

Quantitative Measurement of the GATA, HNF and IRF Transcription Factor Families

Traditionally, transcription factor activity has been studied using either Electrophoretic Mobility Shift Assays (EMSA), immunoblotting or reporter gene assays. These methods are time-consuming and at best provide only semi-quantitative results. Moreover, determination of transcription factor subunit activity requires the investment of significant amounts of both time and money, or may not even be possible. Now, TransAM™ Family Kits make it straightforward to measure the activity of families of transcription factors in one simple experiment and in less than 5 hours*. Plus, the TransAM Family Kits can be used on all sample types, including cell lines and tissues, giving you unsurpassed flexibility.

The TransAM method

Each TransAM Family Kit includes two 96-well plates in which multiple copies of a double-stranded oligonucleotide have been immobilized. When nuclear or whole-cell extract is added, the various members of the transcription factor family of interest bind to the consensus-binding site on the oligonucleotide. Primary antibodies specific for the activated forms of each family member are then added to individual wells, followed by incubation with secondary HRP-conjugated antibody and developing reagent. Reading the plate on a spectrophotometer provides a quantitative, colorimetric readout (Figure 1, page 4).

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An Improved Method to Study DNA Damage and Repair

Active Motif's new DNA Repair Kits offer an improved method for studying DNA repair protein activity. Current techniques used to study DNA damage and repair include time-consuming EMSAs and Western blots. Our new DNA Repair Kits are DNA-binding ELISAs that eliminate radioactivity and provide quantitative results in less than five hours. Studying DNA repair has never been easier.

DNA repair proteins act to maintain genome integrity by recognizing, binding to and repairing damaged DNA. Damage recognition proteins such as G/T binding protein (GTBP, also called MSH6) and Ku70/86 bind as heterodimers to DNA with specific

lesions. GTBP recognizes mismatched G/T base pairs in double-stranded DNA and triggers excision and repair. In contrast, Ku70/86 binds directly to DNA ends and repairs double-stranded DNA breaks. Deficiencies in the activity of these proteins is linked to the development of many pathological diseases, including cancer. Yet, despite the growing interest in studying the relationship between DNA damage and repair, there is a lack of convenient, sensitive assays that are suitable for processing large numbers of samples. To overcome this, we are introducing a new line of high-throughput assays to quantify DNA repair proteins, beginning with kits for GTBP and Ku70/86 (Figure 1).

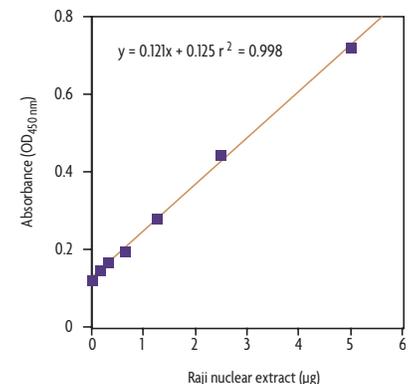


Figure 1: Monitoring Ku70 with the Ku70/86 DNA Repair Kit. Different amounts of nuclear extract from Raji cells were tested for activity using the Ku70/86 DNA Repair Kit.

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Fast, Reproducible Normalization Method for More Meaningful Results

Active Motif's new GAPDH Whole-cell and Lamin Nuclear Normalization Kits make it fast and easy to normalize your samples prior to downstream applications, such as PAGE, Western blot and ELISA analysis. This reduces inaccuracies by ensuring that you are comparing equivalent amounts of protein in each sample. The Whole-cell and Nuclear Normalization Kits quantify the housekeeping proteins GAPDH and Lamin, which are widely used for normalization as they are present in high levels in the cytoplasm and nucleus, respectively. The kits utilize an ELISA-based assay that is more reproducible than other methods. They also feature a convenient 96-stripwell format, so you can process multiple samples quickly, at any level of throughput.

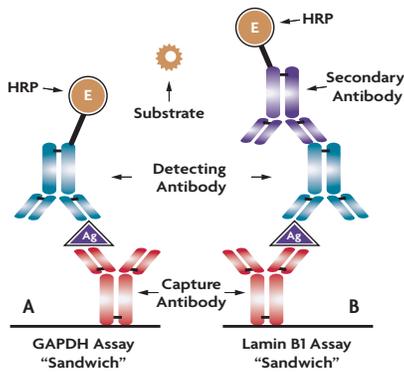


Figure 1: Sandwich ELISA schematics.

