MOTIF VATIONS

THE NEWSLETTER OF ACTIVE MOTIF — MAY 2004 • VOLUME 5 • NUMBER 2

ACTIVE MOTIF®

Innovative Tools for Cell & Molecular Biology

Faster Phospho-specific Analysis using Cell-based ELISAs

Active Motif's Fast Activated Cell-based ELISA (FACE™) Kits enable you to detect phosphorylation events faster and easier than ever before. Conventional techniques such as in-gel kinase assays and Western blotting are costly, time-consuming and provide you with only semi-quantitative results. Moreover, because additional protein modifications can occur during cell extraction procedures, results obtained using these techniques may not reflect actual *in vivo* conditions. FACE cell-based assays eliminate the need for extracts, radioactivity, electrophoresis and membrane blotting, providing you with quantitative results in record time.

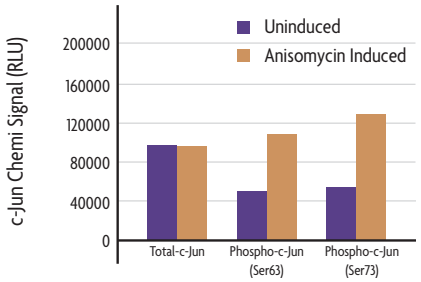

Figure 1: Monitoring c-Jun phosphorylation using FACE.
The FACE c-Jun (S63) and c-Jun (S73) Chemi Kits were used to assay the levels of total and phosphorylated c-Jun contained within uninduced and anisomycin-induced NIH/3T3 cells.

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Simple, Efficient Delivery of Proteins into Cells

Chariot™ is a revolutionary delivery reagent that transports biologically active proteins, peptides and antibodies directly into mammalian cells in about 2 hours with a typical efficiency of 65-95%. After delivery, living cells can be assayed to determine the effects of the introduced molecules. This makes Chariot ideal for a wide variety of functional studies, including delivery of inhibitory peptides, organelle labeling, screening peptide libraries and transient complementation studies.

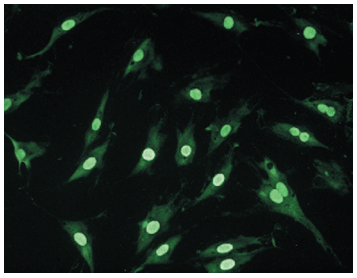

Figure 1: Targeted protein delivery.
50 ng of a 10 kDa nuclear protein labeled with Lucifer yellow at the C-terminus was complexed with Chariot and delivered into HS-68 cells. Unfixed cells were observed 90 minutes post-delivery.

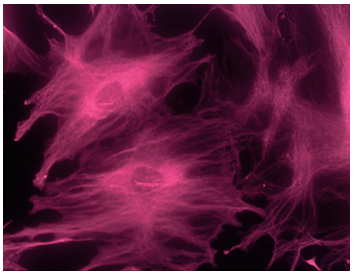

Figure 2: Delivery of Actin antibody.
Chariot was complexed with a 1/1000 dilution of Actin antibody and used to label Actin filaments in human fibroblast (HS-68) cells. Unfixed cells were observed 2 hours post-delivery.

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IN THIS ISSUE

Complete Solution for More Successful Chromatin Immunoprecipitation (ChIP)

Chromatin immunoprecipitation (ChIP) is a powerful tool for analyzing genome regulation because it combines the specificity of immunoprecipitation with the sensitivity of PCR. However, ChIP is a complicated, multi-step procedure that can be technically challenging and yield results that are difficult to interpret. To help eliminate these problems, Active Motif's ChIP-IT™ Kit combines nearly everything needed to successfully perform ChIP. The ChIP-IT Kit includes antibodies, controls, reagents and a comprehensive protocol that have been proven to work in ChIP. This provides a complete solution that makes it possible to successfully obtain and validate your results.

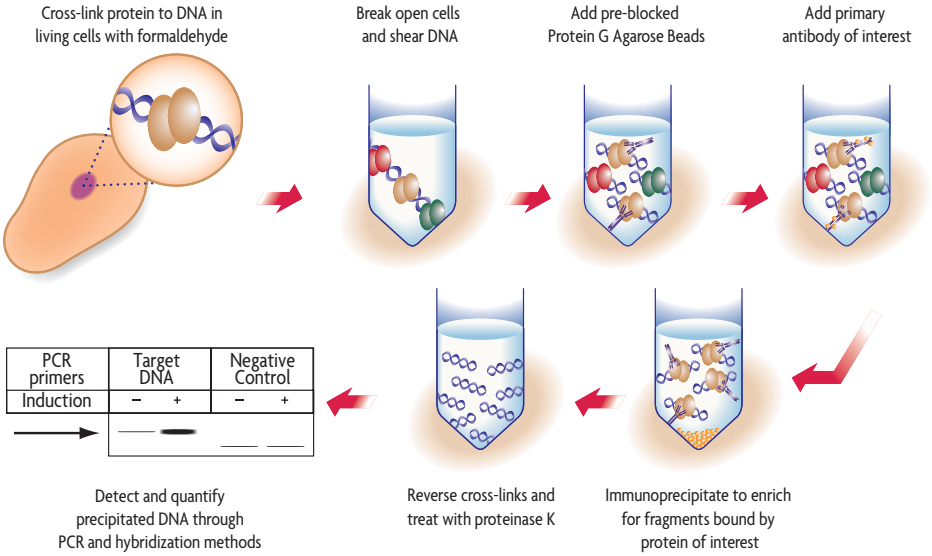


Figure 1: Schematic of chromatin immunoprecipitation. In ChIP, protein/DNA interactions are fixed, and the DNA is then sheared and precipitated using an antibody. After reversing the cross-links, the DNA is purified and then screened to determine which genes were bound by the protein of interest.

What is ChIP; why is it used?

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

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

What factors are important when performing ChIP?

