MOTIF VATIONS

THE NEWSLETTER OF ACTIVE MOTIF — MARCH 2004 • VOLUME 5 • NUMBER 1

ACTIVE MOTIF®

Innovative Tools for Cell & Molecular Biology

Chromatin Immunoprecipitation Made Easy

Active Motif's ChIP-IT™ Kit is the most effective product available today for chromatin immunoprecipitation (ChIP). The kit contains a comprehensive protocol and optimized buffers, inhibitor cocktails, antibodies and controls, all of which have been validated in actual ChIP experiments. Plus, ChIP-IT Kit antibodies are directed against general transcription factors, so you get a more accurate analysis of promoter activity than is possible with histone-based ChIP. If you are performing chromatin immunoprecipitation, using the ChIP-IT Kit will improve your results and save you time.

The ChIP method

In the ChIP-IT method, intact cells are fixed using formaldehyde, which cross-links and therefore preserves protein/DNA interactions. The DNA is then sonicated into small, uniform fragments and the DNA/protein complexes are immunoprecipitated using an antibody directed against a DNA-binding protein of interest. Following immunoprecipitation, the cross-links are reversed and the DNA fragments are screened to determine which gene or groups of genes were bound by the protein of interest (see Figure 1, page 4).

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Quickly Quantitate Estrogen Receptor Activity

Active Motif's new Nuclear Receptor ERα ELISAs make it fast and easy to accurately measure the effects and tissue selectivity of agonists and antagonists on Estrogen Receptor alpha activity. Unlike traditional assays, which are time consuming and expensive, the Nuclear Receptor ELISAs utilize a unique peptide-capture system that provides you with quantitative results in about three hours (Figure 1). And, the method is flexible enough to use on multiple sample types, including both cellular samples and recombinant proteins.

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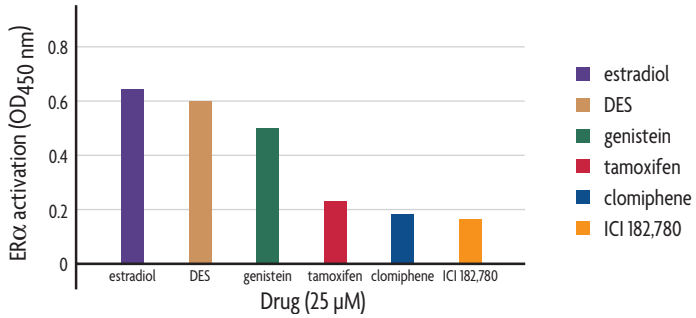

Figure 1: ERα agonism/antagonism response of estradiol, diethylstilbestrol (DES), genistein, tamoxifen, clomiphene and ICI 182,780.
Fifteen µg of nuclear extract from the breast cancer cell line MCF-7 are incubated in the presence of test compounds (25 µM final concentration) in wells of the Nuclear 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.)

IN THIS ISSUE

Deliver Biologically Active Proteins Into Mammalian Cells

The Chariot™ delivery reagent 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 the ideal tool for a variety of functional studies.

Targeted delivery

Chariot is a peptide that forms a non-covalent complex when combined 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).

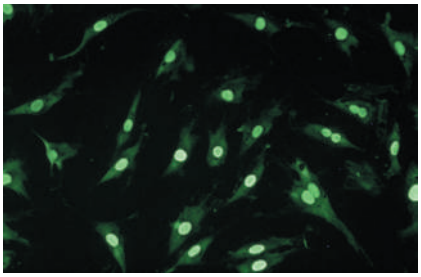

Figure 1: Targeted protein delivery.
50 ng of a 10 kDa nuclear protein labeled with Lucifer yellow at the C-terminus was complexed with Chariot and delivered into HS-68 cells. Unfixed cells were observed 90 minutes post-delivery.

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 G_1 phase. Over 90% of cells receiving a Chariot-$p27^{kip1}$ complex were unable to progress beyond G_1 phase (Figures 2A & 2B), demonstrating the efficient delivery of active $p27^{kip1}$.

Deliver large proteins

Chariot can efficiently deliver a broad range of macromolecules directly into mammalian cells. To demonstrate, Chariot was used to deliver functional β-galactosidase protein (a tetramer composed of four identical, 119 kDa subunits) into HeLa cells (Figure 3).

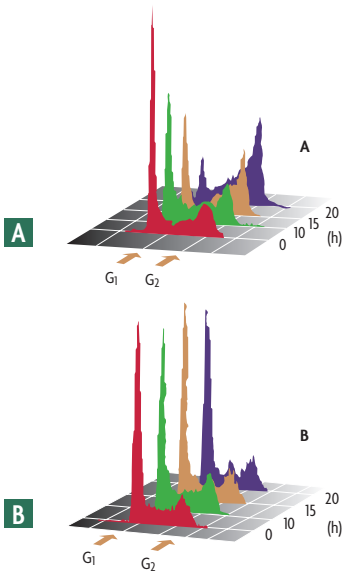

Figure 2: Chariot delivery of functional protein.
HS-68 cells arrested in G_0 phase by serum deprivation for 48 hours were released by addition of serum for 3 hours. Flow cytometry performed 0, 10, 15 and 20 hours after addition of Chariot alone and a Chariot-$p27^{kip1}$ complex indicate that cells receiving Chariot alone progressed into G_1 phase (A), while over 90% of the cells receiving the Chariot-$p27^{kip1}$ complex remained in G_1 phase (B). Data generously provided by Dr. Gilles Divita, Biophysics Dept., CNRS, Montepellier, France.

What can Chariot do for you?

Direct delivery of active protein makes it easy to perform many studies that are not possible using DNA transfection and expression. Chariot results have been extensively published; successful 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. The method has proven to be effective on both adherent and suspension cells, as well as *in vivo*. 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.

