MOTIF VATIONS

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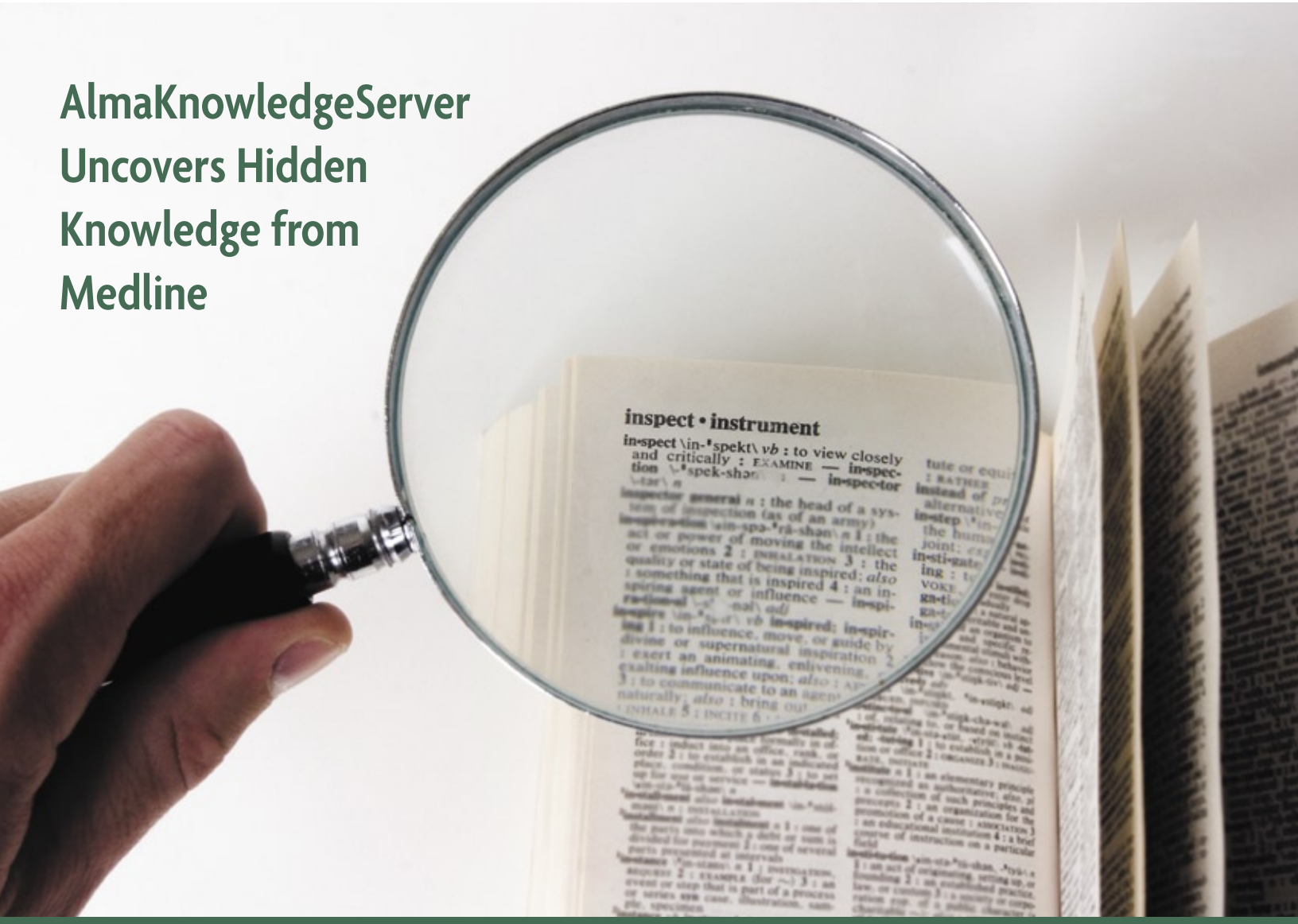
ACTIVE MOTIF®

Tools to Analyze Cellular Function

AlmaKnowledgeServer Uncovers Hidden Knowledge from Medline



IN THIS ISSUE

NEW: Specific, Flexible Labeling of Proteins

Active Motif's new LigandLink™* Universal Labeling technology is an innovative tool for performing site-specific labeling of proteins in living cells. With LigandLink, it is now possible to create a single fusion protein that can be labeled with a variety of tags, making it ideal for use in FRET and co-localization studies, where the requirement for varying fluorophores is high.

Improved co-localization studies

The ability to label proteins in living cells is key to understanding the dynamics and functions of proteins. Classically, these studies have been performed by fusing a gene of interest to a fluorescent protein, such as green fluorescent protein (GFP), and transfecting the construct into cells. These approaches have significantly expanded our understanding of protein function within the cell.

> " LigandLink's ease of use makes it an ideal tool for co-localization experiments where multiple labeling colors are required."

However, protein function is commonly regulated via protein-protein interactions, creating the need for multiple labeling systems. Using conventional approaches, this means you would need to clone a series of constructs containing your gene of interest fused to different fluorescent proteins. This has made such studies time-consuming and labor intensive. In contrast, LigandLink is a novel tool that makes it possible to create a single clone that can be labeled in cells with different tags. This makes LigandLink an ideal tool for co-localization studies, where multiple labeling colors are required.

Why use LigandLink?

