits make it simple to capture and quantifiably measure the amount of a specific protein in your sample."

Convenient format saves time

FunctionELISA & NR Sandwich Kits are faster and simpler than Western blots because your samples are read on a spectrophotometer (or on a luminometer for IκBα). This means that you don't need to run, blot and develop gels. In just hours, you'll have accurate, quantitative results that are easier to interpret than blots (Figure 2). Plus, the convenient 96-well format makes it possible to process multiple samples quickly, at your level of throughput.

Sandwich ELISA advantages

- Quantitative results in just hours
- ELISA format eliminates the need to run, blot and develop gels
- Easily analyze multiple samples
- Ready-to-use format with Capture Antibody precoated on the plate
- Assay both cell and tissue samples

Complete kits ensure your success

Our Sandwich ELISA Kits contain all of the reagents required to rapidly quantify your protein. FunctionELISA Kits even include recombinant protein for producing a standard curve. For your convenience, the Capture Antibody is supplied immobilized in a 96-well assay plate, so you won't waste time with overnight incubations. For complete details, please visit our website at www.activemotif.com.

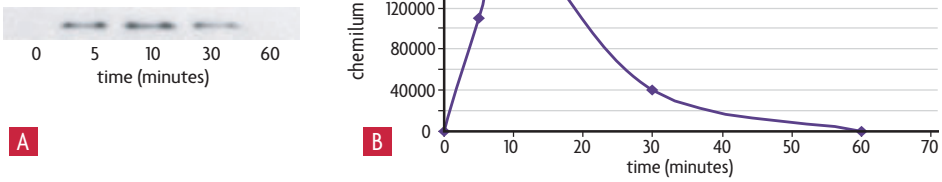

Figure 2: FunctionELISA provides a more quantitative measurement of IκBα phosphorylation than Western blot.
Jurkat cells were induced with 1 nM TNF-α and harvested at the indicated time points. Whole-cell extracts were assayed in Western blot analysis (A) using Phosphorylated-IκBα mAb (Cat. No. 40904), and with the FunctionELISA IκBα Kit (B).

Product	Format	Catalog No.
FunctionELISA™ Cytochrome c	1 x 96 rxns	48006
	5 x 96 rxns	48506
FunctionELISA™ IκBα	1 x 96 rxns	48005
	5 x 96 rxns	48505
FunctionELISA™ TRAIL	1 x 96 rxns	48010
	5 x 96 rxns	48510
NR Sandwich AR	1 x 96 rxns	49196
	5 x 96 rxns	49696
NR Sandwich ERα	1 x 96 rxns	49296
	5 x 96 rxns	49796
NR Sandwich PR	1 x 96 rxns	49396
	5 x 96 rxns	49896

Positive Control Recombinant Proteins and Cell Extracts for All Your Needs

Active Motif offers you an extensive line of recombinant proteins against a variety of transcription factors and cell-signaling related proteins that are sure to suit your research needs. These proteins are great for use as positive controls in Western blot, EMSA, kinase assays and as standards in ELISAs such as our TransAM Kits.

Finding a good positive control is essential for any experiment. With Active Motif's line of recombinant proteins, you can feel confident of your results as each protein has undergone extensive testing to ensure its functionality.

Cellular extract controls

In addition to our recombinant proteins, Active Motif offers a broad line of ready-to-use nuclear, cytoplasmic and whole-cell extracts that are prepared using extraction protocols that have been optimized to produce high yields of cell signaling proteins. In addition, many of our extracts are prepared from cells that have been stimulated or treated to specifically induce activation of hard-to-detect transcription factors. Be sure to check out our online search tool to find your extract of choice either by keyword or tied to the antibody for which you need a positive control.

Figure 1: Active Motif's cell extract search engine. Active Motif's online cell extract search engine enables you to find cell extracts by cell type, application, catalog number, keyword, stimulation condition or antibody of choice.

Product	Format	Catalog No.
Recombinant AKT1 protein	5,000 units	31105
Recombinant ATF-2 protein	25,000 units	31106
Recombinant BRCA1 protein	2,000 units	31113
Recombinant c-Fos protein	5,000 units	31115
Recombinant c-Jun protein	5,000 units	31116
Recombinant c-Myc protein	5,000 units	31117
Recombinant CREB protein	25,000 units	31107
Recombinant CTF1 (NF-1) protein	5,000 units	31118
Recombinant eIF2α protein	25,000 units	31108
Recombinant ER protein	4,000 units	31119
Recombinant FXR protein	10,000 units	31120
Recombinant GR protein	5,000 units	31121
Recombinant IκBα protein	25,000 units	31109
Recombinant JNK2α1 protein	5,000 units	31110
Recombinant JNK2α2 protein	5,000 units	31111
Recombinant LXRα protein	10,000 units	31122
Recombinant LXRβ protein	10,000 units	31123
Recombinant NFκB p50 protein	5,000 units	31101
Recombinant NFκB p65 protein	5,000 units	31102
Recombinant p53 protein	5,000 units	31103
Recombinant p300 protein	4,000 units	31124
Recombinant PPARα protein	10,000 units	31125
NEW: Recombinant PPARα-LBD protein	10,000 units	31141
Recombinant PPARβ(δ) protein	10,000 units	31126
NEW: Recombinant PPARβ(δ)-LBD protein	10,000 units	31142
Recombinant PPARγ protein	10,000 units	31127
NEW: Recombinant PPARγ-LBD protein	10,000 units	31143
Recombinant pRB protein	5,000 units	31128
NEW: Recombinant PXR protein	10,000 units	31144
Recombinant Rad51 protein	10,000 units	31129
Recombinant RAR-α protein	5,000 units	31130
Recombinant RAR-β protein	5,000 units	31131
Recombinant RAR-γ protein	5,000 units	31132
Recombinant RXR-α protein	10,000 units	31133
Recombinant RXR-β protein	5,000 units	31134
Recombinant RXR-LBD protein	10,000 units	31135
Recombinant Sp1 protein	5,000 units	31136
Purified Sp1 protein	2,000 units	31137
Recombinant STAT1 protein	25,000 units	31112
NEW: Recombinant STAT3 protein	10,000 units	31140
Recombinant TRα1 protein	10,000 units	31138
Recombinant TRβ1 protein	10,000 units	31139

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