Why be normal?

Before performing many cell biology assays, the protein concentration of each sample must be quantified, commonly using a Bradford or Lowry protein assay. "Equal" amounts of each sample are then assayed. However, these methods can yield variable results depending on the protein composition of the extract and its buffer type. This can introduce significant errors in subsequent assays because, in fact, unequal amounts of the different samples are being assayed. Unlike these methods, Active Motif's normalization kits use an antibody-based detection method, so your results are not altered by the protein makeup of your sample or its buffer composition.

Simple, specific method

Active Motif's new normalization kits provide a simple, specific method for protein normalization. They utilize the "Sandwich" ELISA method, which uses two antibodies, each recognizing a distinct epitope on the protein. Each kit includes an ELISA plate coated with the first antibody, called the Capture Antibody. This is used to capture either GAPDH or Lamin B1 from the sample. A second antibody, called the Detecting Antibody, is used to detect the protein bound by the Capture Antibody. Horse-radish peroxidase (HRP) is then used for colorimetric detection. In the GAPDH Kit, HRP is directly conjugated to the Detecting Antibody, while the Lamin Kit utilizes an HRP-conjugated secondary antibody (Figures 1A & 1B). Each kit provides quantifiable and reproducible results through the generation of standard curves using the included GAPDH (Figure 2) or Lamin B1 protein standard. Using the curve, the amount of GAPDH or Lamin B1 per sample can then be used to normalize your assay results.

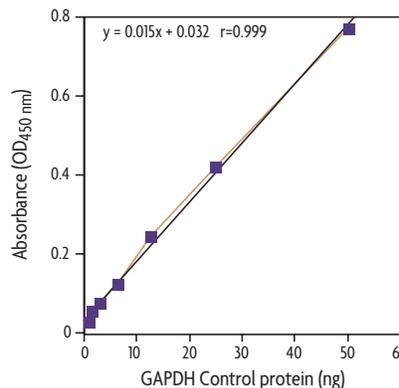


Figure 2: Production of a GAPDH Standard Curve. The GAPDH Whole-cell Normalization Kit was used with known amounts of the GAPDH Control protein to generate a standard curve.

Verify the integrity of your samples

The Lamin Nuclear Normalization Kit can also be used to verify the integrity of your samples. Because Lamin B1 is found only in the nucleus, its presence in cytoplasmic extracts would indicate contamination of the extract by nuclear proteins. To demonstrate, nuclear and cytoplasmic extracts were made from NIH/3T3 cells using our Nuclear Extract Kit (page 7). The absence of Lamin B1 in the cytoplasmic extract confirms the integrity of the extracts (Figure 3).

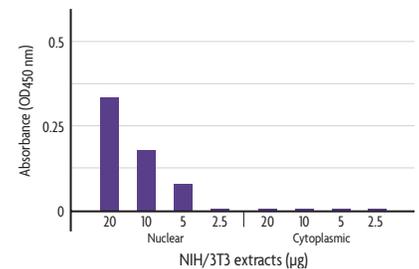


Figure 3: Assaying cytoplasmic and nuclear extracts for Lamin B1. The Lamin Nuclear Normalization Kit was used to assay cytoplasmic and nuclear extracts that were isolated using the Nuclear Extract Kit.

Get better results every experiment

Active Motif's new normalization kits are a simple way to improve the accuracy of your cell biology assays. They eliminate the variability introduced into your research by inaccurate protein determination methods. In many instances, the normalization kits make it possible for you to perform your assays without even determining the protein concentration of your samples. With normalization this easy, there's no longer a reason to not improve all of your results.

| Product | Format | Catalog No. |
|------------------------------------|-------------|-------------|
| GAPDH Whole-cell Normalization Kit | 1 x 96 rxns | 48007 |
| | 5 x 96 rxns | 48507 |
| Lamin Nuclear Normalization Kit | 1 x 96 rxns | 48008 |
| | 5 x 96 rxns | 48508 |

Ultra-sensitive Chemiluminescent Assay for MAPK Phospho-proteins

Fast Activated Cell-based ELISA (FACE™) Kits are an innovative alternative to classical methods for monitoring protein phosphorylation. FACE Kits enable modification-specific analysis directly within the cell, without the need for time-consuming cell extractions, gel electrophoresis or membrane blotting. In addition to the highly popular colorimetric version, FACE Kits are now available in a new ultra-sensitive, chemiluminescent format. Even the smallest change in protein phosphorylation can be detected simply and quickly with the new FACE Chemi Kits.