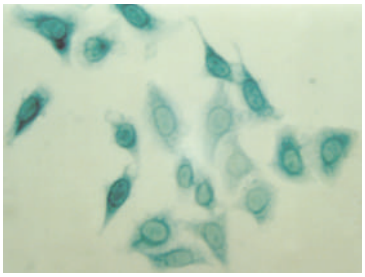

Figure 3: Staining of HeLa cells.
One µg of β-galactosidase protein, which is included with Chariot for use as a positive control, was complexed with Chariot for 30 minutes and delivered into HeLa cells. Cells were fixed and stained 2 hours post-delivery using Active Motif's β-Galactosidase Staining Kit.

Advantages

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

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 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* 100 rxns	30025 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.

The Answer for Assaying Transcription Factor Activation

TransAM™ Kits are DNA-binding ELISAs that facilitate the study of transcription factor activation in mammalian tissue and cell culture extracts. The method is faster and more sensitive than other commonly used methods, while eliminating the use of radioactivity. Many different kits are available for studying both individual transcription factors as well as transcription factor families.

How does TransAM work?

Transcription factors bind to their DNA target(s) after they've been activated, commonly by phosphorylation. TransAM Kits take advantage of this property by supplying a 96-well plate in which multiple copies of a specific double-stranded oligonucleotide are immobilized. When sample is added, the activated transcription factor binds to the oligonucleotide at its consensus-binding sequence. Primary antibody directed against the bound form of the transcription factor is then added, followed by an HRP-conjugated secondary antibody. The colorimetric change is measured with a spectrophotometer, which provides a sensitive, quantitative measurement of the activated transcription factor.

How is TransAM an improvement over existing methods?

Two commonly used methods to study transcription factor activation are gelshift/EMSA and reporter gene assays. TransAM has a number of advantages over each of these procedures. With respect to gelshift, TransAM assays are 10-fold more sensitive,

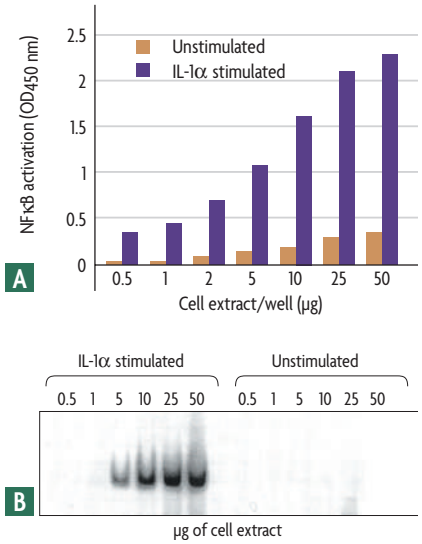

Figure 1: TransAM assays are more sensitive than gelshift and provide quantitative results.
Human fibroblast WI-38 cells are stimulated with IL-1α for 30 minutes. Increasing amounts of whole-cell extract are assayed using the TransAM NFκB p50 Kit (A) or gel retardation (B).

so you get more accurate results while using less sample. TransAM eliminates the use of radioactivity, as well as the need to run, blot, expose and develop gels. This saves you a considerable amount of time; TransAM is complete in less than 5 hours, while with gelshift you'll wait days for results. In addition, the qualitative data provided by TransAM is easier to interpret and compare than blots (Figure 1). TransAM also overcomes the problems associated with the inconsistency of reporter gene assays. Because transfection is a variable process and gene expression is subject to many confounding factors, reporter assays must be repeated many times to obtain statistically reliable data. And, unlike TransAM, reporter assays will not work with tissue samples.

What types of samples can be assayed with TransAM?

TransAM assays are typically used with nuclear extracts; most commonly, extracts are made from cells in their normal state as well as after some form of stimulation that activates the transcription factor of interest. The results are then compared to determine the extent of activation caused by the treatment. For NFκB proteins, whole-cell extract can be used. Active Motif also sells a number of recombinant proteins that can be used in TransAM assays to generate a standard curve (see page 10).

Which transcription factors can be studied with TransAM?

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.

What is in a TransAM Kit?

All kits come with the appropriate 96-well plate(s) and antibody(ies), as well as all buffers required to assay your sample. Positive control extract(s) is also included to help ensure results. For complete details, return the enclosed reply card or visit our website at www.activemotif.com. There, you can download product manuals, as well as a list of publications citing the use of TransAM.

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

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Analyze the enhanceosome

Gene promoter regions commonly possess multiple known or putative transcription factor binding sites. Using sequential or multiple ChIP experiments to analyze the active transcription factor sites and the transcription factor interactions that occur within this region can be time consuming and yield variable, conflicting results. The ChIP-IT Kit, however, makes enhanceosome analysis faster and more reproducible. Each ChIP-IT Kit contains all of the buffers, inhibitor cocktails, controls and blocking reagents needed for shearing, pre-clearing, immunoprecipitation, washing, elution and purification of DNA. This means that sequential and/or multiple ChIPs can be performed with ease, using consistent reagents and controls that have been proven to work. No other kit available today can offer you such a complete solution.

Proven controls ensure your results

Successful ChIP depends on sonicated DNA of the correct length, antibody that is effective at immunoprecipitating the protein/DNA complexes and a PCR primer set that specifically flanks the region of DNA bound by the protein of interest. However, it can be extremely difficult to determine whether "pull down" of the DNA or its subsequent PCR screening was successful or not, unless you already have an antibody and PCR primers that have been proven to be effective in ChIP.

To help minimize the time and effort spent validating antibodies and PCR primer sets, the ChIP-IT Kit includes a TFIIB antibody that has been shown to immunoprecipitate TFIIB-bound DNA specifically. The kit also contains positive and negative control PCR primers directed against DNA that either is or is not bound by TFIIB (Figure 2). Collectively, the ChIP-IT Kit's proven reagents make it faster and easier for you to validate your own antibodies and primers. For more successful ChIP, try the ChIP-IT Kit.