Successful ChIP depends on a variety of different factors: proper fixation of the protein/DNA interactions, preparing sheared DNA of the correct length, and having both an antibody that works in immunoprecipitation as well as a PCR primer set that specifically amplifies the region of DNA bound by the protein of interest. A variety of specialized buffers, protease inhibitor cocktails and blocking reagents are also needed to ensure that the protein/DNA interactions are preserved and to minimize non-specific enrichment and reduce background. ChIP experiments are likely to be unsuccessful should any of these steps or solutions fail. However, using the ChIP-IT Kit means you won't have to worry about this, as all reagents are rigorously QC'd and proven to work in ChIP.

How does the ChIP-IT Kit make ChIP more successful?

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

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

Sensitive, Specific Transcription Factor Analysis

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, 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. TransAM™ Kits, however, make it straightforward to measure the activity of entire families of transcription factors in one simple experiment that is complete in less than 5 hours. Plus, TransAM Kits can be used on all sample types, including cell lines and tissues, giving you unsurpassed flexibility.

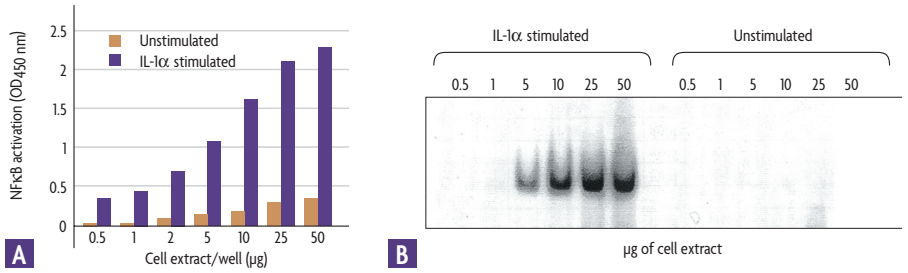


Figure 1: TransAM NFκB is more sensitive than gelshift.
Human fibroblast WI-38 cells are stimulated with IL-1α for 30 minutes. Increasing amounts of whole-cell extract are assayed using either the TransAM NFκB p50 Kit (A) or gel retardation (B).

Assay more relevant sample types

Many important disease states are known to be associated with aberrant changes in cellular gene expression. Consequently, transcription factors, which regulate gene expression, are of great interest both for their potential as therapeutic targets and as biological markers of disease states. Reporter gene assays expressed transiently or from a stable cell line are commonly used as a primary screen in drug discovery and biomarker research. However, because it can be difficult to relate cell culture-based data to actual *in vivo* experiments, the value of reporter assays is limited. And, reporter assays rely on the intracellular delivery of vectors, which can be time-consuming and inefficient. In contrast, TransAM Kits can selectively bind activated transcription factors in cellular extracts isolated from either tissue or cell culture samples. This means that TransAM Kits are ideal for use in both cell culture-based screening and in more biologically relevant, *in vivo* experiments. Why use reporter assays when you can access the most versatile transcription factor assay available?

TransAM advantages

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

A better transcription factor assay

EMSA has long been considered the "gold standard" for measuring transcription factor activation. However, EMSAs are time consuming, radioactive and lack sensitivity. Not only this, but they yield limited data, which is prone to non-specific effects that can complicate data interpretation. Fortunately, science and consequently the technology around it, is constantly evolving and improving. TransAM Kits are one such improvement. The highly sensitive ELISA-based approach of TransAM makes obtaining and interpreting results simple, safe, fast and consistent. Plus, you can easily analyze entire families of transcription factors in a single experiment, while using a fraction of the sample needed to obtain results using EMSA (Figure 1). Why not try the new "gold standard" of transcription factor measurement today?

The latest additions

Active Motif has developed a number of kits to study both individual transcription factors as well as transcription factor families (see table below). TransAM Family Kits contain multiple antibodies so that you can simultaneously profile the activation levels of several different family members. The new TransAM MAPK Kit will even enable you to profile the activity of multiple families of transcription factors, which are regulated by the MAPK kinase cascade. For better transcription factor analysis, try TransAM Kits.

TransAM Product Line

TransAM™ AP-1 Family	TransAM™ AP-1 c-Fos	TransAM™ CREB	TransAM™ MyoD	TransAM™ p53
TransAM™ GATA Family	TransAM™ AP-1 c-Jun	TransAM™ pCREB	TransAM™ NF-YA	TransAM™ PPARγ
TransAM™ HNF Family	TransAM™ AP-1 FosB	TransAM™ Elk-1	TransAM™ NFATc1	TransAM™ Sp1
TransAM™ IRF Family	TransAM™ AP-1 JunD	TransAM™ ER	TransAM™ NFκB p50	TransAM™ Sp1/Sp3
TransAM™ MAPK Family	TransAM™ ATF-2	TransAM™ HIF-1	TransAM™ NFκB p50 Chemi*	TransAM™ STAT3
TransAM™ NFκB Family	TransAM™ c-Myc	TransAM™ HNF-1	TransAM™ NFκB p65	
TransAM™ STAT Family	TransAM™ C/EBP α/β	TransAM™ MEF2	TransAM™ NFκB p65 Chemi*	

* TransAM Chemi Kits require the use of a luminometer.

continued from page 1 — Faster Phospho-specific Analysis using Cell-based ELISAs

How does FACE work?
FACE Kits are easy to use and require less than 2 hours of hands on time. In FACE, cells are cultured in the provided 96-well plates and stimulated to induce the pathway of interest. The cells are then rapidly fixed with formaldehyde to preserve protein modifications, including phosphorylation. Each well is then incubated with a primary antibody specific for the activated protein of interest. This is followed by secondary HRP-conjugated antibody and developing reagent, which provides a sensitive colorimetric or chemiluminescent readout. FACE Kits are supplied complete with everything needed to perform one 96-well assay with the phosphorylated antibody and one 96-well assay with the total antibody for the protein of interest. Finally there's a kit that makes it easy for you to compare activated to native protein levels at the same time (Figure 1, page 1 and Figure 2C).