Regulation of cellular protein activity via phosphorylation has been shown to be critical for normal functioning of the majority of cellular pathways. Indeed, many disease states such as cancer and autoimmune responses have been attributed to the aberrant control of protein phosphorylation. Yet despite widespread interest, measurement of protein phosphorylation has remained low-throughput and labor intensive. FACE Kits are designed to overcome the limitations of classical assays and at the same time improve the quality of your results.

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 fixed, which preserves protein modifications, including phosphorylation. Each well is then incubated with a primary antibody specific for the activated protein of interest. Subsequent incubation with secondary HRP-conjugated antibody and developing solution provides an easily quantified, colorimetric or chemiluminescent readout. The signals are then normalized for cell number using the provided Crystal Violet solution and plotted. FACE Kits are easy to use and require less than two hours hands on time, giving you convenience not available with other phosphorylation assays.

Flexibility with chemiluminescent detection
Colorimetric ELISA measurement of protein phosphorylation offers a more convenient alternative to Western blotting and in-gel kinase assays. However, use of colorimetric detection can make it difficult to maintain linearity within the assay readout, meaning that small changes in protein phosphorylation can be lost due to sub-optimal detection. The new FACE Chemi Kits, however, use chemiluminescence, so the assay detection limits can be adjusted to maintain linearity and ensure that your detection sensitivity is appropriate to your sample type. This means that FACE Chemi Kits can accurately monitor even the smallest changes in protein phosphorylation. For experiments that require maximum sensitivity, try the new FACE Chemi Kits.

Reproducible - for more meaningful results
FACE Kits are highly reproducible, which is vital for getting the most meaningful results possible when measuring small changes in the amounts of phosphorylated protein. To demonstrate, FACE Chemi assays were performed on three different samples of macrophage 4/4 cells that had been treated in an identical manner with PMA to

induce ERK phosphorylation (Figure 1). The combination of the high reproducibility and increased throughput of FACE Chemi Kits means that you'll get the most meaningful phospho-specific results available.

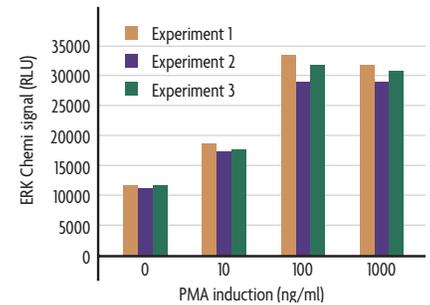


Figure 1: Reproducible assay of phosphorylated ERK.
Murine macrophage 4/4 cells were cultured in 96-well plates and serum starved for 16 hours. Cells were then stimulated with Phorbol 12-myristate 13-acetate (PMA) for 10 minutes and fixed. Levels of phospho-ERK were assayed in triplicate using the FACE ERK1/2 Chemi Kit. Data was plotted after normalization for cell number (performed through use of Crystal Violet).

FACE Kits are available for monitoring the cellular levels of phosphorylated p38, JNK, AKT, ERK1/2 and EGFR, with many other kits to be released throughout 2003. For a better way to monitor protein phosphorylation, use FACE.

| Product | Format | Catalog No. |
|--------------------|-------------|-------------|
| FACE™ AKT Chemi | 1 x 96 rxns | 48220 |
| | 5 x 96 rxns | 48720 |
| FACE™ EGFR Chemi | 1 x 96 rxns | 48250 |
| | 5 x 96 rxns | 48750 |
| FACE™ ERK1/2 Chemi | 1 x 96 rxns | 48240 |
| | 5 x 96 rxns | 48740 |
| FACE™ JNK Chemi | 1 x 96 rxns | 48210 |
| | 5 x 96 rxns | 48710 |
| FACE™ p38 Chemi | 1 x 96 rxns | 48200 |
| | 5 x 96 rxns | 48700 |

continued from page 1 — Quantitative Measurement of the GATA, HNF and IRF Transcription Factor Families

Proven specificity – for improved accuracy

To accurately study transcription factor activation, you must be able to determine which of the complex mixture of related heterodimers is involved in pathway regulation. To ensure that this is possible, all antibodies supplied in TransAM Family Kits are tested for cross-reactivity with other family members and shown to recognize only the isoform of interest. In addition, TransAM Family Kits are tested for specificity by assaying in the presence of an excess of oligonucleotide containing a wild-type or mutated consensus-binding site (Figure 2). This competitive assay proves that the transcription factor isoform detected is binding specifically to the probe that has been immobilized to the TransAM plate. With the guaranteed specificity of the TransAM Family Kit antibodies, you'll get the accuracy you want for all your experiments.