- **Spend less time on cloning and clone validation** – clone only once, but obtain different functionalities by using different LigandLink labels
- **Superior fluorescence** – synthetic dyes have improved spectral properties vs. naturally fluorescent proteins
- **Small label** – unlikely to interfere with protein function
- **Label your protein in living cells** – LigandLink labels are cell permeable

The LigandLink method

In the LigandLink method, the gene of interest is cloned in frame with the gene for *E. coli* dihydrofolate reductase (eDHFR) in the pLL-1 vector (Figure 1). The vector is then transfected into mammalian cells and used to express the fusion protein. Twenty-four hours post-transfection, the protein of interest can be labeled simply by adding the LigandLink ligand of choice to the cell medium (Figure 2). Depending on the cell type and the label used, cells can be imaged in as little as 10 minutes.

User-friendly vector

The LigandLink vector, pLL-1 (Figure 1), is easy to use. It has a CMV promoter to ensure high-level expression of eDHFR fusion proteins, with Neomycin for selection of stable cell lines. In addition, the multiple cloning site (MCS) was designed to facilitate cloning, whatever method you use. In addition to many popular restriction sites, the MCS includes three blunt-cutting restriction enzymes towards the 3´ end, each in a different reading frame with the eDHFR gene. This enables a number of PCR and restriction enzyme cloning strategies.

Prokaryotic protein for a cleaner signal

The LigandLink protein is derived from the *E. coli* DHFR protein and binds rapid-

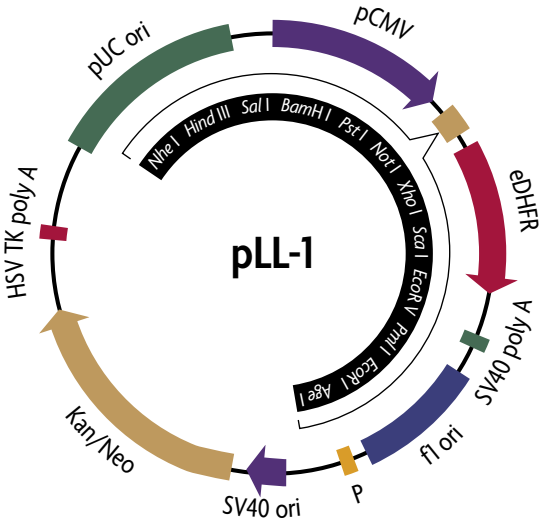


Figure 1: The LigandLink pLL-1 vector.

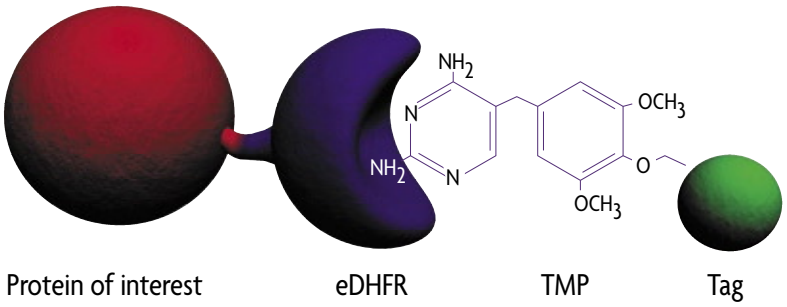


Figure 2: Specific protein labeling using LigandLink.
The gene of interest is cloned into pLL-1 in frame with the vector's *E. coli* dihydrofolate reductase (eDHFR) gene. After transfection into cells, the protein of interest is expressed as a fusion to eDHFR. Addition of cell-permeable LigandLink Label to the medium results in rapid, specific binding of the label by the fusion protein.

ly and specifically to the LigandLink TMP (trimethoprim) ligand. This is because TMP is an antibiotic that was designed to specifically inhibit the bacterial eDHFR protein, without interacting with mammalian proteins. The result is high specificity binding ($K_i \approx 1$ nm) with minimal background (Figure 3). In addition, the eDHFR fusion protein is significantly smaller than naturally fluorescent proteins, such as GFP (18 kDa vs. 27 kDa for GFP), which means that cloned protein activity is less likely to be affected.

Superior and flexible dye options

Once a gene of interest is cloned into a classical fluorescent protein-fusion vector, it can be time consuming and laborious to change the fluorescent fusion.

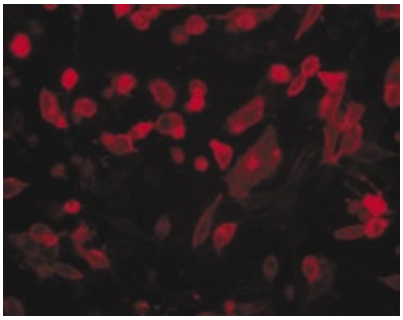


Figure 3: Membrane-localized eDHFR labeling by LigandLink Hexachlorofluorescein.
The myristoylation/palmitoylation sequence from Lyn was cloned into pLL-1 and transfected into CHO cells. Twenty-four hours post-transfection, 10 nM LigandLink Hexachlorofluorescein was added to the cells; 2 hours later, the above image was taken.

In addition, native fluorescent proteins are limited by their intrinsic physical properties and may suffer from high levels of photobleaching, low quantum yield or a number of other physical limitations. The dyes used in LigandLink have been specifically engineered to possess superior physical properties that are ideal for use in common applications such as FRET and co-localization research (Figure 4).

Pre-made translocation vectors

The LigandLink Universal Labeling technology is ideal for studying translocation events because of the highly permeable nature of the TMP ligand and the simple way in which proteins are labeled in *in vivo* environments. To make it even easier, we have already prepared a line of pre-made vectors containing transcription factors such as NFκB, STAT and p53 that are ready to transfect into the mammalian cell line of your choice.