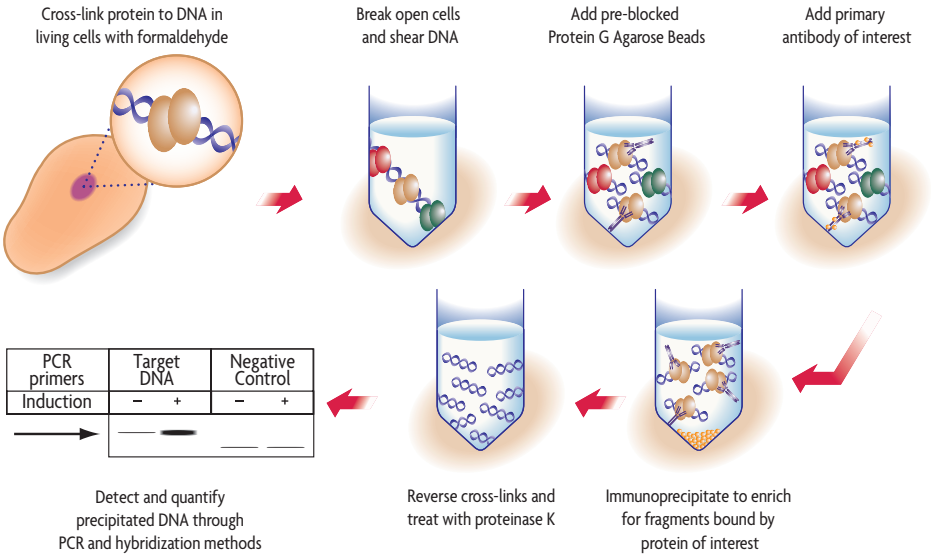

Figure 1: Schematic of chromatin immunoprecipitation. In ChIP, protein/DNA interactions are fixed, and then the DNA is sheared and precipitated using an antibody. After reversing the cross-links, the DNA is purified and then screened to determine which genes were bound by the protein of interest.

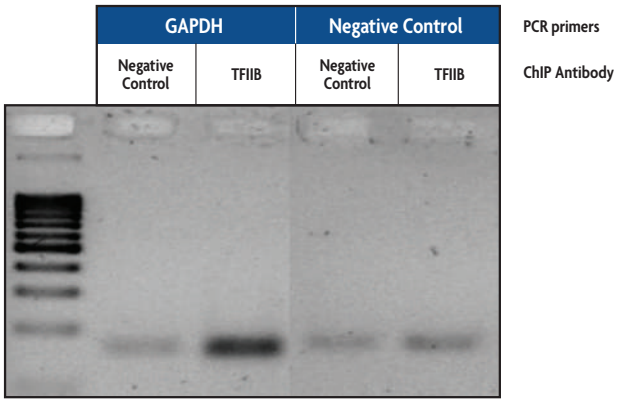

Figure 2: PCR amplification of immunoprecipitated DNA. U-937 cells were fixed for 10 minutes with 1% formaldehyde and then ChIP was performed using the ChIP-IT Kit. The kit's GAPDH PCR primers flank the TFIIB binding site in the GAPDH promoter region. These primers give a strong PCR product when used on templates enriched for the GAPDH promoter region, *e.g.* DNA immunoprecipitated with TFIIB antibody.

The Negative Control primers flank a region of DNA that should not be bound by TFIIB. These primers are used on immunoprecipitated DNA to confirm that antibody-enrichment of a target DNA is due to specific immunoprecipitation of the protein target, rather than a non-specific precipitation of total DNA. **Note:** the Negative Control primers give an amplification product because chromatin immunoprecipitation is an enrichment of DNA bound by a particular protein, not a complete purification of the DNA of interest. If enough PCR cycles are used, it is always possible to get a PCR product for a given target locus.

DNA immunoprecipitated with the TFIIB antibody and the Negative Control antibody were used in PCRs performed with the GAPDH primers. As shown, the GAPDH target was effectively immunoprecipitated by the TFIIB antibody (Lane 3) but not by the Negative Control antibody (Lane 2). This demonstrates that the DNA enrichment was due to the TFIIB antibody, and was not simply a result of non-specific sticking to the IgG or Protein G beads.

PCRs performed on these same templates with the Negative Control primers produced an equivalent amount of PCR product (Lanes 4 and 5), demonstrating that the TFIIB antibody did not simply bind non-specifically to chromatin.

Collectively, these data validate that the ChIPs performed with the ChIP-IT Kit and the TFIIB antibody enable efficient and specific enrichment of TFIIB target DNA, and they demonstrate that the ChIP-IT Kit reagents are suitable for use with any ChIP-proven antibody.

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

TimeLogic® DeCypherBLAST™ – The Solution for Fast, Flexible Genome Analysis

The processes used to identify genes and annotate genomic sequences can be compute-intensive. Large-scale comparisons to annotated genomes are required, and these analyses cannot be handled by publicly available servers. The solution is DeCypherBLAST™, which brings tremendous processing power to your sequence annotation projects through the use of software and hardware components that are installed in a single computer.

DeCypherBLAST enables you to:

- Process complete genome comparisons without the use of an expensive-to-maintain cluster of computers
- Utilize familiar BLAST tools with flexible alignment methods
- Configure the results format for easy data integration and faster analysis
- Complete more sequence annotations per day than other methods

High-performance similarity searching
TimeLogic's DeCypher System dramatically increases the speed of BLAST analyses. In a recent study, DeCypherBLAST was utilized to compare 451 EST sequences (384,000 bases) from wheat (*Triticum*) with NCBI's **nt** database. The search was completed in under 20 minutes on the DeCypher System, but would require approximately 1 day to complete on the same 2-CPU computer without DeCypher.

DeCypherBLAST's speed advantage is even greater when performing larger searches (*i.e.* 100,000 or more sequences). For example, comparison of human chromosome 19 to the complete mouse genome can be completed in as little as 3 hours on a single server.

More optimal local alignment options
DeCypherBLAST includes a variety of alignment methods that provide greater sensitivity and flexibility than NCBI BLAST, enabling you to:

- Create accurate alignment representations across nucleic and protein data
- Optimize short sequence alignments (*i.e.* proteins)
- Span introns across genomic data
- Easily identify sequence patterns by locking the alignment to the 5´ or 3´ end of your query

Flexible result formats improve data integration
DeCypherBLAST streamlines your analysis by providing options for the output of your data files. You can specify the field order and generate result files in Tab- or comma-delimited formats, which speeds and simplifies importing the data into your proprietary databases.