The complete solution for all your needs

Reporter gene assays expressed either transiently, or from a stable cell line, are commonly used to monitor transcription

factor activation. However, it can be difficult to relate this data to actual *in vivo* experiments, which can limit more relevant, downstream research. TransAM Family Kits provide a complete solution that is well suited for both model systems and *in vivo* studies. Don't waste time with DNA transfections or stable cell line construction, which can't be used *in vivo*; TransAM Kits are designed to meet all your needs.

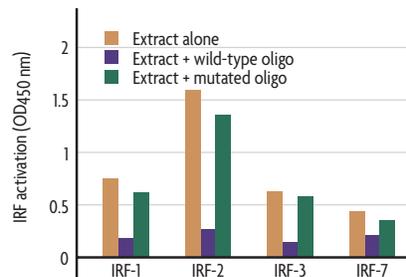


Figure 2: Specificity of TransAM Kits.

TransAM IRF assays are performed in the absence or presence of competitor oligonucleotides that contain either a wild-type or mutated IRF consensus-binding site. IRF-1, IRF-2 and IRF-3 activity was assayed using 5 µg/well nuclear extract from U-937 cells stimulated with TPA + IFN γ , while IRF-7 activity was assayed using 5 µg/well nuclear extract from Jurkat cells stimulated with PHA.

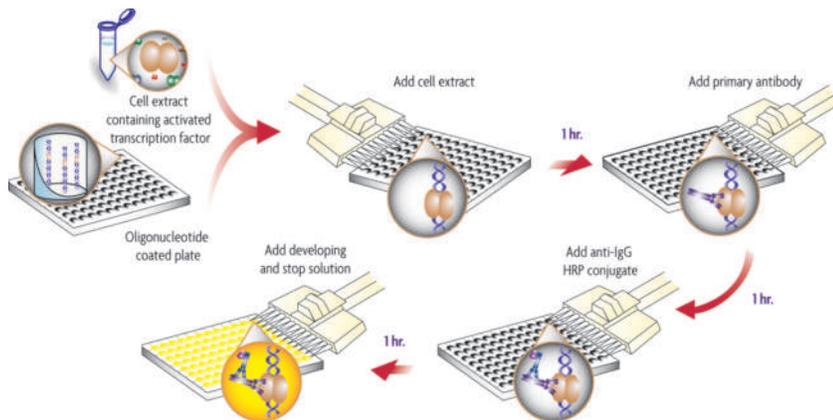


Figure 1: Flowchart of the TransAM procedure.

| Product | Format | Catalog No. |
|----------------------|--------------------|-------------|
| TransAM™ AP-1 Family | 2 x 96-well plates | 44296 |
| TransAM™ GATA Family | 2 x 96-well plates | 48296 |
| TransAM™ HNF Family | 2 x 96-well plates | 46296 |
| TransAM™ HNF-1 | 1 x 96-well plate | 46196 |
| | 5 x 96-well plates | 46696 |
| TransAM™ IRF Family | 2 x 96-well plates | 45296 |
| TransAM™ NFκB Family | 2 x 96-well plates | 43296 |
| TransAM™ STAT Family | 2 x 96-well plates | 42296 |

TransAM IRF Family

Interferon Regulatory Factors (IRFs) are a family of transcription factors that play a critical role in host defense. The function and activity of the individual IRFs can depend both on their activation state as well as that of other IRF family members. The ability of the TransAM IRF Family Kit to simultaneously profile the activation states of IRF-1, -2, -3 and -7 makes it easy to understand the critical role of each isoform in the regulation of immune function and oncogenesis.

TransAM HNF Family

Hepatocyte Nuclear Factors (HNFs) are primarily required for cellular differentiation and metabolism. Unlike most transcription factor families, this group of evolutionary conserved proteins does not have a common consensus-binding site. This makes assaying HNF family activity using classical EMSA or reporter assays cumbersome and time consuming. The new TransAM HNF Family Kit contains multiple binding sites in each well, making it quick and easy to monitor HNF-1, -3 α , -3 β and -4 α activity in a single assay. In addition, a TransAM HNF-1 Kit is available for researchers who want to monitor this important regulator of liver function separately.