FACE advantages

- **Cell-based format** – no extracts, gels, blotting or radioactivity
- **Fast** – requires less than 2 hours of hands on time
- **Flexible** – high-throughput chemi and colorimetric formats
- **Accurate** – sensitive readout means less well-to-well variation
- **Quantitative** – provides more meaningful results

Try FACE Kits today
FACE Kits offer you a fast, sensitive and quantitative alternative to traditional assays. New kits are available for studying the activation of ATF-2, c-Jun, EGFR, ErbB-2, GSK3β and MEK1/2. To get to the most up-to-date information available on FACE Kits, visit our website at www.activemotif.com.

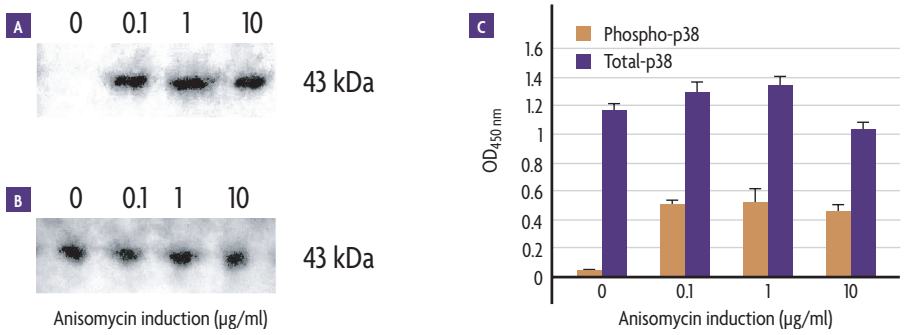


Figure 2: Phospho- and total-p38 MAPK assays.
Macrophage 4/4 cells were grown in 10 cm dishes to 80% confluency, serum-starved for 16 hours and stimulated with anisomycin for 15 minutes. Cell lysates were made and Western blots performed using phospho- (A) and total-p38 (B) antibodies. For FACE, 4/4 cells were grown in 96-well plates, stimulated as above, fixed and then assayed in triplicate using the FACE p38 Kit (C). Data were corrected for cell number through use of the kit's Crystal Violet Dye.

Product	Format	Colorimetric Kit Catalog No.	Chemiluminescent Kit Catalog No.
FACE™ AKT	1 x 96 rxns 5 x 96 rxns	48120 48620	48220 48720
FACE™ ATF-2	1 x 96 rxns 5 x 96 rxns	48115 48615	48215 48715
FACE™ c-Jun (S63)	1 x 96 rxns 5 x 96 rxns	48125 48625	48225 48725
FACE™ c-Jun (S73)	1 x 96 rxns 5 x 96 rxns	48135 48635	48235 48735
FACE™ EGFR (Y992)	1 x 96 rxns 5 x 96 rxns	48150 48650	48250 48750
FACE™ EGFR (Y1173)	1 x 96 rxns 5 x 96 rxns	48190 48690	48290 48790
FACE™ ErbB-2 (Y877)	1 x 96 rxns 5 x 96 rxns	48130 48630	48230 48730
FACE™ ErbB-2 (Y1248)	1 x 96 rxns 5 x 96 rxns	48105 48605	48205 48705
FACE™ ERK1/2	1 x 96 rxns 5 x 96 rxns	48140 48640	48240 48740
FACE™ GSK3β	1 x 96 rxns 5 x 96 rxns	48170 48670	48270 48770
FACE™ MEK1/2	1 x 96 rxns 5 x 96 rxns	48180 48680	48280 48780
FACE™ JNK	1 x 96 rxns 5 x 96 rxns	48110 48610	48210 48710
FACE™ p38	1 x 96 rxns 5 x 96 rxns	48100 48600	48200 48700

High-Quality Transcription Factor Antibodies for Westerns, EMSA & ChIP

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

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

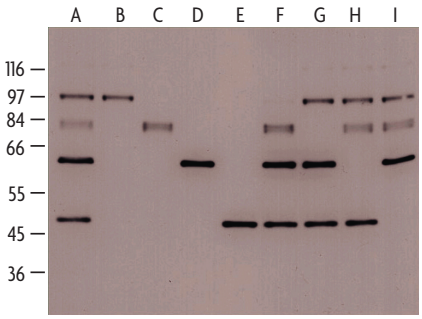


Figure 1: Western blot with Active Motif antibodies.
Using nuclear extract from Raji cells, antibodies for Sp1 (B), c-Rel (C), YY1 (D) and Pax-5 (E) were tested individually or as a mixture (A) to reveal their respective antigens. The mixed antibodies were simultaneously incubated with one peptide at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

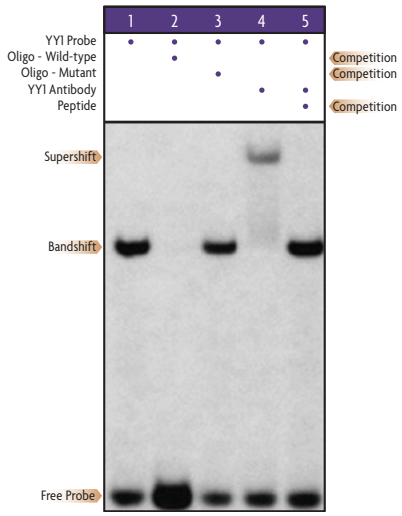


Figure 2: Highly specific YY1 mobility shift.
The Nushift YY1 Kit (Catalog No. 37043) detects YY1 using nuclear extracts prepared from K-562 cells. Specificity is determined by use of the wild-type and mutated competitor oligonucleotides and neutralizing peptide.