Integration with other DeCypher modules
Another advantage of DeCypherBLAST is its strong integration with other bioinformatics methods. For example, if you are performing BLAST but also wish to do protein annotation, DeCypherBLAST can simultaneously generate files of sequences for each "hit" in the BLAST search. Processing these files with CLUSTALW produces a multiple sequence alignment that can be imported directly into TimeLogic's DeCypherHMM™ to generate a model that can be used to search for additional domain family members that may have been missed by the BLAST search.

Keep pace with the data deluge
With over 800 organisms currently being sequenced, data available for sequence comparisons is growing at a tremendous rate. DeCypherBLAST has been designed for easy scalability, to effectively utilize growing resources for a deeper understanding of the genes you study. As your analysis needs increase, simply add an additional DeCypher Engine to double your system performance.

DeCypherBLAST enables you to perform extensive analyses of your sequence data, which provides you with greater confidence in your annotations. Its cost-effective performance, powerful alignment methods and turnkey installation lets you begin exploratory bioinformatics studies immediately.

Solution pricing starts from $40,000 and academic discounts are available. To learn more about our DeCypher products for rapid, low-cost genomic analysis, contact us today by calling toll-free at 877-222-9543, returning the enclosed reply card or by logging on at www.timelogic.com.

TimeLogic®
biocomputing solutions

continued from page 1 — Quickly Quantitate Estrogen Receptor Activity

Estrogen Receptor regulation
Activation of ERα is dependent on ligand binding to the receptor. Agonist and antagonist ligands both bind to ERα but have opposite effects on conformation. When an agonist is bound, a hydrophobic groove is exposed that enables binding of co-activator proteins (Figure 2A). In contrast, upon antagonist binding, co-activators are unable to bind. As inappropriate activation of ERα is associated with numerous diseases, there is a need for a convenient assay that examines the effects and tissue selectivity of various compounds on ERα activity. To fill that void, we developed the Nuclear Receptor ELISAs.

The Nuclear Receptor ELISA method
Nuclear Receptor ELISAs are simple and efficient and can be used with both cellular extracts and recombinant proteins. The Nuclear Receptor ERα ELISAs are designed to specifically capture the ligand-activated form 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 a sample that contains ERα is added to a well, the ligand-activated ERα binds to this Capture Peptide. Each well is then incubated with a primary antibody that is specific for ERα. Subsequent incubation with HRP-conjugated secondary antibody and developing solution provides an easily quantified colorimetric or chemiluminescent readout (Figure 2B).

Improved 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 inefficient cloning or cell transfections. Inconsistencies due to variable transfection of reporter plasmids and the need to construct stable cell lines are also eliminated. Nuclear Receptor ERα ELISAs are complete in mere hours, rather than days, have increased specificity, and provide quantitative results. Studying ERα has never been easier.

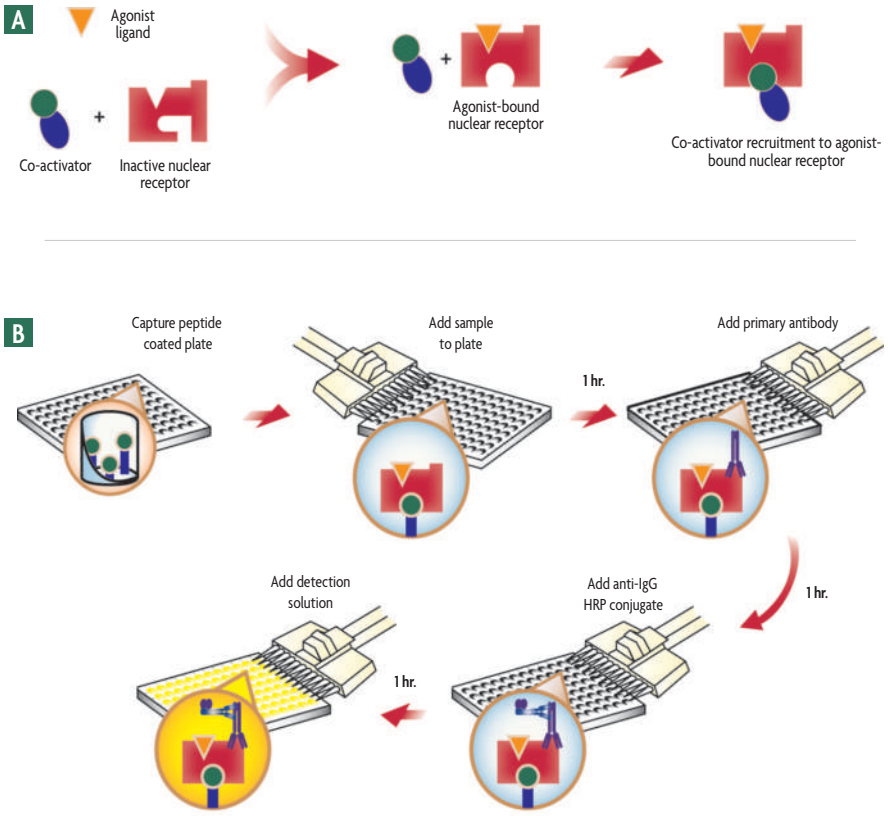

Figure 2: A. Ligand Activation of Nuclear Receptor in the cell. B. Schematic of the Nuclear Receptor ELISA.

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

Two types of detection
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 detection limits can be adjusted to ensure that the sensitivity is appropriate for the sample type being assayed.

What's available
Nuclear Receptor ELISAs are currently available for studying ERα. Additional kits for studying other nuclear receptors will be released throughout the year. Be sure to check out 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

Cell-based Method for ErbB-2 (Her2/neu), GSK3β and EGFR Phosphorylation Analysis

Fast Activated Cell-based ELISA (FACE™) Kits provide a simple, innovative alternative to classical methods for monitoring protein phosphorylation. With FACE, modification-specific analysis can be performed without time-consuming cell extractions, gel electrophoresis or membrane blotting. Plus, in addition to their convenience, FACE Kits generate better 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 rapidly fixed, which preserves activation-specific protein modifications. Each well is then incubated with a primary antibody specific for the protein modification of interest. Subsequent incubation with secondary HRP-conjugated antibody and developing solution provides a colorimetric or chemiluminescent readout that is quantitative and reproducible (Figure 1). FACE Kits also contain a primary antibody for the native non-modified protein, so you can monitor both native and activated protein levels in the same experiment (Figure 2).