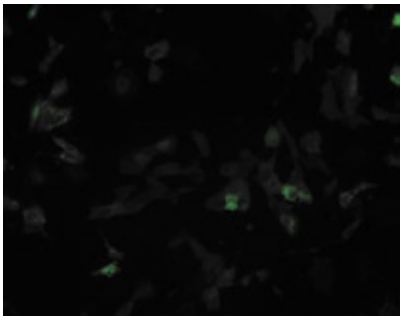


Figure 4: Translocation of NFκB p65 to the nucleus.
The LigandLink pLL-1-NFκB p65 Kit was used to express and monitor the translocation of NFκB into the nucleus. HeLa cells were stimulated with TPA + CI and images were captured 2 hours after stimulation.

Try LigandLink today

LigandLink will improve the way you label proteins in living cells. The highly specific and stable LigandLink tags are ideal for use in FRET and co-localization research because once a protein of interest is expressed as a LigandLink fusion, you can easily change the properties of its tag by adding a different LigandLink label to the medium. Find out more about the new LigandLink system by visiting www.activemotif.com.

Product	Format	Catalog No.
LigandLink™ pLL-1 Kit	1 kit	34001
LigandLink™ pLL-1-AKT1 Kit	1 kit	34002
LigandLink™ pLL-1-AKT2 Kit	1 kit	34003
LigandLink™ pLL-1-NFκB p65 Kit	1 kit	34004
LigandLink™ pLL-1-p53 Kit	1 kit	34005
LigandLink™ pLL-1-STAT1 Kit	1 kit	34006
LigandLink™ Fluorescein Label	300 rxns	34101
LigandLink™ Hexachlorofluorescein Label	300 rxns	34104

* Patent pending.

Detect Protein Phosphorylation Directly Inside the Cell

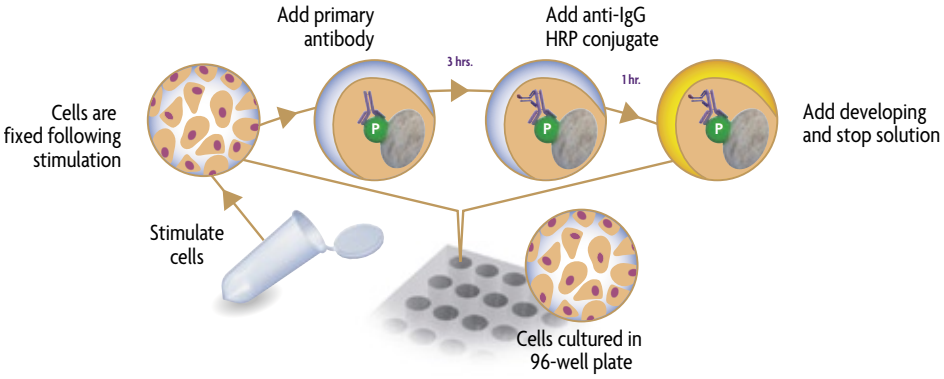
Fast Activated Cell-based ELISA (FACE™) Kits provide you with a simple, sensitive, cell-based method for monitoring protein phosphorylation directly in the cell without the need for cell extracts, gels or membrane blotting.

The FACE method – an "in-cell" Western

In FACE, cells are cultured as desired 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).

No more cell extractions

Because FACE Kits are cell-based, you no longer need to prepare cell extracts and perform Westerns to determine the phosphorylation state of your protein. Plus, as FACE Kits enable you to fix your cells directly after stimulation, further protein modifications that can occur during the extraction process are eliminated. This means you get more accurate results, in a fraction of the time, as FACE Kits require less than 2 hours of hands on time to perform.



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.

FACE™ Product Line			
FACE™ AKT (S473)	FACE™ ATF-2 (T71)	FACE™ Bad (S112)	FACE™ c-Jun (S63)
FACE™ c-Jun (S73)	FACE™ c-Src (Y418)	FACE™ EGFR (Y992)	FACE™ EGFR (Y1173)
FACE™ ErbB-2 (Y877)	FACE™ ErbB-2 (Y1248)	FACE™ ERK1/2 (T202/Y204 & T185/Y187)	FACE™ FAK (Y397)
FACE™ FKHR (T24)	FACE™ GSK3β (S9)	FACE™ JAK1 (Y1022/Y1023)	FACE™ JNK (T183/Y185)
FACE™ MEK1/2 (S217/S221)	FACE™ NFκB Profiler (S468 & S536)	FACE™ p38 (T180/Y182)	FACE™ PI3 Kinase p85
FACE™ STAT2 (Y689)	FACE™ STAT4 (Y693)	FACE™ STAT6 (Y641)	

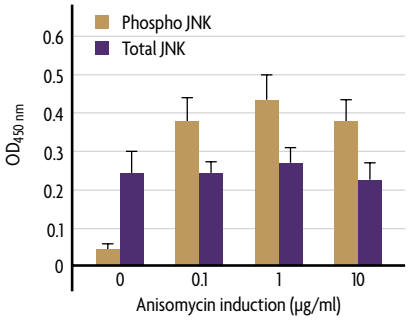


Figure 1: Monitoring the effects of anisomycin treatment on JNK phosphorylation.
The FACE JNK assay was used to assay the levels of phosphorylated JNK in response to different concentrations of anisomycin treatment on macrophage 4/4 cells.

FACE advantages

- **Cell-based method** – no extracts, gels or membrane blotting
- **Fast** – requires less than 2 hours of hands on time
- **Flexible** – use suspension or adherent cells
- **Total & Phospho Antibodies** – compare both phosphorylated and native protein levels in the same kit
- **Quantitative results**

"FACE Kits enable detection of phosphorylated proteins within the cell, eliminating the need for cell extractions, gels & Western blotting."

A variety of kits to choose from

FACE Kits are available for many kinases and receptors, as indicated in the table at left. All kits are conveniently provided in both colorimetric and ultra-sensitive chemiluminescent formats. For more information about the FACE Kits, please visit www.activemotif.com/face.