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

Antibodies for ChIP
Chromatin immunoprecipitation is one of proteomics' powerful new tools for analyzing genome regulation (see page 2). However, one of the drawbacks of performing ChIP is finding antibodies that are proven to work in this technique. Although not all of our antibodies have been tested for use in ChIP, a high proportion have been validated in EMSA using our Nushift Kits. This is a good indication that the antibody will also function in ChIP, which increases your chances of getting the antibody you need for ChIP the first time. Currently our antibodies against AP-2, ATF-6, E2F-1, E2F-6, GATA-1, HDAC3, HDAC4, IRF-3, Pax-5 and Sp1 are ChIP positive. As this list is growing, be sure to check our website for up-to-date information.

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

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

continued from page 1 — Simple, Efficient Delivery of Proteins into Cells

How does Chariot work?

Chariot delivers antibodies, peptides and proteins into cells through a 4-step process (Figure 1):

1. Association of Chariot to your macromolecule to form a stable complex;
2. Membrane uptake of the complex at the external cell surface;
3. Insertion into the cell membrane, conformational change and pore formation;
4. Release of the complex into the cytoplasm, followed by complex dissociation.[1]

Non-covalent delivery

Many protein delivery systems require that you fuse a carrier protein to your macromolecule. However, this can change the folding characteristics of your protein and, ultimately, its function. Because Chariot uses non-covalent, charged interactions to "cage" proteins, it does not affect protein folding or function. The result is the delivery of highly active, native proteins.

Independent of the endosomal pathway

Experiments at 4°C have shown that Chariot delivery is independent of the endosomal pathway.[2] This is important because endocytosis can cause protein modifications, and may also prevent release of the protein into the cytoplasm, both of which will effect the success of your experiment. In contrast, the Chariot complex enters the cell through an interaction with the cell membrane. When the Chariot complex crosses the cell membrane, the complex is destabilized, leaving unmodified antibody, protein or peptide free to perform its function.

Don't just take our word for it

There is a lot of exciting research taking place with Chariot; much of it would have not been possible without the help of this innovative technology. The ability to delivery functionally active protein directly into mammalian cells makes it easy to perform studies that are not possible using conventional methods. Our website offers an extensive list of publications describing such novel research, as well as data that has been furnished to us by respondents to our Chariot survey (see table above). Details of two recent, innovative publications are shown below:

Cell line or model	Cell type	Macromolecule delivered	Delivery 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 (*in vivo*)	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%
Thyrocytes	Primary human	Protein	90%
WI-38	Human lung fibroblast	Protein, peptide, antibody	95%
WISH	Human placenta carcinoma	Protein	30%

"Direct Activation of Bax by p53 Mediates Mitochondrial Membrane Permeabilization and Apoptosis" was published in *Science* in February 2004. It describes delivery of wheat germ agglutinin by Chariot, thereby blocking p53 accumulation in the nucleus.[3]

The *American Journal of Respiratory Cell & Molecular Biology* published "Alveolar Wall Apoptosis Causes Lung Destruction and Emphysematous Changes" in 2003. Here, an *in vivo* model was used to study apoptosis through delivery of Caspase-7 protein into living mouse lung tissue by Chariot.[4]

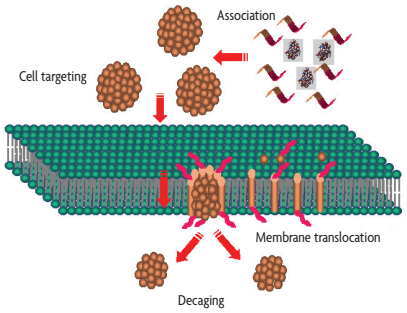


Figure 1: Schematic model for translocation of the Chariot complex. Figure generously provided by Dr. Gilles Divita, Biophysics Dept., CNRS, Montpellier, France.[1]

To get ideas on what you might do with Chariot, please visit our website at www.activemotif.com.

1. Deshayes *et al.* (2004) *Biochemistry* 43:1449-1457.
2. Morris *et al.* (2001) *Nature Biotech.* 19:1173-1176.
3. Chipuk *et al.* (2004) *Science* 303:1010-1014.
4. Aoshiba *et al.* (2003) *Am. J. Respiratory Cell & Mol. Bio.* 28:555-562.

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

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

More Effective Gene Silencing

Active Motif's custom gripNAs™ offer a highly effective alternative to traditional antisense- and siRNA-based gene silencing. gripNA probes utilize a modified antisense approach that offers high-affinity binding with unsurpassed sequence discrimination. This minimizes non-specific interactions that can cause unintended, mutant phenotypes. Plus, delivery is simple using either a lipid-based approach or Chariot™ II. gripNAs have proven to be effective at silencing genes in Zebrafish, mammalian cells and *Xenopus*. And because gripNAs are resistant to nuclease degradation, they are stably maintained in the cell, enabling them to silence genes over a prolonged period.

New antisense for specific gene silencing

The use of antisense molecules has long been viewed as a promising strategy for gene-specific silencing. However, successful gene silencing using classical DNA-antisense technology has been limited by the rapid degradation of probes by intracellular nucleases, insufficient target affinity and non-specific side effects. In order to overcome these limitations, several novel oligonucleotide chemistries have been developed. Peptide nucleic acids (PNAs) are a novel form of DNA-analogs that possess several characteristics that are ideal for gene silencing. PNAs are resistant to nucleases and display high-affinity and sequence-specific binding. However, PNAs have also proved to be inefficient tools for gene silencing, due mostly to their lack of cell permeability, tendency to self-aggregate and poor solubility. Active Motif's new gripNAs are a novel form of negatively charged PNA that overcome the limitations of classical PNAs. They are highly soluble, can be efficiently delivered using multiple approaches and have been shown to be highly effective at gene silencing (Figure 1).