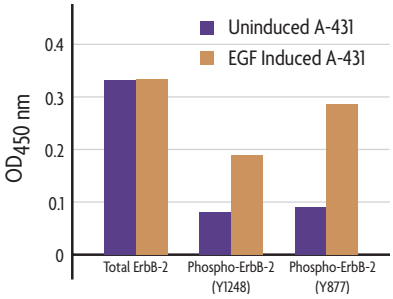

Figure 2: Monitoring total- and phospho-ErbB-2 using FACE. The FACE ErbB-2 (Y1248) and ErbB-2 (Y877) Kits were used to assay the levels of total and phosphorylated ErbB-2 contained within untreated or EGF treated A-431 cells.

Reproducible, scaleable assay

Typically, phospho-specific analyses are performed on small sample numbers because the classical methods of study, such as Western blot and in-gel kinase assays, are labor intensive and time-consuming. This forces you to run multiple experiments on different days and then to compare the results. However, because the variability of these methods is high and the induction of phosphorylation is typically low, this can lead to statistically poor data. Fortunately, FACE Kits make running more samples as simple as adding an extra tip to your multi-channel pipettor. And, because FACE Kits are highly reproducible, you'll get results that are more statistically relevant.

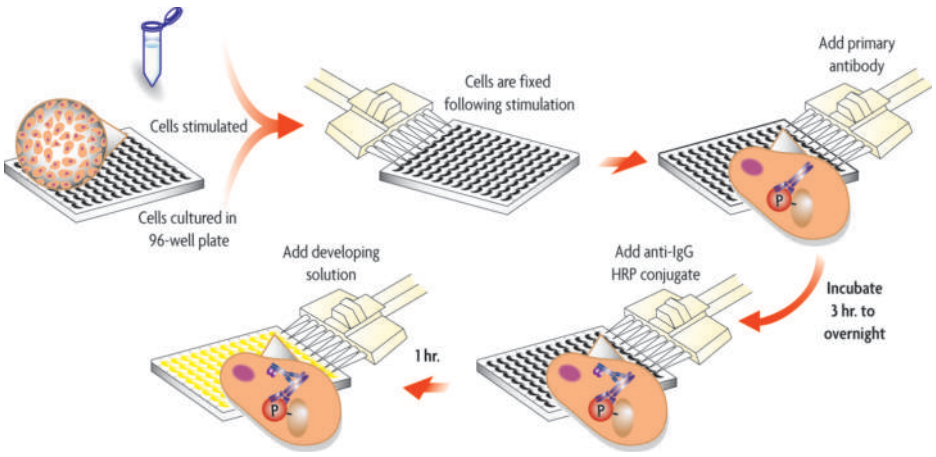

Figure 1: Flow Chart of the FACE Process. Cells are grown, stimulated and fixed in the same 96-well plate. Addition of primary and secondary antibodies detects phosphorylated protein.

Latest additions to the FACE line

FACE Kits are now available for monitoring activation of two different phosphorylation sites on ErbB-2 (Her2/neu) and EGFR, as well as one site on GSK3β. Existing FACE Kits include those for monitoring phosphorylated p38, JNK, AKT and ERK1/2, with many other kits on the way. To obtain an up-to-date list of available FACE Kits, return the enclosed reply card or visit our website at www.activemotif.com.

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™ 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™ 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

Sandwich ELISAs for Fast, Accurate Protein Quantification and Normalization

Both Active Motif's FunctionELISA™ Kits and its Normalization Kits utilize the Sandwich ELISA technique to capture and accurately quantify the amount of a specific protein in your sample. This method is faster and simpler to perform than other techniques used to study proteins, like Western blot. And, it enables you to make quantitative rather than just qualitative protein measurements.

Specific, quantitative assay

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

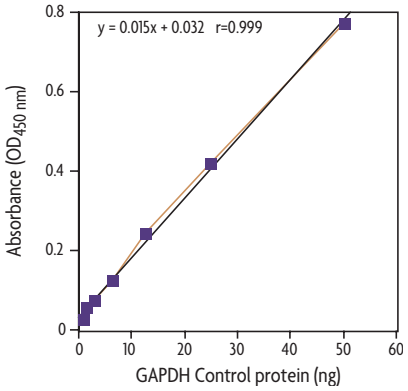

Figure 1: Production of a GAPDH Standard Curve. The GAPDH Whole-cell Normalization Kit was used with known amounts of the GAPDH Control protein to generate a standard curve.

Convenient format saves time

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

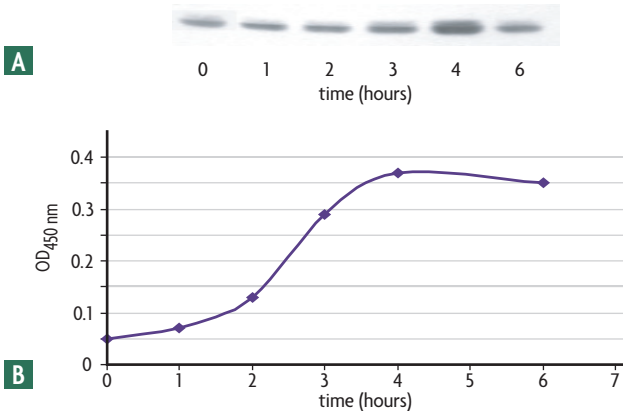

Figure 2: Time course of TRAIL induction. Jurkat cells treated with 1 nM TNF-α were harvested at various time points. Cell extracts were assayed by Western blot analysis (A) using TRAIL mAb (Cat. No. 40966) and through the use of FunctionELISA TRAIL Kit (B).