NEW: Highly Specific Kinase Activity Assays

Active Motif's KineActive™ activity assays enable the study of highly conserved members of kinase families in cell and tissue extracts by providing you with unmatched sensitivity and specificity in a quantitative, non-radioactive, high-throughput format.

Detect only the kinase you want

Many kinase families, such as Src, contain members that phosphorylate a common target. Therefore, to accurately measure the activity of a specific kinase, it is necessary to immunoprecipitate it from others that can act upon the same peptide substrate. The high specificity of the KineActive Kits enable the study of conserved kinase families with ease. To illustrate, recombinant proteins for Src, Yes, Lyn, Fyn, Hck, Lck, Brk and FGR were incubated in KineActive and tested for cross-reactivity using the Src, Yes and Lyn primary antibodies used in the kits. As the data clearly illustrates, the antibodies used in KineActive are specific for only the kinase of interest (Figure 1).

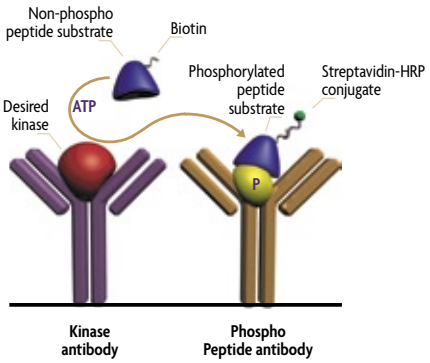
"KineActive provides specific detection of activated kinases in a fast, non-radioactive format."

Advantages

- Quantitative
- Highly specific (see Figure 1)
- Non-radioactive detection
- High-throughput stripwell format
- Includes sample preparation reagents and protein standards
- Improved sensitivity
- Assay cell and tissue extracts or purified protein

Improved technique

Traditionally, kinase activity has been studied through the use of radioactive immunoprecipitation-based kinase assays that can be time-consuming and lack sensitivity. In contrast, KineActive Kits make it simple to assay kinase activity in less than 5 hours in a non-radioactive, 96-stripwell format (See Flow Chart). Plus, because KineActive Kits are very sensitive, you can assay all sample types, including cell lines, tissues and purified proteins. Try KineActive today for an improved method to study kinase activity.



Flow chart of KineActive process.
In KineActive, sample containing the desired kinase is added to a 96-well antibody capture plate. This is followed by addition of kinase antibody, which binds to the plate and specifically captures the kinase of interest. After washing, a biotinylated, non-phosphorylated peptide substrate is added in the presence of ATP. The immobilized kinase phosphorylates this peptide, which is captured by the addition of a second antibody directed against the phosphorylated peptide. Addition of Streptavidin-HRP and developing solution provides a quantitative colorimetric readout.

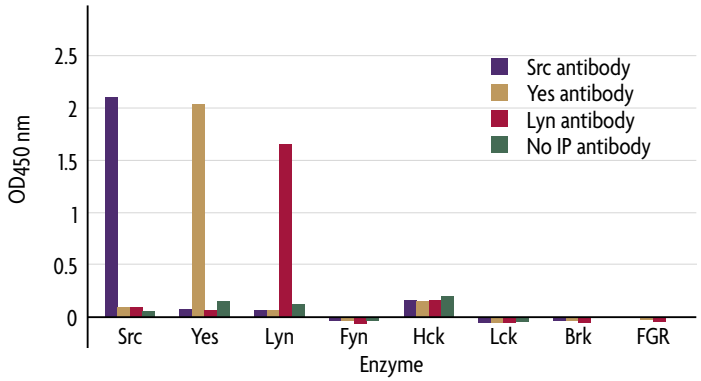


Figure 1: Specific detection of Src family members using KineActive.
Recombinant proteins for Src, Yes, Lyn, Fyn, Hck, Lck, Brk and FGR were incubated in KineActive and tested for specificity using the Src, Yes and Lyn primary antibodies. As the data clearly illustrates, the antibodies used in KineActive are specific for only the factor of interest. Note: the enzymes for Fyn, Hck, Lck, Brk and FGR were tested for activity using radioactive kinase assays in separate experiments.

Product	Format	Catalog No.
KineActive™ Lyn	1 x 96 rxns	56096
KineActive™ Src	1 x 96 rxns	56196
KineActive™ Yes	1 x 96 rxns	56296

Application Note: Quantitation of Activated Transcription Factors

To date, screening large numbers of drug compounds to determine effects on transcription factor activation has been limited by a lack of suitable assays. Existing methods such as Westerns, EMSA and reporter assays lack sensitivity & reproducibility and require substantial processing. In this study, the TransAM™ Kit, an ELISA-based method that assays transcription factor activation[1], is used to examine translocation and DNA-binding activity of transcription factors. Competition assays were also performed.

Materials & Methods

Detection of DNA-binding by ELISA

The TransAM™ NFκB p50 Kit was used to assay DNA-binding activity of NFκB p50 in nuclear and cytoplasmic extracts made from both unstimulated and TNF stimulated HeLa cells. For competitive binding experiments, 5 µg of nuclear extract from stimulated cells were assayed in the presence of wild-type or mutated competitor oligonucleotides (oligos). Each TransAM Kit contains a 96-well plate comprised of twelve 8-well strips. Each well is supplied coated with oligo that contains a consensus-binding site for NFκB (5´-GGGACTTTCC-3´). This sequence is also present in the wild-type competitor oligo. The mutated competitor oligo contains three mutated bases, destroying the NFκB binding site. The p50 and secondary antibodies, as well as Wash & Binding Buffers and Developing Solutions were supplied in TransAM.