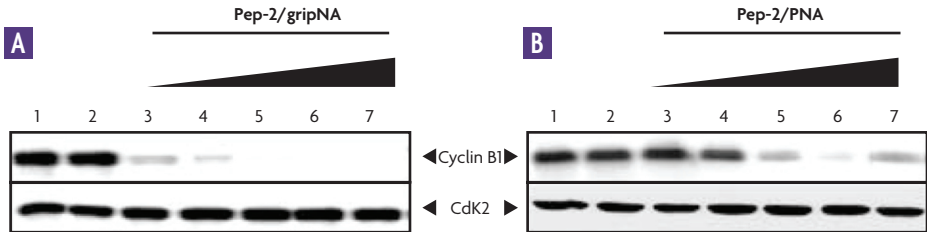


Figure 1: Inhibition of expression by gripNAs and PNAs directed against cyclin B1.
Increasing concentrations (lane 3: 50 nM, lane 4: 100 nM, lane 5: 500 nM, lane 6: 1 µM, lane 7: 2 µM) of anti-cyclin B1 gripNA (A) or PNA (B) were incubated with Pep-2 at a molar ratio 1:25 at 37°C for 1 hour, then overlaid onto cultured HS-68 cells. Cyclin B1 protein levels were analyzed by Western blotting after 24 hours. Cdk2 protein was used as a control to normalize protein levels. The level of endogenous cyclin B1 in untreated cells is shown in lane 1 of each panel, while lane 2 shows addition of gripNA or PNA alone, without Pep-2. Data generously provided by Dr. Gilles Divita, Biophysics Dept., CNRS, Montpellier, France.[2]

Hit your target only

A recurring problem of many gene silencing reagents is a lack of specificity. This can cause unintended phenotypes because these reagents may bind and silence expression of more than just the intended target. *In vivo* mismatch discrimination experiments have shown that gripNA binding is reduced or eliminated by the presence of a single base-pair mismatch. Recent experiments performed in Zebrafish embryos indicate that this specificity can improve your results. While wild-type gripNA and morpholino probes were comparable in potency at silencing the *chordin*, *uroD* and *no tail* genes, the intentional inclusion of 2 and 4 base-pair mismatches in the morpholino probes caused non-specific effects that were not triggered by comparable gripNA probes.[1]

Order anytime

Active Motif's custom gripNA synthesis service can provide you with better materials for your gene silencing experiments. All the information needed to design, order and use gripNA probes can be accessed at www.activemotif.com/gripna. Our simple, online ordering system makes it easy to place your gripNA order 24 hours a day, 7 days a week. gripNA probes can be ordered with or without Chariot II. In addition, you can add a 3´ modification (Biotin, FITC or primary amine) to your probe. Each gripNA is verified by mass spectrophotometry and supplied with a fluorescently labeled positive control for human CREB. Log on now and order your custom gripNA today.

1. Urtishak *et al.* (2003) *Developmental Dynamics* 228(3).
2. Morris *et al.* (2004) *Gene Therapy* (Feb 12, e-pub ahead of print).

Product	Format	Catalog No.
Custom gripNA™ Probe	200 nmol	24001
Custom gripNA™ Probe w/Chariot™ II	200 nmol	24002
Primary Amine Modification		24004
Biotin Modification		24005
Fluorescein Modification		24006
Chariot™ II	96 rxns	24008
gripNA™ Human CREB Positive Control	25 nmol	24007
gripNA™ Chordin Positive Control	5 nmol	24009

Fast, Quantitative Measurement of DNA Repair Protein Activity

Active Motif's DNA Repair Kits provide a fast, user-friendly format for studying DNA damage and repair protein activity. This is because our DNA Repair Kits utilize the DNA-binding properties of DNA repair proteins to selectively capture activated protein, which is then detected and quantified using the kit's highly characterized antibodies. Assaying protein activity using the DNA Repair Kits is fast and simple, which saves you both time and money.

In recent years, a clear link has been established between cancer and genome instability. Defects within the cellular recombination and repair machinery can have disastrous effects to the cell and lead to cancer. This makes DNA repair proteins ideal drug targets and creates a need for new sensitive, accurate and convenient assays for monitoring DNA repair protein activity. To meet this need, Active Motif has developed its innovative DNA Repair Protein Kits.

Advantages

- Non-radioactive, colorimetric method
- 10X more sensitive than EMSA
- Results in under 5 hours
- Works with cells and tissues
- High-throughput compatible

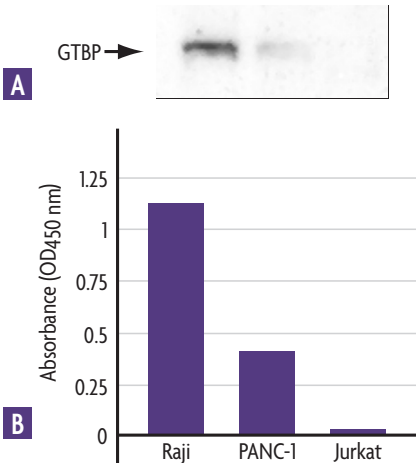


Figure 2: Measurement of GTBP activity in different cell lines using Western blot and the new GTBP DNA Repair Kit.
Raji, PANC-1 and Jurkat nuclear extracts were assessed for GTBP activity using Western blot (A) and the new GTBP DNA Repair Kit (B).