Verify the integrity of your samples

The Normalization Kits can be used to verify your sample's integrity (Figure 3) and to reduce inaccuracies in other downstream assays. By normalizing for the levels of the housekeeping proteins GAPDH and Lamin B1, you can be sure that you're comparing equivalent amounts of your samples.

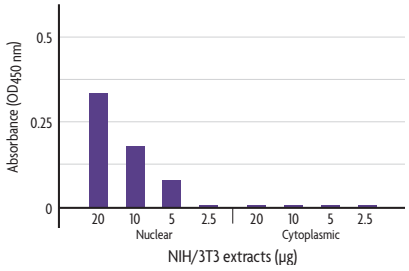

Figure 3: Assaying cytoplasmic and nuclear extracts for Lamin B1. The Nuclear Extract & Lamin Normalization Kit was used to assay for Lamin B1 in extracts from NIH/3T3 cells. As Lamin B1 is a nuclear protein, its absence verifies the integrity of the cytoplasmic extract.

Advantages

- Quantitative results in just hours
- No need to run, blot and develop gels
- Easily analyze multiple samples

Complete kits ensure your success

FunctionELISA and Normalization Kits contain all of the reagents required to rapidly quantify your protein, including recombinant protein for producing a standard curve. For your convenience, the Capture Antibody in these kits is supplied immobilized in a 96-well assay plate, so you won't waste time with overnight incubations. In addition, the Normalization Kits contain the same components found in our popular Nuclear Extract Kit, so you can prepare quality extracts and normalize their results. For complete details on these kits, please return the enclosed reply card or log on to our website at www.activemotif.com.

Product	Format	Catalog No.
FunctionELISA™ Cytochrome c	1 x 96 rxns 5 x 96 rxns	48006 48506
FunctionELISA™ IκBα	1 x 96 rxns 5 x 96 rxns	48005 48505
FunctionELISA™ TRAIL	1 x 96 rxns 5 x 96 rxns	48010 48510
Nuclear Extract & Lamin Normalization Kit	1 x 96 rxns 5 x 96 rxns	48008 48508
GAPDH Whole-cell Normalization Kit	1 x 96 rxns 5 x 96 rxns	48007 48507

Colorimetric Quantitation of Replication Protein A

Active Motif's RPA DNA Repair Kit makes quantitation of Replication Protein A (RPA) activity easy to perform. That's because our DNA Repair Kits are DNA-binding ELISAs that eliminate radioactivity and provide quantitative results in less than five hours. This makes studying RPA regulation faster and more sensitive than traditional assays like gelshifts and Westerns. Plus, the DNA Repair Kits can be used on all sample types, including cell lines and tissues, giving you unsurpassed flexibility.

Why study RPA?

RPA is composed of three subunits, RPA70, RPA32 and RPA14, which bind to ssDNA during DNA replication, recombination and repair. Once bound, RPA organizes and protects the ssDNA. Deficiencies in RPA activity can lead to cell cycle arrest and apoptosis. Despite the growing interest in studying this important regulator of DNA metabolism, there is a lack of convenient, sensitive assays that are suitable for modern high-throughput research. That's why the scientists at Active Motif have developed the new RPA DNA Repair Kits.

Advantages

- 10X more sensitive than EMSA
- Works in human, hamster and yeast
- Two primary antibodies to study RPA32 and RPA70
- Linear over a broad sample range

The DNA Repair Kit method

Each RPA DNA Repair Kit includes a 96-well plate in which multiple copies of a single-stranded oligonucleotide have been immobilized. When nuclear extract is added, the RPA protein binds to this oligonucleotide. Each well is then incubated with a primary antibody that is specific for either RPA32 or RPA70. Addition of a secondary HRP-conjugated antibody and developing solution provides an easily quantified colorimetric readout (Figure 1). The easy-to-follow protocol makes it simple for you to monitor both the RPA32 and RPA70 subunit activity under different growth and stimulation conditions.

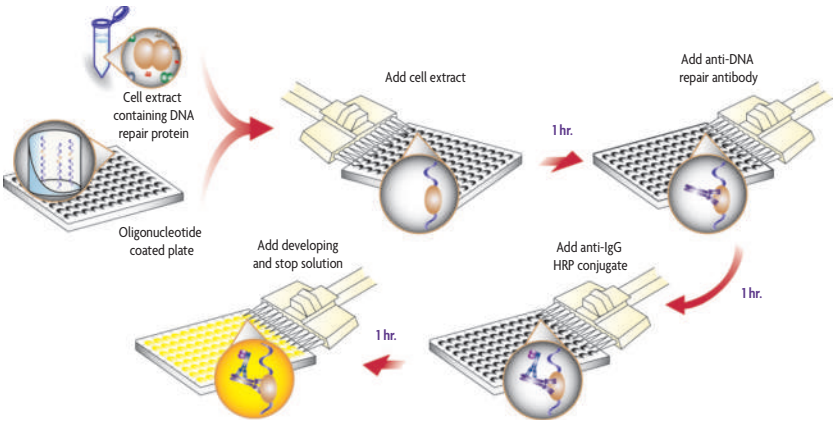

Figure 1: Flowchart of the RPA DNA Repair Kit procedure.

Flexible format, accurate results

RPA DNA Repair Kits are accurate over a wider range of sample concentrations than other methods because they utilize colorimetric detection, which is linear over a broader sample range (Figure 2). This saves you time and money, as fewer samples will need to be diluted and reassayed to obtain accurate results. Plus, the kit comes in a convenient 96-stripwell format, which provides you with the flexibility to use from 1 to 96 wells at a time, depending on the needs of your experiment.

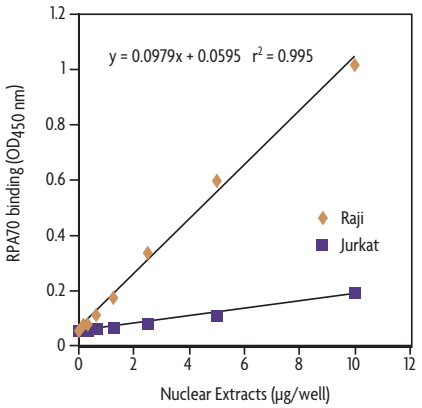

Figure 2: Quantitation of RPA70 with the RPA DNA Repair Kit. Various amounts of nuclear extract from Raji and Jurkat cells were assayed for RPA70 activity using the new RPA DNA Repair Kit.