TransAM GATA Family

The GATA family of transcription factors contains six zinc-finger binding proteins that regulate differentiation and cell proliferation. GATA proteins are grouped into two subfamilies. The new TransAM GATA Family Kit measures GATA-1, -2, and -3, the hematopoietic subfamily. As hematopoietic cells are difficult to transfect, they are often studied as primary isolates, making reporter gene assays and EMSA impractical. The TransAM method, however, can be used easily on primary isolates, so it is ideal for GATA research.

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

continued from page 1 — An Improved Method to Study DNA Damage and Repair

How it works

The DNA Repair Kit method is simple and efficient and can be used with both tissue and cell culture extracts. Each kit includes a 96-well plate in which multiple copies of a specific double-stranded oligonucleotide have been immobilized. The oligonucleotide in the GTBP Kit contains a G/T mismatch, while that in the Ku70/86 Kit simply has a blunt end. When cellular extract is added, the repair protein of interest binds to the oligonucleotide. Each well is then incubated with a primary antibody that is specific for the repair protein being studied. Addition of a secondary HRP-conjugated antibody and developing solution provides an easily quantified colorimetric readout (Figure 2). This allows you to easily monitor the regulation of DNA repair proteins in a particular cell line, animal model or tumor biopsy.

Advantages

- Non-radioactive, colorimetric method
- Quantitative results in less than 5 hours
- 10X more sensitive than gelshift
- High-throughput compatible
- Ability to assay both cells and tissues

Precise, quantitative results

Being able to precisely measure small changes in DNA repair protein activity within any sample is important. Although traditional methods such as Western blot are sensitive, they are typically more of a qualitative rather than quantitative tool. Active Motif's DNA Repair Kits, however, provide a colorimetric readout that is easily quantified, so you can detect even the slightest change in protein activity. To illustrate, GTBP activity was measured using Active Motif's GTBP DNA Repair Kit and compared to a Western blot of the same sample. Assays were performed on Raji, PANC-1 and Jurkat nuclear extracts. The GTBP DNA Repair Kit results are clearly more quantitative than Western blot (Figure 3).

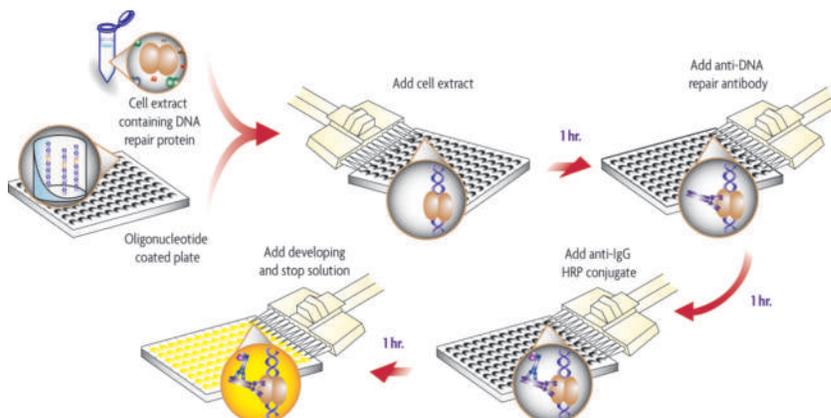


Figure 2: Flowchart of the DNA Repair Kit procedure.

Improved accuracy

The DNA Repair Kits are accurate over a wider range of sample concentrations than other methods because they use colorimetric detection, which is linear over a broader sample range (Figure 1, page 1). This saves time and money, as fewer samples will need to be diluted and reassayed to obtain accurate results. Get the most out of your experiments by using the new DNA Repair Kits.

What's available

DNA Repair Kits are currently available for studying GTBP and Ku70/86. The GTBP Kit contains an antibody specific for GTBP, while the Ku70/86 Kit contains two antibodies, specific for either Ku70 or Ku86. Be sure to check our website for complete kit configurations, downloadable manuals and to learn about new additions to the DNA damage and repair product line.