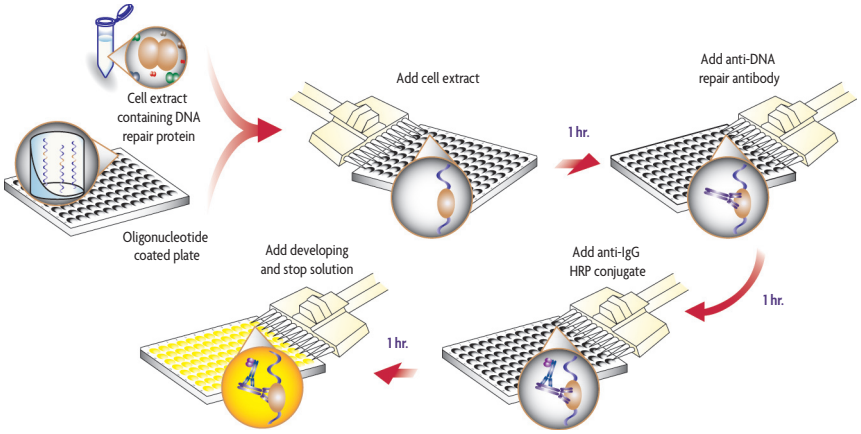


Figure 1: Flowchart of the DNA Repair Kit procedure.

The DNA Repair Kit method

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 oligonucleotide have been immobilized. 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 HRP-conjugated secondary antibody results in an easily quantified colorimetric readout (Figure 1).

Easily quantitate your data

Being able to measure small changes in DNA repair protein activity is important. While traditional methods such as Western blot are sensitive, they typically provide qualitative, rather than quantitative data. 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 and compared using Active Motif's GTBP DNA Repair Kit and by Western blot. The GTBP DNA Repair Kit results are clearly more quantitative than Western blot (Figure 2).

All you need

DNA Repair Kits come complete with optimized reagents including positive control nuclear extract to simplify the way you study interactions between damaged DNA and repair proteins. Don't waste time with inefficient Western blots or EMSAs; use DNA Repair Kits today.

What's available

Active Motif's DNA Repair Kit product line is continually growing. Currently available kits include those for studying Ku70/86, GT Binding Protein (GTBP) and Replication Protein A (RPA). Please give us a call or visit our website for complete kit configurations, downloadable manuals and to learn about new additions to the DNA Repair Kit product line.

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

Quickly Quantitate Estrogen Receptor Activity

Active Motif's Nuclear Receptor ERα ELISAs are a simple solution for monitoring the effects of agonists and antagonists on the activity of Estrogen Receptor alpha (ERα). The assays utilize a unique peptide-capture system that provides quantitative results in only three hours, while enabling you to study both cellular samples and recombinant proteins.

Estrogen Receptor regulation

ERα activation is dependent upon ligand binding to the receptor; this causes the receptor to dimerize and bind to estrogen response elements (EREs) located within the promoters of estrogen-responsive genes. While both agonist and antagonist compounds can bind to ERα, they have opposite effects on ERα conformation. When an agonist is bound, a hydrophobic groove is exposed that allows the binding of co-activator proteins such as SRC1 and RIP140. In contrast, when an antagonist is bound the co-activators needed for transcription of target genes are unable to bind. Because inappropriate activation of ERα has been associated with numerous diseases, there is an urgent need to identify ligands that modulate its activity.

The Nuclear Receptor ELISA method

The Nuclear Receptor ERα ELISAs are designed to measure the effects that various agonist and antagonist ligands have on the activation of ERα. Each kit contains a 96-stripwell plate that is coated with a sequence-optimized peptide that includes the consensus-binding motif of the ERα co-activators. When sample containing ERα and an agonist or antagonist ligand is added to a well, the ligand-activated ERα binds to the Capture Peptide. Incubation with primary antibody specific for ERα followed by HRP-conjugated secondary antibody and developing solution provides an easily quantified colorimetric or chemiluminescent readout. The result is a quantitative measurement of the effect of the agonist or antagonist on ERα activation (Figure 1).

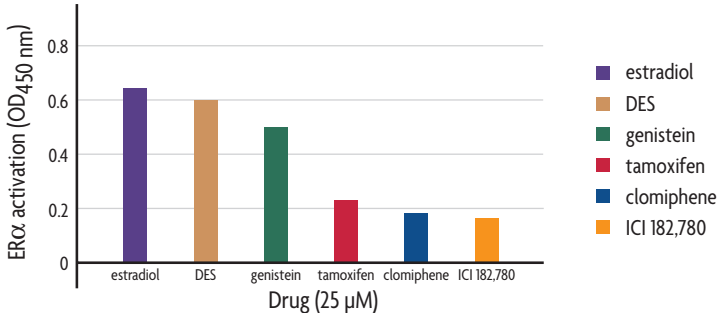


Figure 1: ERα agonism/antagonism response of estradiol, diethylstilbestrol (DES), genistein, tamoxifen, clomiphene and ICI 182,780. Fifteen µg of nuclear extract from the breast cancer cell line MCF-7 are incubated in the presence of test compounds (25 µM final concentration) in wells of the Nuclear Receptor ERα ELISA plate. Only ligand-activated ERα can bind to the Capture Peptide that is immobilized in the plate. The bound ERα is specifically detected with an ERα antibody. Secondary antibody and detection solution are then used to quantitate ligand-activated ERα. Note that in MCF-7 cells estradiol, DES and genistein are ERα agonists while tamoxifen, clomiphene and ICI 182,780 are ERα antagonists. (DES, genistein, clomiphene and ICI 182,780 results are provided for demonstration purposes only; these compounds are not included in the Nuclear Receptor ERα ELISA Kits.)

Faster, more reproducible technique

Nuclear Receptor ERα ELISAs are a marked improvement over other methods used to study ERα activation. Unlike cell proliferation or reporter gene assays, Nuclear Receptor ERα ELISAs do not require the cloning and transfection of reporter plasmids or the construction of stable cell lines. Nuclear Receptor ERα ELISAs are complete in hours, rather than days, have increased specificity and provide quantitative results. Moreover, the method is far more reproducible, which is crucial when comparing results from a large number of different compounds. With the Nuclear Receptor ERα ELISAs, studying ERα has never been easier.