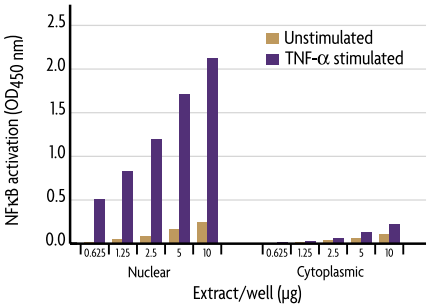


Figure 1: TransAM measures DNA-binding activity. Nuclear and cytoplasmic extracts were assayed using the TransAM NFκB p50 Kit. Because activated NFκB translocates to the nucleus, only nuclear extract from stimulated cells should contain activated NFκB.

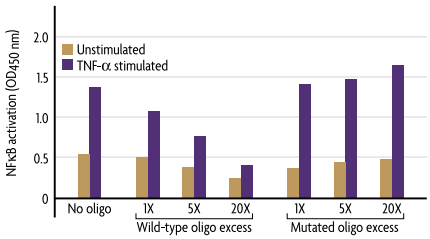


Figure 2: Specificity of TransAM Kits. TransAM assays are performed in the presence of wild-type and mutated competitor oligonucleotides using 10 µg/well of cellular extract.

The TransAM procedure

1. Bind transcription factor to consensus site. 30 µl of Binding Buffer was added to each well. For competitive binding experiments, Binding Buffer contained 1, 5 or 20 pmol of wild-type or mutated competitor oligos. Increasing amounts of HeLa nuclear and cytoplasmic extracts were diluted in 20 µl Lysis Buffer and added to wells being used. After a 1 hour room temperature (RT) incubation, wells were washed 3X with Wash Buffer.

2. Binding of primary antibody. 100 µl of a 1:1000 dilution of NFκB p50 rabbit pAb was added to each well and incubated at RT for 1 hour. After incubation, wells were washed 3X with Wash Buffer.

3. Binding of secondary antibody. 100 µl of a 1:1000 dilution of HRP-conjugated secondary antibody was added to each well and incubated at RT for 1 hour. After incubation, wells were washed 3X with Wash Buffer.

4. Colorimetric reaction. 100 µl TMB substrate was added to each well for 10 minutes before adding 100 µl Stop Solution. Optical density was then read at 450 nm using a spectrophotometer. All results were expressed as averages of duplicates assayed after subtracting the blank values.

Results

Activation & Translocation of NFκB

Figure 1 demonstrates TransAM measures DNA-binding activity of NFκB. The linearity shows that the signal is directly proportional to the quantity of transcription factor. The assay also confirms that stimulation results in both translocation and activation of DNA-binding activity of NFκB as only nuclear extracts from stimulated cells test positive.

Assay Specificity

The specificity of the transcription factor/DNA interaction is confirmed through use of the competitor oligos. Figure 2 demonstrates that increasing amounts of wild-type competitor oligo reduce the signal of the nuclear extract. In contrast, addition of a large excess of mutated competitor oligo does not affect the signal strength.

Conclusion

Because TransAM specifically measures transcription factor bound to its target DNA, it quantifies transcription factor activation. While the extreme complexity of transcriptional regulation dictates that multiple study systems and numerous assay methods be employed in drug discovery efforts, TransAM's unique features (high-throughput, compatible with both cells and tissues, high-sensitivity) make it a powerful addition to existing methods for studying transcription factor activation. TransAM Kits are now available for 30 different factors. Visit www.activemotif.com/transam.

Reference: 1. Renard P. *et al.* (2001) *Nucleic Acids Research* 29(4): E21.

ChIP-IT™ Simplifies Chromatin Immunoprecipitation

Active Motif's kits for chromatin immunoprecipitation (ChIP) simplify your ChIP experiments by providing you with ready-to-use reagents, including shearing and reaction buffers, positive control antibodies and PCR primers, DNA purification columns and a comprehensive protocol to ensure success.

How ChIP works

Chromatin immunoprecipitation is a powerful tool for analyzing genome regulation. In ChIP-IT, intact cells are fixed using formaldehyde, which cross-links and preserves protein/DNA interactions. The DNA is then sheared into small, uniform fragments and protein/DNA complexes are immunoprecipitated using antibodies directed against the protein(s) of interest. Next, the cross-links are reversed and DNA fragments are purified and screened to determine which gene, or group of genes, were bound by the protein of interest.

"ChIP-IT improves ChIP experiments by providing ready-to use reagents and proven controls in a single kit."

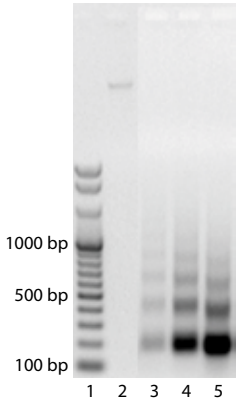


Figure 1: Analysis of DNA sheared using the Enzymatic Shearing Kit.
HeLa cells were fixed for 10 minutes with 1% formaldehyde and then chromatin was prepared using the Enzymatic Shearing Kit protocol. Chromatin was sheared with the enzymatic shearing cocktail for 5, 10 & 15 minutes and the reaction was stopped.
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 (optimized).
Lane 5: HeLa DNA treated for 15 minutes.

ChIP-IT advantages

- Easy – includes critical buffers, Protein G and purification columns
- 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

A successful ChIP begins with correctly shearing DNA into 200-1000 bp fragments. This can be achieved using either enzymatic digestion or sonication. Sonication is an effective method for shearing DNA but can be difficult to optimize. In contrast, enzymatic digestion can be used to quickly and easily digest DNA into fragments suitable for ChIP with very little effort. For your convenience, ChIP-IT Kits are available with either sonication or enzymatic shearing.