Try it today

Our DNA Repair Kits will simplify the way you study interactions between damaged DNA and repair proteins. These easy-to-use kits are flexible enough to use in a variety of functional studies, such as examining the specificity of repair proteins for a particular DNA damage. And, the increased sensitivity means you can accurately detect small changes in activity levels. If you're tired of using radioactive probes, try one of our DNA Repair Kits today.

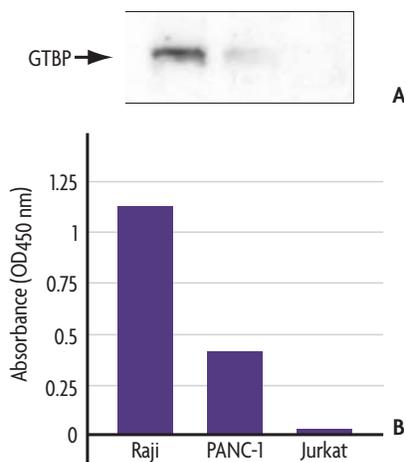


Figure 3: Measurement of GTBP activity in different cell lines using Western blot and the new GTBP DNA Repair Kit. Jurkat, PANC-1 and Raji nuclear extracts were assessed for GTBP activity using Western blot (A) and the new GTBP DNA Repair Kit (B).

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

Efficient Delivery of Biologically Active Proteins

Chariot™ is a revolutionary delivery reagent that efficiently transports biologically active proteins, peptides and antibodies directly into cultured cells in less than two hours. Typical delivery efficiencies are 65-95%. After delivery, living cells can be assayed immediately to determine the effects of the introduced material, without the need for fixing. This makes Chariot ideal for functional studies.

Simple delivery method

Chariot is a peptide that forms a non-covalent complex when combined with purified protein, peptide or antibody. This protects the protein from degradation during internalization. Upon addition to cells, the complex is rapidly internalized, where it dissociates. The delivered protein is biologically active and free to proceed to its cellular target.

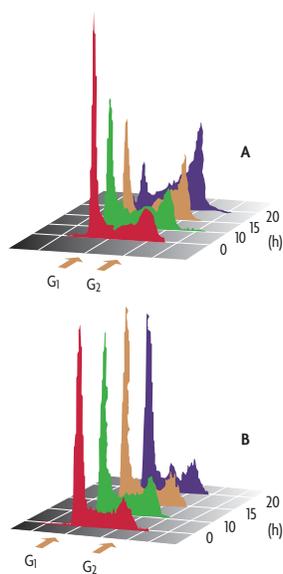


Figure 1: Chariot delivery of functional protein.

HS-68 cells arrested in G₁ phase by serum deprivation for 48 hours were released by addition of serum for 3 hours. Flow cytometry performed 0, 10, 15 and 20 hours after addition of Chariot alone and a Chariot-p27^{kip1} complex indicate that cells receiving Chariot alone progressed into G₂ phase (A), while over 90% of cells receiving the Chariot-p27^{kip1} complex remained in G₁ phase (B). Data generously provided by Dr. Gilles Divita, Biophysics Dept., CNRS, Montpellier, France.

| Cell line or model | Cell type | Macromolecule delivered | Transfection efficiency (%) |
|--------------------------------|--------------------------------------|----------------------------|-----------------------------|
| 3T3-L1 | Mouse fibroblast | Antibody | 80% |
| A549 | Human lung carcinoma | Antibody | 95% |
| Arabidopsis | Primary plant protoplasts | Protein | Not provided |
| COS-7 | Monkey kidney | Protein, oligopeptide | 80% |
| CV-1 | Monkey kidney | Antibody | 55% |
| HCA2 | Human fibroblasts | Protein | 90% |
| HeLa | Human cervix carcinoma | Protein, peptide, antibody | 95% |
| HMSC | Primary human mesenchymal stem cells | Peptide | 80% |
| HS-68 | Human foreskin fibroblast | Protein, peptide, antibody | 95% |
| IMR90 | Human fibroblast | Protein, peptide | 80% |
| Jurkat | Human T-cell leukemia | Protein, peptide, antibody | 75% |
| Mono Mac 6 | Primary human monocyte | Protein | 50% |
| Mouse hepatocytes | Primary liver | Protein | 95% |
| Mouse model (<i>in vivo</i>) | Alveolar wall tissue | Protein | 88% |
| Neural retina cells | Primary chicken | Protein | 80% |
| NIH/3T3 | Mouse embryo | Protein, peptide | 80% |
| NRK | Normal rat kidney | Antibody | 30-60% |
| PC-12 | Rat pheochromocytoma | Protein, peptide | 80% |
| Sensory neurons (DRG) | Primary chick | Antibody, peptide | 80% |
| Sensory neurons (DRG) | Primary rat | Protein | 80% |
| Thyocytes | Primary human | Protein | 90% |
| WI-38 | Human lung fibroblast | Protein, peptide, antibody | 95% |
| WISH | Human placenta carcinoma | Protein | 30% |