A complete solution

Active Motif's RPA DNA Repair Kits come complete with optimized reagents including a positive control nuclear extract to simplify the way you study interactions between damaged DNA and repair proteins. Plus, the kit supplies antibodies against both RPA32 and RPA70 so you can examine the differences between these two subunits side by side. Don't waste time with inefficient Western blots — use RPA DNA Repair Kits today.

Product	Format	Catalog No.
RPA DNA Repair Kit	1 x 96 well-plate	51296
	5 x 96 well-plates	51796
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

Use Silica Resin for More Specific Protein Purification

Specific protein purification is simple with Active Motif's Ni-TED™ Protein Purification System. Unlike traditional resins made of agarose, Ni-TED uses a silica resin that makes purification of 6xHis proteins better than ever before.

Stop non-specific binding

Protein purification using agarose-based nickel columns is frequently plagued by the co-purification of untagged proteins. This is caused by their non-specific binding to the agarose matrix. In contrast, Ni-TED resin is made of silica, which has fewer side chains than agarose. This increases the selectivity of Ni-TED and reduces the non-specific binding of cellular proteins. The result is a much cleaner purification of your target protein (Figure 1).

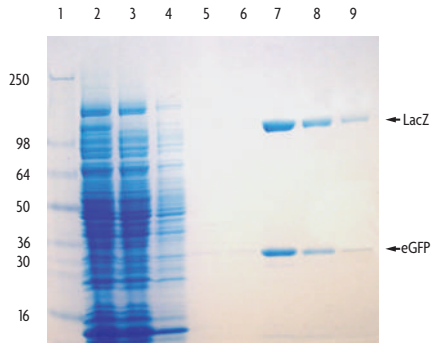

Figure 1: Dual expression in the Cascade™ Expression System.
lacZ and eGFP were cloned into the pCHROMO and pCHROMO2 vectors, respectively, and integrated into TAP *E. coli*. Five hours after induction (2 mM salicylate), the two proteins were co-purified using Ni-TED Silica, then run on a 4-20% Tris-Glycine gel.

Lane 1: Protein standard (kDa)
Lane 2: Lysate
Lane 3: Flow-through
Lanes 5: Washes
Lanes 6-9: Elutions

One-step protocol

Purification using Ni-TED is complete in one simple step and works for 6xHis-tagged proteins produced in any expression system. You can elute your proteins under both native and denaturing conditions using either competition with imidazole or by a simple reduction in pH.

Flexible formats

Great for small- or large-scale purifications, Ni-TED is available in a variety of formats to meet your purification needs. Ni-TED Spin Columns for protein isolations from small samples (0.1-10 ml of culture). The Spin Columns feature a silica-based membrane with a binding capacity of 50 µg that is ideal for proteins or peptides of < 100 kDa. For larger-sized samples, Ni-TED Silica is supplied in prepackaged columns or in bulk for purifications from culture volumes of up to 100 ml. Ni-TED Silica will bind up to 3 mg of His-tagged protein per gram of resin and is suitable for isolating proteins of any size. For quick, efficient purification, try Ni-TED today.

Product	Format	Catalog No.
Ni-TED™ Spin Columns	25 rxns 100 rxns	97025 97100
Ni-TED™ Spin Columns (w/solutions)	25 rxns 100 rxns	98025 98100
Ni-TED™ Silica (0.3 g resin in prepacked columns)	5 columns 20 columns	97005 97020
Ni-TED™ Silica	1 g	97001

Recombinant Proteins for Your Research

Active Motif now offers a complete line of recombinant proteins that are ideal for use in many different biological applications. The NFκB p50, NFκB p65, p53 and CREB proteins are currently optimized for use in our TransAM ELISAs (see page 2). 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.

Recombinant Protein Product Line			
Recombinant AKT1 protein	Recombinant CTF1 (NF-1) protein	Recombinant LXR protein	Recombinant RAD51 protein
Recombinant ATF-2 protein	Recombinant eIF2α protein	Recombinant NFκB p50 protein	Recombinant RAR protein
Recombinant BRCA1 protein	Recombinant ER protein	Recombinant NFκB p65 protein	Recombinant RXR protein
Recombinant BRCA2 protein	Recombinant FXR protein	Recombinant p300 protein	Recombinant RXR-LBD protein
Recombinant c-Fos protein	Recombinant GR protein	Recombinant p53 protein	Recombinant Sp1 protein
Recombinant c-Jun protein	Recombinant IκBα protein	Recombinant p53 R273H protein	Recombinant STAT1 protein
Recombinant c-Myc protein	Recombinant JNK2α1 protein	Recombinant PPAR protein	Recombinant TR protein
Recombinant CREB protein	Recombinant JNK2α2 protein	Recombinant pRB protein	

mTRAP™ Isolates More mRNA

mTRAP™ Kits isolate high yields of quality mRNA from mammalian cells, tissue and total RNA. mTRAP Maxi, Midi and 96 Kits optimize isolation from different sample sizes, while mTRAP Total isolates mRNA from total RNA. All mTRAP Kits utilize Active Motif's Poly T gripNA™ Probe, which has a higher affinity and specificity for mRNA than oligo dT. The result is more mRNA per sample, with reduced ribosomal RNA and genomic DNA contamination.

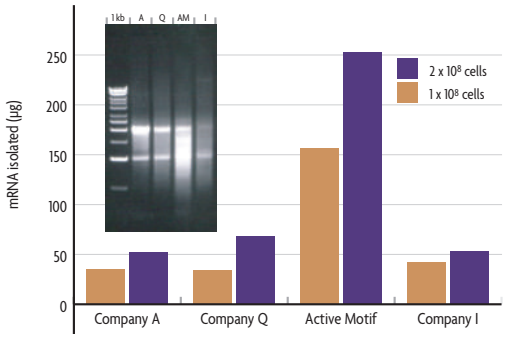

Figure 1: Higher yields of pure mRNA.
mRNA was isolated from 1 and 2 x 10^8 HeLa cells using mTRAP Maxi and three other suppliers' kits. Eluted mRNA was quantified by spectrophotometer and plotted. Two µg of mRNA from each 1 x 10^8 sample was run on a 0.8% agarose gel. mTRAP-isolated mRNA shows no genomic DNA and less rRNA contamination, with a far greater percentage of the sample being actual mRNA.