Nuclear Receptor ELISA advantages

- Quantitative analysis of agonist/antagonists effects
- Results in ~3 hours
- Specific, antibody-based measurement
- High-throughput compatible
- Complete kit with proven controls

Choice of sensitivity

To meet your research demands, the Nuclear Receptor ERα ELISAs are available in both colorimetric and chemiluminescent formats. The colorimetric kit utilizes a standard ELISA-plate reader, while the ultra-sensitive Chemi kit requires the use of a microplate luminometer. The flexible measurement parameters of chemiluminescence mean that you can adjust the detection limits to ensure that the sensitivity is appropriate for the sample type that you are assaying.

Get it today

Nuclear Receptor ELISAs make studying the effects of agonist and antagonist ligands on receptor activation both faster and more reproducible. In addition to the ERα ELISAs, we will be releasing new Nuclear Receptor ELISAs for studying other nuclear receptors throughout 2004. Please give us a call or visit our website for complete kit configurations, downloadable manuals and to learn about new additions to this innovative product line.

Product	Format	Catalog No.
Nuclear Receptor ERα ELISA	1 x 96-well plate 5 x 96-well plates	49096 49596
Nuclear Receptor ERα Chemi ELISA	1 x 96-well plate 5 x 96-well plates	49097 49597

TimeLogic® DeCypher® Helps Unlock Key Bioinformatics Questions

The DeCypher® Solutions combine innovative software and reconfigurable hardware for high-performance sequence analysis:

TimeLogic®
biocomputing solutions

- **DeCypherBLAST**™ – rapid genomic and protein sequence comparisons
- **DeCypherHMM**™ – powerful Hidden Markov Model analysis for rapid protein classification
- **DeCypherSW**™ – rigorous Smith-Waterman methods find optimal alignments across frameshifts and introns for homology modeling, microarray probe design and siRNA studies
- **GeneDetective**™ – align cDNAs or amino acids to genomic DNA for fast, accurate identification of gene models and splice variants

What is DeCypher?
DeCypher allows you to extend the process of experimental design from your bench to the computer. DeCypher employs a comprehensive set of ultra-fast analysis tools that let you experiment with varied parameter settings—or completely different algorithms—to define the best possible sequence annotations.

The turnkey DeCypher solutions are capable of the large-scale analysis routinely required for genome comparisons. Installed within a single computer, they allow you to complete sequence comparisons at the speed of hundreds to thousands of CPUs. DeCypher features both a web interface & a command line client, enabling all users to execute powerful bioinformatics analyses.

How would I use DeCypher to predict the function of a gene? DeCypher enables you to quickly process comprehensive BLASTX searches of your gene sequences against a well-curated protein database of known proteins. For high-scoring matches, you can assume a similar protein function. To reinforce your conclusions, it's possible to compare your protein sequence with an annotated Hidden Markov Model database such as PFAM. A match to a specific protein family can imply similar function. DeCypher's HMM-Framesearch method is also available for automatic translation of your nucleic sequences and frameshift tolerant alignments while assigning protein families.

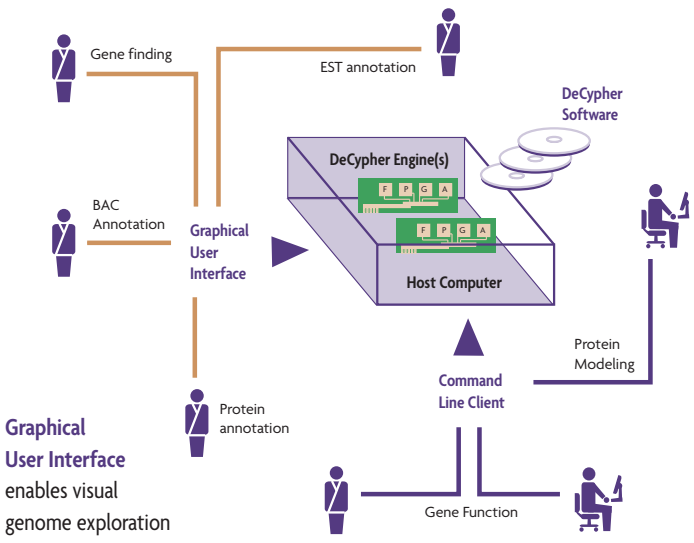
How can I use DeCypher to rapidly identify exons and splice variants? TimeLogic's new GeneDetective™ solution allows you to map ESTs or amino acid sequences to genomic data to build sophisticated gene models. The system uses multiple sequence alignment methods to accurately describe exons and introns, then delivers a zoomable, graphical view of your gene model for exploration of splice variants.

Can DeCypher be used for protein structure modeling? For homology modeling, DeCypher's BLAST and robust Smith-Waterman methods can be used to find sequences with known protein structure by comparison with Protein Data Bank (PDB) sequences. If no significant homology to PDB is found, DeCypherHMM can efficiently identify protein structural families reported in databases such as SCOP.

How can DeCypher be used to identify distantly-related sequences?
DeCypherHMM enables you to build your own Hidden Markov Model database from a collection of similar proteins from different organisms. This model can be used to search for remote homologs through comparison with a large sequence database. DeCypherHMM's performance enables you to begin exploring results from extremely-large HMM comparisons in minutes.

DeCypherBLAST and DeCypherSW can also be used for comparing distantly related genomes by processing protein-space comparisons of nucleic data.

To learn more about these methods, or about the DeCypher solutions' speed and capability, please contact TimeLogic today at 877-222-9543 or at www.timelogic.com.



Graphical User Interface enables visual genome exploration

Command Line Clients permit sophisticated analysis pipelines.

Rapid Gene Isolation using RecA

The RecActive™ Kit for gene enrichment offers a fast, flexible and efficient method for isolating target DNA from complementary DNA (cDNA) or genomic DNA (gDNA) libraries. With up to 10,000-fold enrichment of your target gene(s) in only two rounds of purification, RecActive is the most effective gene isolation technology available.