Order a ChIP-IT Kit 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, visit www.activemotif.com/chip.

Save on Antibodies for ChIP

Finding antibodies that have been proven to work in ChIP is not easy. Although our list of ChIP-validated antibodies is growing (see Table 1), we need your help. That's why in the months of May and June we are offering 50% off the purchase of any antibody that is purchased in conjunction with one of our ChIP-IT Kits. Visit www.activemotif.com to browse our antibody search engine.

Antibody	Cat. No.	Price ($US)
AP-2 pAb	39304	280
c-Jun pAb	39309	280
C/EBPα pAb	39306	280
DNMT1 mAb	39204	280
DNMT3A mAb	39206	280
DNMT3B mAb	39207	280
E2F-1 pAb	39313	280
E2F-6 mAb	39509	280
GATA-1 pAb	39025	280
HBP-1 mAb	39511	280
HDAC3 pAb	40968	280
HDAC4 pAb	40969	280
HDAC5 pAb	40970	280
HDAC6 pAb	40971	280
IRF-3 pAb	39033	280
JunB pAb	39326	280
JunD pAb	39328	280
p53 pAb	39334	280
Pax-5 pAb	39336	280
PPARγ pAb	39338	280
RNA pol II mAb	39097	280
Sp1 pAb	39058	280
TRF2 pAb	39223	280

Table 1: ChIP-validated antibodies available.

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

MethylDetector™ – Fast & Efficient DNA Methylation Analysis

Active Motif's MethylDetector™ Bisulfite Modification Kit makes DNA methylation analysis fast and efficient by combining optimized reagents for performing DNA conversion with time-saving DNA purification columns and positive control PCR primers to help you validate your results.

Proven controls ensure success

DNA methylation analysis typically involves using bisulfite to convert unmethylated cytosines to uracils, while leaving methylated cytosines unchanged. The DNA is then amplified by PCR and analyzed by sequencing or restriction digest. However, bisulfite conversion can be technically challenging, and confirming the process was successful before sample analysis is preferred. MethylDetector provides positive control PCR primers that are specific for bisulfite-converted DNA, so you can confirm the procedure worked before starting further costly analysis (Figure 1).

Advantages

- **Flexible** – use high G/C content sequences and uncut DNA
- **Reproducible** – 99% conversion efficiency
- **Efficient** – reagents and protocol with proven controls
- **Easy** – DNA purification columns mean no more precipitations

Try MethylDetector today

MethylDetector will speed and simplify your methylation analysis. Learn more at www.activemotif.com.

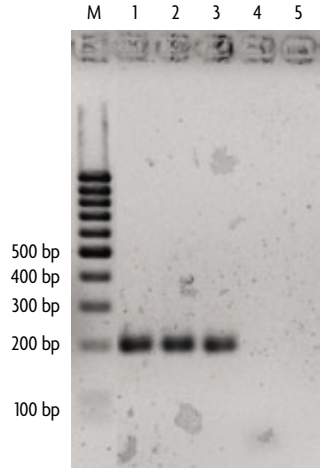


Figure 1: Agarose gel analysis of PCR products generated with MethylDetector.
Three different DNA conversions were performed (Lanes: 1-3) and compared to an unconverted DNA control (Lane: 5) and to a no DNA control (Lane: 4). The presence of PCR product in only the converted samples demonstrates the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

Product	Format	Catalog No.
MethylDetector™	50 rxns	55001

Co-Immunoprecipitation of Nuclear Protein Complexes

Performing co-immunoprecipitation of nuclear protein complexes has just gotten easier as Active Motif's Nuclear Complex Co-IP Kit provides optimized reagents that maintain DNA-binding protein complexes and improve results.

Optimized method and reagents

Co-immunoprecipitation (Co-IP) is often used to analyze protein/protein interactions. However, traditional Co-IP methods are not optimal for studying DNA-binding protein complexes as these complexes are unstable. The Nuclear Complex Co-IP Kit helps maintain these complexes by providing optimized reagents for both nuclear extract preparation and immunoprecipitation, including both high and low stringency buffers, as well as additional salt and detergent to modify stringency as required.

Advantages

- Simple and efficient
- Optimized extraction procedure preserves nuclear protein complexes
- Easily alter IP stringency to detect interactions of varying strengths

Order the new Co-IP Kit today

The Nuclear Complex Co-IP Kit enables you to study tightly bound or weak protein complexes with equal ease. Visit www.activemotif.com.

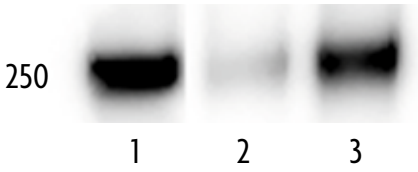


Figure 1: Western blot analysis of the IP'd p33 subunit of the RNA pol II complex.
For IP experiments, the stringency of the IP High Buffer was increased by supplementing with NaCl and Detergent. 100 µg of HeLa nuclear extract was used per IP and incubated with either 2 µg p33 antibody or no antibody. Following the IP, Western blot using RNA pol II mAb was used to detect the p33/RNA pol II complex (lane 3). This demonstrates the Co-IP was successful in maintaining the protein complex.

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

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

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).

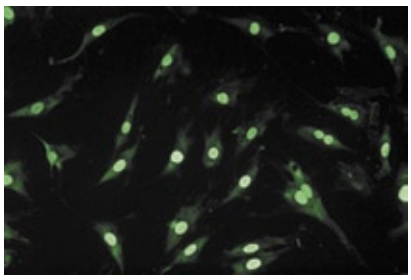


Figure 1: Targeted protein delivery.
50 ng of a 10 kDa nuclear protein that was labeled with Lucifer yellow at the C-terminus was complexed with Chariot and delivered into HS-68 cells. Unfixed cells were observed 90 minutes post-delivery.