Deliver biologically active proteins

The ability of Chariot to deliver biologically active compound was demonstrated using p27^{kip1}, a 27 kDa cyclin-dependent kinase inhibitor that causes cell-cycle arrest in G₁ phase. Over 90% of cells receiving Chariot-p27^{kip1} complex were unable to progress beyond G₁ phase (Figures 1A & 1B), demonstrating efficient delivery of active p27^{kip1}.

What can Chariot do for you?

Many papers have been published in scientific journals citing the use of Chariot. Successful delivery has been shown in a variety of cell types, including hard-to-transfect

cells like neuronal and plant cells. Recently, the first paper showing usage of Chariot *in vivo* was published. The table above was compiled from data furnished to us by respondents to our Chariot survey. For the complete survey, or to download a list of papers that cite Chariot, visit our website.

Chariot delivers results

Chariot speeds and simplifies a variety of functional studies because it efficiently delivers biologically active proteins, peptides and antibodies directly into cells, even hard-to-transfect and non-dividing cells. For a new way to study your protein, try Chariot.

| Product | Format | Catalog No. |
|----------|----------|-------------|
| Chariot™ | 25 rxns | 30025 |
| | 100 rxns | 30100 |

Fast and Simple Mitochondrial Fractionation

The Mitochondrial Fractionation Kit isolates highly enriched mitochondrial and cytosolic fractions from mammalian cell lines. This simplifies the study of protein translocation

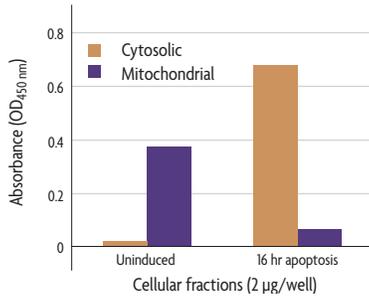


Figure 1: Monitoring cytosolic and mitochondrial cytochrome c levels. HeLa cells were grown to 90-95% confluence and treated with 10 nM Actinomycin D to induce apoptosis. Cell samples were harvested prior to treatment as well as 16 hours post-treatment. The Mitochondrial Fractionation Kit was used to isolate cytosolic and mitochondrial fractions from each sample. Two µg of each fraction was assayed using the FunctionELISA™ Cytochrome c Kit to monitor changes in the mitochondrial and cytosolic cytochrome c levels.

events that occur during apoptosis and in 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.

Precise fractionation

To demonstrate its utility, Active Motif's Mitochondrial Fractionation Kit was used to isolate mitochondrial and cytosolic fractions from both uninduced and apoptotic HeLa cells. The FunctionELISA™ Cytochrome c Kit was used to determine the levels of cytochrome c in the mitochondrial and

cytosolic fractions because the release of cytochrome c from mitochondria is a key indicator of apoptosis. As expected, nearly all cytochrome c in uninduced cells was found in the mitochondrial fraction, while nearly all the cytochrome c in the apoptotic sample was in the cytosol (Figure 1).

Upstream quality for downstream success

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

| Product | Format | Catalog No. |
|---------------------------------|---|----------------|
| Mitochondrial Fractionation Kit | 100 rxns | 40015 |
| FunctionELISA™ Cytochrome c | 1 x 96-well plate 5 x 96-well plates | 48006 48506 |

High Yields of Specifically Segregated Extracts

Active Motif's Nuclear Extract Kit enables you to isolate high yields of active nuclear, whole-cell and cytoplasmic extracts from mammalian cell and tissue samples. Its use eliminates the need to optimize your own reagents and protocols, so you get error-free results every time.

Nuclear Extract Kit advantages

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

Results you can rely on

Accurate research often depends on precisely isolating proteins from a specific cellular compartment. If nuclear extract contains cytoplasmic proteins or vice-versa, your results may not be valid. The Nuclear Extract Kit helps ensure that proteins remain segregated in their particular fraction.