Isolate genes without hybridization or PCR
Conventional methods for isolating clones from cDNA and gDNA libraries require the preparation and hybridization of a large number of filters from library master plates. This is time-consuming, labor-intensive and, depending on the method, often requires the use of radioactivity. PCR amplification is faster, but can introduce errors into the amplified DNA. Furthermore, PCR is not effective for isolation of full-length genes or splice variants. In contrast, RecActive utilizes the natural properties of RecA recombinase from *E. coli* to form stable, multi-stranded hybrids that can be easily purified, removing the need for membrane hybridization or PCR amplification.

Save time by enriching for your DNA
With the RecActive technique, a short sequence (200-600 bp) corresponding to your target DNA is prepared as a biotinylated DNA probe and coated with RecA. When added to the pooled library DNA, the RecA catalyzes formation of stable multi-stranded complexes consisting of probe DNA and homologous target sequences. Streptavidin-coated magnetic beads are then used to enrich for clones that are bound by the biotinylated probe (Figure 1). After enrichment, the purified DNA is transformed into bacteria and plated. As only enriched DNA is transformed, you save time and materials by working with only the DNA you need.

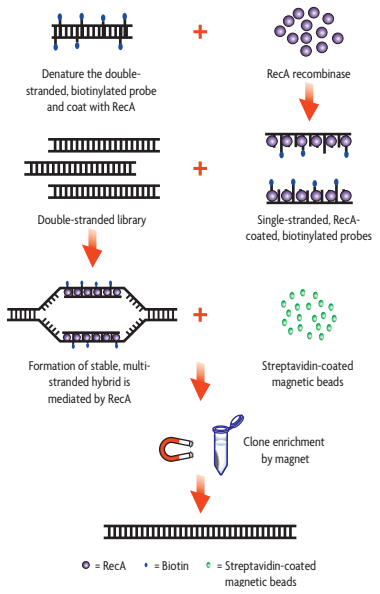


Figure 1: Flowchart of the RecActive process.
A short (200-600 bp) double-stranded, biotinylated probe is produced by PCR using the Biotin-dNTP Mix. The probe is denatured and coated with RecA. Each strand of the probe then finds and hybridizes to its complementary sequence in the DNA library. After removal of RecA, streptavidin-coated magnetic beads are used with a magnet to enrich for the homologous clone(s).

Ensure you isolate all the splice variants
The RecActive method is very effective at isolating splice variants. Traditional PCR methods will not amplify unknown splice variants unless the splice sites are internal to the PCR primers. However, RecActive enriches for all clones that share homology to the 200-600 bp piece of probe DNA. That means that you will enrich for all splice variants contained within your library, irrespective of the splice site.

Proven performance
To demonstrate the powerful results possible with the RecActive Kit, a "library" was constructed by combining white and blue colony-forming plasmids at a ratio of 1:10,000. Following a single enrichment using RecActive, 65% of transformants were white colony-forming clones (Figure 2).

Gene enrichment from model species
Model systems are widely used for drug discovery and other modern research projects due to their ease of handling. However, functionally related genes from other species must then be isolated for efficient drug discovery and basic research to be performed. Often the DNA homology between these related genes is too low to enable effective use of hybridization or PCR-based approaches. By altering the binding conditions, RecA can catalyze formation of probe:target complexes with up to 30% heterology. This makes RecActive the system of choice for isolating interspecies and/or interfamily genes.

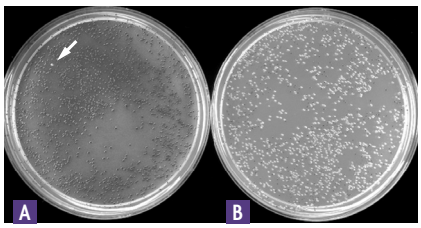


Figure 2: Gene specific enrichment.
RecActive enrichment of White Plasmid from a library containing a ratio of 1 White:10,000 Blue plasmids.

Find your gene of interest today
For the simplest, most versatile gene enrichment tool available, try the RecActive Kit today. For complete details, please give us a call or visit our website.

Product	Format	Catalog No.
RecActive	10 rxns*	52010
	5 x 10 rxns	52050

Recombinant Proteins for Your Research

Active Motif now offers a growing line of recombinant proteins that are ideal for use in many different biological applications. The NFκB p50, NFκB p65, p53, c-Myc and CREB proteins are currently optimized for use in our TransAM ELISAs (Figure 1, see page 3). Complete information on the recombinant proteins, including detailed technical data sheets that specify protein length, the species it was produced in, method of purification, *etc.*, can be found at www.activemotif.com.

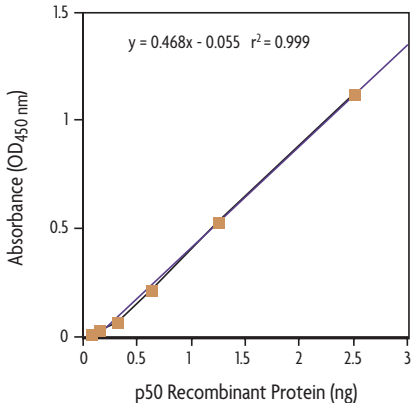


Figure 1: NFκB p50 Standard Curve.
A NFκB p50 standard curve was generated using the recombinant NFκB p50 protein and the TransAM™ NFκB p50 Kit.

Recombinant Protein Line			
AKT1	ER	p53	RARγ
ATF-2	FXR	p53 R273H	RXRα
BRCA1	GR	p300	RXRβ
BRCA2	IκBα	PPARα	RXR-LBD
c-Fos	JNK2α1	PPARβ(δ)	Sp1
c-Jun	JNK2α2	PPARγ	STAT1
c-Myc	LXRα	pRB	TRα1
CREB	LXRβ	Rad51	TRβ1
CTF1 (NF-1)	NFκB p50	RARα	
eIF2α	NFκB p65	RARβ	

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