Lane 1: 1 kb DNA Ladder
Lane 2: mRNA isolated using Company A's kit
Lane 3: mRNA isolated using Company Q's kit
Lane 4: mRNA isolated using mTRAP™ Maxi Kit
Lane 5: mRNA isolated using Company I's kit

The mTRAP advantage

gripNAs are a novel class of negatively charged peptide nucleic acids (PNAs) that bind to nucleic acids with high affinity and specificity. This enables mTRAP Kits to employ low-salt Lysis, Wash and Elution Buffers that destabilize mRNA secondary structures as well as any weak, non-specific interactions that may have formed between nucleic acids and proteins with the Poly T gripNA Probe. The result is significantly higher yields of pure mRNA with lower levels of rRNA contamination compared to oligo dT-based methods (Figure 1). The higher affinity of Poly T gripNA Probe also enables isolation of mRNA that have shorter poly A tails, resulting in a more representative sample. This makes mTRAP-isolated mRNA ideal for production of cDNA probes for microarrays, cDNA libraries or in Northern blots.

Advantages

- Isolates more mRNA per sample than oligo dT-based methods
- Reduces ribosomal RNA and genomic DNA contamination
- Captures mRNA with secondary structure and shorter tails, for a more representative mRNA population
- Higher purity mRNA means better cDNA probes for microarray analysis

mTRAP Maxi and mTRAP Midi

The mTRAP Maxi Kit provides 6 large-scale mRNA isolations (Figure 1). Each reaction can isolate mRNA from 0.5-2.0 x 10^8 cells or 0.4-1 gram of tissue and will yield 100-200 µg mRNA, depending on cell type.

The mTRAP Midi Kit provides 24 medium-sized mRNA isolations. Each reaction can isolate mRNA from 0.5-1.0 x 10^7 cells or 50-200 mg tissue and will yield 10-20 µg mRNA, depending on cell type.

mTRAP 96

The mTRAP 96 Kit is supplied in a 96-well format designed for high-throughput usage and is ideal for use with arrays and RT-PCR. Each reaction can isolate mRNA from 0.5-1.0 x 10^6 cells or 10-20 mg tissue and yields 100-300 ng mRNA, depending on cell type.

mTRAP Total

The mTRAP Total Kit was created for single-pass enrichment of mRNA from total RNA (Figure 2). Reagents and plasticware are included for 12 mRNA isolations. Each reaction can isolate mRNA from up to 500 µg total RNA and will yield 10-15 µg mRNA.

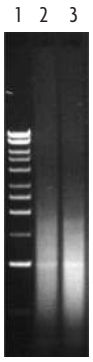

Figure 2: Pure mRNA from total RNA in one passage.
mRNA was isolated from 500 µg of total RNA in duplicate using mTRAP Total, quantified by spectrophotometry and run on a 0.8% agarose gel.

Lane 1: 1 kb DNA Ladder
Lanes 2-3: 2 µg mRNA isolated in one pass with the mTRAP Total Kit.

Complete package

mTRAP Kits come complete with all of the plasticware and reagents needed to perform your mRNA isolations. And, with increased yields and purity, you'll have more confidence using your mRNA in microarrays, Northerns, RPA and RT-PCR applications. For higher yields of cleaner mRNA, switch to mTRAP today.

Product	Format	Catalog No.
mTRAP™ Maxi	6 rxns 5 x 6 rxns	23006 23506
mTRAP™ Midi	24 rxns 5 x 24 rxns	23024 23524
mTRAP™ 96 mTRAP™ 96 w/MAG-96 MAG-96 magnetic stand	3 x 96 rxns 3 x 96 rxns 1 stand	23096 23097 90096
mTRAP™ Total	12 rxns 5 x 12 rxns	23012 23512

Efficient, Affordable Competent *E. coli*

RapidTrans™ are high-efficiency competent *E. coli* supplied in a convenient, 96-tube tray. Ideal for cloning, plasmid preparation and library construction, RapidTrans cells are affordably priced and packaged in a format that provides maximum flexibility while eliminating waste. Each tube contains 50 µl of cells for one transformation reaction. In contrast to other 96-well formats that require thawing all 96 reactions at one time, RapidTrans enables the use of as few or as many reactions as needed, without thawing the other cells. This eliminates the reduced efficiencies and waste caused by repeated freeze/thaw cycles.

Strain & competency availability
RapidTrans TAM1 competent *E. coli* can be purchased with or without the F´ and λ pir+ genotype modifications depending on your needs. For applications that require higher efficiencies, some RapidTrans strains are available in an Extra Competent format that supplies an efficiency of > 3 x 10^8 cfu/µg.

Advantages

- Freedom to use anywhere from 1 to 96 reactions eliminates wasted reactions
- Choice of > 1 x 10^8 or > 3 x 10^8 cfu/µg
- Economical pricing

Additional information
To learn more about RapidTrans Competent *E. coli,* including specific genotype information, please return the enclosed reply card or log on to our website at www.activemotif.com/rapidtrans.

Product	Format	Catalog No.
RapidTrans™ TAM1-F´ Competent *E. coli*	1 x 96 rxns 5 x 96 rxns	10096 10596
RapidTrans™ TAM1-F´ Extra Competent *E. coli*	1 x 96 rxns 5 x 96 rxns	10099 10599
RapidTrans™ TAM1 Competent *E. coli*	1 x 96 rxns 5 x 96 rxns	11096 11596
RapidTrans™ TAM1 Extra Competent *E. coli*	1 x 96 rxns 5 x 96 rxns	11099 11599
RapidTrans™ TAM1 λ pir+ Competent *E. coli*	1 x 96 rxns 5 x 96 rxns	11097 11597

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