Specific segregation for accurate results

To demonstrate the importance of using a quality extraction procedure, nuclear and cytoplasmic extracts were prepared from unstimulated and TNF-α-stimulated HeLa cells using our Nuclear Extract Kit and assayed for activated NFκB p50 using the

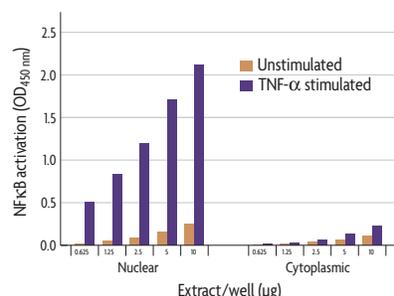


Figure 1: NFκB p50 activity in nuclear and cytoplasmic extracts. Increasing amounts of nuclear and cytoplasmic extracts isolated with the Nuclear Extract Kit are assayed using TransAM™ NFκB p50.

TransAM™ NFκB p50 DNA-binding assay (Figure 1). As activated NFκB is not present in the cytoplasm, only nuclear extract from the stimulated cells should test positive. This is precisely what was seen, demonstrating the specificity of the kit.

Quality in means quality out

Extracts prepared using the Nuclear Extract Kit are ideal for use in any experiment that requires nuclear, whole-cell or cytoplasmic extract. In addition, Active Motif offers over 100 different ready-to-use extracts from a variety of cell lines and tissue types (page 8). Whether you choose to make them using our kit or purchase them from us, the use of high-quality extracts will improve the accuracy of your downstream experiments. To get reproducible results, try the Nuclear Extract Kit.

| Product | Format | Catalog No. |
|---------------------|----------------------|----------------|
| Nuclear Extract Kit | 100 rxns 400 rxns | 40010 40410 |

Specific Antibodies & Quality Positive Control Extracts

Highly characterized transcription factor antibodies

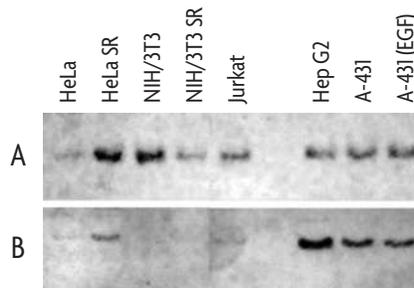
Active Motif offers one of the broadest lines of mono- and polyclonal antibodies for cell signaling and transcription factor-related research. These antibodies are ideal for performing Western blots, ELISAs and supershift/electrophoretic mobility shift assays (EMSA).

With over 200 antibodies to choose from, evaluation of cell cycle regulators and transcription factors is easier than ever before. Our antibodies are highly characterized using appropriate cellular extracts, so you can be sure of their quality. And, to make using our antibodies as simple as possible, each antibody is accompanied by a detailed, lot-specific protocol.

Whether you are researching AP-1, C/EBP, NF κ B/Rel, Rb, Sp1 or STAT, we can help. Don't waste your time and money on custom antibody services when we may already have the antibodies you need.

Ready-to-use positive control extracts

Many types of cell biology research require high-quality cell and tissue extracts. However, there's no longer a need to isolate these extracts yourself. Active Motif offers quality extracts available from a variety of cell types and tissue sources. Many of our extracts have been stimulated or treated to specifically induce transcription factor activation. All of our extracts are tested for activity and cross-contamination by Western blot (Figure 1) or with our TransAM™ transcription factor ELISAs (page 1). So, why spend time developing your own positive control when we have already done it for you?



Find the right positive control & antibody

To make your life easier, we have tested our extracts to determine which antibodies test positive in each extract. This allows you to quickly pick the right positive control for your antibody of interest.

Active Motif's website has an easy-to-use search engine; you can search for antibodies and extracts, download their data sheets and determine which extracts are appropriate for use as a positive control.

To use this valuable tool and save time while finding the antibodies and positive controls you need, logon to our website at www.activemotif.com.

Figure 1: Western blot analysis using Active Motif antibodies & extracts. HeLa, HeLa SR (1 hour serum response), NIH/3T3, NIH/3T3 SR (1 hour serum response), Jurkat, Hep G2, A-431 and A-431 (EGF) nuclear extracts were loaded at 9 μ g per well and separated by SDS-PAGE. The membranes were then analyzed by Western blot using NF- γ B pAb (Panel A) and C/EBP β pAb (Panel B).

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