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).

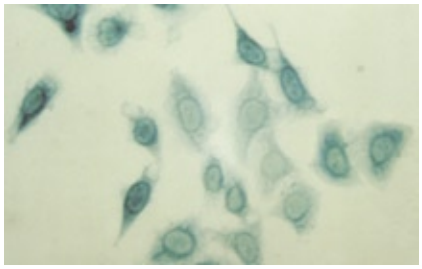


Figure 2: Chariot delivery of β-galactosidase.
One µg of a 119 kDa subunit of β-galactosidase was complexed with Chariot for 30 minutes and delivered into HeLa cells. Cells were fixed and stained with X-gal 2 hours post-delivery.

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.

NEW: Effective Fluorescent Labels for Bioanalysis

Active Motif's Chromeo™ Dyes improve labeling of biomolecules for cell culture experiments because they exhibit excellent photostability, pH tolerance, low cell toxicity and can be used with most excitation sources.

Recent advances in fluorescent chemistry and fluorescent detection equipment have facilitated a rapid increase in the development of synthetic dyes for analysis of biomolecules. Fluorescent dyes are widely used for cell identification, flow cytometry and cellular imaging of localization and movement of subcellular structures. For a fluorescent dye to be useful in fluorescence microscopy it must possess many key features: brightness of conjugates, photostability, solubility and pH insensitivity. In addition, detection limits that are significantly improved compared with colorimetric-based detection platforms is essential.

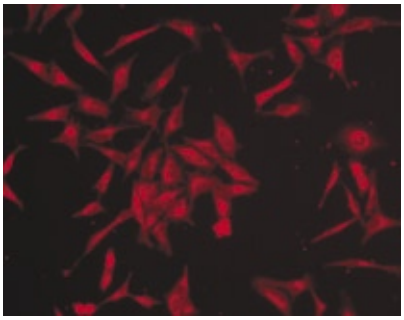


Figure 1: Cytosolic staining of HeLa cells using Chromeo 494.

Excellent fluorescent properties

The Chromeo Dyes, Chromeo 494, Chromeo 546 and Chromeo 642, exhibit many key features that make them ideal for any fluorescent application including superior luminescence properties and broad Stokes shifts (Table 1). Chromeo Dyes are also suitable for use with many common excitation sources such as diode lasers, LEDs, tungsten and Xenon arc lamps. In addition, Chromeo 494 has a very large Stokes shift of 124 nm, making it an ideal partner for multiplexing with other, short-shifted 488-excitable dyes.

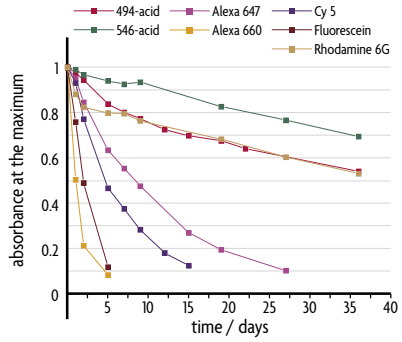


Figure 2: Photostability test.
Dyes were dissolved in PBS (22 mM, pH 7.2) and the solutions were allowed to stand at room temperature in daylight. Absorbance at the respective maximum was measured over a period of 36 days.

Chromeo™ Dye advantages

- Convenient spectral properties
- High quantum yields
- pH insensitive
- Available as reactive NHS-Esters to label amino groups or as carboxylic acid
- Unmatched photostability

Visit the Active Motif Chromeon website

As a leader in providing fluorescent tools for bioanalysis, Active Motif Chromeon also offers several other dyes to meet your research needs, including RuLabels and Py-Dyes. RuLabels are phosphorescent ruthenium probes that emit strong polarized light with a long decay time, which enhances sensitivity and reduces background. The Py-Dyes possess unique physical and spectral properties that make conjugation simple, while greatly reducing background. To find out more about Active Motif Chromeon products visit www.chromeon.com.

Dye	Absorption	Emission	ε L/(mol-cm)	Quantum Yield (%)*	Stokes Shift
Chromeo™ 494	494	628	20,000	25	124 nm
Chromeo™ 546	545	561	96,800	10	16 nm
Chromeo™ 642	642	660	180,000	15	18 nm

Table 1: Chromeo Dye properties. (* when conjugated to BSA)

Product	Format	Catalog No.
Chromeo™ 494 Carboxylic Acid	1 mg 5 mg	15110 16110
Chromeo™ 494 NHS-Ester	1 mg 5 mg	15111 16111
Chromeo™ 546 Carboxylic Acid	1 mg 5 mg	15210 16210
Chromeo™ 546 NHS-Ester	1 mg 5 mg	15211 16211
Chromeo™ 642 Carboxylic Acid	1 mg 5 mg	15310 16310
Chromeo™ 642 NHS-Ester	1 mg 5 mg	15311 16311

You can find additional Chromeon Dyes at www.chromeon.com

Tap into the Knowledge Stored in Medline with AlmaKnowledgeServer

AlmaKnowledgeServer (AKS) is a powerful text mining system that makes it possible for you to uncover relationships that exist in the scientific literature between genes, proteins, chemical compounds and diseases. AKS can even discover links that exist, but that have not yet been published.

Keep current with the latest papers

Researchers read journal articles to learn about new developments in their field. But, with 1000's of journals and more than 15 million abstracts on Medline (plus over 2,000 more added daily), it is physically impossible to keep current, let alone to explore all of the areas that may provide new and useful insights.

The answer is AlmaKnowledgeServer (AKS), a powerful text mining system that, unlike you, reads every paper every day. AKS examines all abstracts added to PubMed, then applies statistics and rules-based analysis to uncover relationships that exist in the scientific literature between genes, proteins, chemical compounds and diseases. This enables you to make better informed decisions about your research in far less time.

Bioentity and synonym recognition

AKS understands and retrieves biological concepts (bioentities) instead of simple words. The bioentities recognized by AKS include genes and proteins, diseases and chemical compounds. AKS has been taught the many synonyms, acronyms and other scientific naming ambiguities that plague traditional searching. For example, searching PubMed for IL-2, IL2, IL 2 or interleukin 2 return different results, though all are the same gene. So, an exhaustive search for a single gene may actually require 5-10 individual searches. With AKS, one search returns all documents that are relevant to IL-2.

Learn by exploring co-occurrences

After a search, AKS ranks the relevant concepts and bioentities. Documents can be explored by viewing sentences in which two or more bioentities occur (Figure 1). This makes it possible to build a significant understanding of the interrelations between bioentities before you've even read any of the papers. You can also read abstracts with co-occurring bioentities highlighted, and apply filters to fine tune your results. And, AKS provides links to Medline, so you can quickly access the papers of interest.

What's a picture worth? 1000's of papers

Because knowledge often comes by seeing data from a different perspective, AKS also makes it possible to view bioentity relationships graphically. Figure 2 diagrams the relationships between co-occurring bioentities. The power of the system is that even tough this visual representation contains the content of thousands of documents, the relationships are easily seen, helping you to obtain new insights with minimal effort.

Information for better decision making

AKS can help provide answers that are important to your research, like which proteins are involved in a disease, or who are the thought leaders in an emerging field? To learn more, give us a call or visit Active Motif's biocomputing division, TimeLogic, at www.timelogic.com.

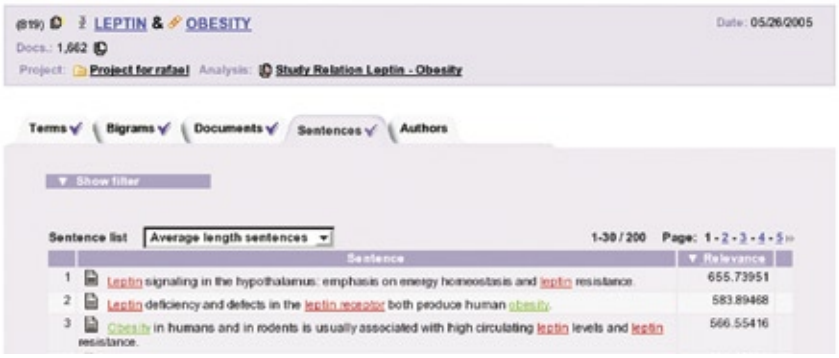


Figure 1: Sentences displaying co-occurrences of "leptin" and "obesity" bioentities.

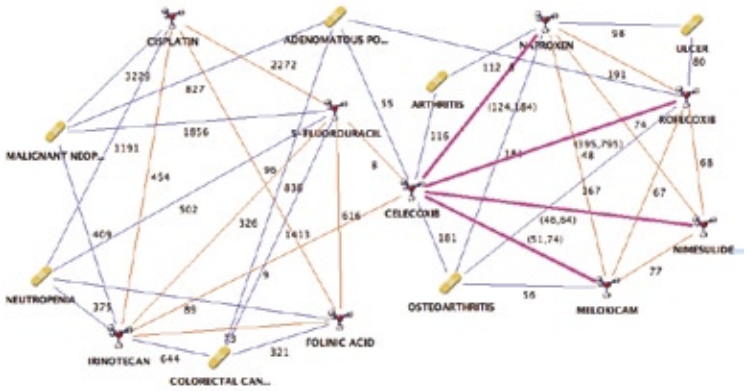


Figure 2: AKS includes a powerful visual environment to map relationships between bioentities.

Optimized Preparation of Cellular Samples

Active Motif provides many kits that are ideal for preparing and quantifying samples from cell or tissues samples. These include our popular Nuclear Extract Kit, Mitochondrial Fractionation Kit, GAPDH Whole-cell Normalization Kit and ProStain™ Protein Quantification Kit.

Nuclear Extract Kit

The Nuclear Extract Kit is ideal for preparing nuclear, cytoplasmic and whole-cell extracts from mammalian cells and tissues because it eliminates the need to optimize reagents while ensuring consistently high yields. The resultant high-quality extracts may be used with Active Motif's TransAM™ Kits, gelshift assays, Western blots, DNA footprinting, as a starting point for transcription factor purification and more.

Mitochondrial Fractionation Kit

The Mitochondrial Fractionation Kit isolates highly enriched mitochondrial and cytosolic fractions from mammalian cells and tissues, simplifying the study of protein translocation events that occur during apoptosis and in many 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.

Protein Quantification & Normalization

After you have prepared your samples, it is also important to either quantitate your samples or normalize them for downstream use. Active Motif's ProStain™ Protein Quantification Kit is a sensitive fluorescent-based assay that offers superior detection limits over traditional protein assays such as Bradford, while also offering resistance to many contaminants. As an alternative to a protein assay, the GAPDH Whole-cell Normalization Kit can be used to normalize protein samples, as GAPDH is constitutively expressed at high levels in almost all tissues.

Product	Format	Catalog No.
Nuclear Extract Kit	100 rxns	40010
	400 rxns	40410
Mitochondrial Fractionation Kit	100 rxns	40015
GAPDH Whole-cell Normalization Kit	96 rxns	48007
ProStain™ Protein Quantification Kit	100 rxns	15001

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ACTIVE MOTIF®
Tools to Analyze Cellular Function