MOTIF VATIONS

THE NEWSLETTER OF ACTIVE MOTIF — JUNE 2005 • VOLUME 6 • NUMBER 2

ACTIVE MOTIF®

Tools to Analyze Cellular Function

Look Inside For Hot New Technologies

IN THIS ISSUE

Your Source for Studying the NFκB Pathway

NFκB is an important cellular regulator that has also been shown to play a role in many disease states such as inflammation, cancer, apoptosis and autoimmune response. To make the study of NFκB easier and more efficient, Active Motif has developed a number of innovative products that are sure to suit your needs.

TransAM™ measures DNA-binding activity
In order to initiate transcription, transcription factors such as NFκB must first bind to their DNA target(s). TransAM Kits take advantage of this property by using a combination of DNA binding and antibody detection to give a specific, quantitative readout of DNA-binding activity from all sample types (Figure 1; see page 3 for more details). TransAM Kits are sensitive, quantitative and fast, making them an ideal replacement for classical methods such as radioactive gelshifts and time-consuming reporter gene assays.

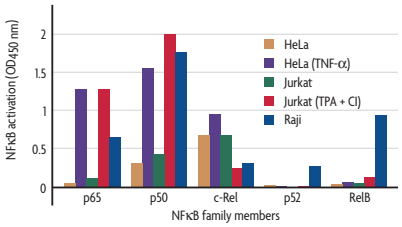

Figure 1: NFκB family profiling of DNA binding.
Nuclear extracts prepared from HeLa, HeLa treated with TNF-α, Jurkat, Jurkat treated with TPA + calcium ionophore (CI) and Raji cells were assayed at 10 µg/well for p65, p50, c-Rel, p52 and RelB activity using the TransAM NFκB Family Kit.

Detection of phospho-proteins
Because many of the proteins involved with the NFκB pathway are activated by phosphorylation, it is important to be able to quickly and easily quantitate phosphorylation events. FACE™ Kits are cell-based ELISAs that offer a convenient alternative for monitoring the phosphorylation of the NFκB pathway members AKT, JNK, PI3K, GSK and NFκB p65. FACE Kits enable analysis directly within the cell, which eliminates the need for preparing extracts, running gels and performing Western blots. All FACE Kits provide both a total and a phosphorylated antibody for the protein being studied, and all kits are available in both colorimetric and chemiluminescent formats (Figure 2). See page 6 for more information on FACE Kits.

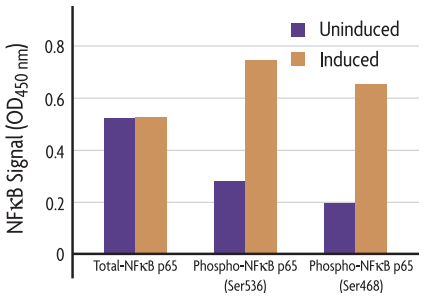

Figure 2: Monitoring phosphorylation of NFκB p65 at multiple sites.
The FACE NFκB p65 Profiler Kit was used to assay levels of total and phosphorylated NFκB p65 in uninduced and TNF-α + Calyculin A induced HeLa cells. Data was plotted after correction for cell number (performed through use of the kit's Crystal Violet reagent).

In addition, Active Motif offers a Sandwich ELISA to study IκBα phosphorylation. FunctionELISA™ IκBα provides a quantitative method for monitoring phosphorylation of the low-level IκBα protein in a sensitive chemiluminescent ELISA (Figure 3).

Monitor gene promoter regulation
Chromatin immunoprecipitation (ChIP) is a powerful tool used to study protein/DNA interactions. To make ChIP experiments more successful, Active Motif offers its complete line of ChIP-IT™ Kits. Depending on your needs, you can buy all of the critical components you need in a single kit, including antibodies, buffers and PCR primer controls that have been proven to work in ChIP. Or, if you are a more experienced user, you can use ChIP-IT Kits that do not contain control antibodies and PCR primers. This makes our ChIP-IT product line the most effective and versatile available. Look on page 7 to learn about our exciting new products for ChIP.

Antibodies & recombinant proteins
Active Motif's extensive line of antibodies for NFκB, toll-like receptors (TLRs) and IKK provide superior performance and reliable results. In addition, Active Motif offers a number of recombinant proteins for use as positive controls, in *in vitro* screening studies and as protein standards in our ELISAs.

To find out more about Active Motif's tools for studying the NFκB pathway, simply visit www.activemotif.com.

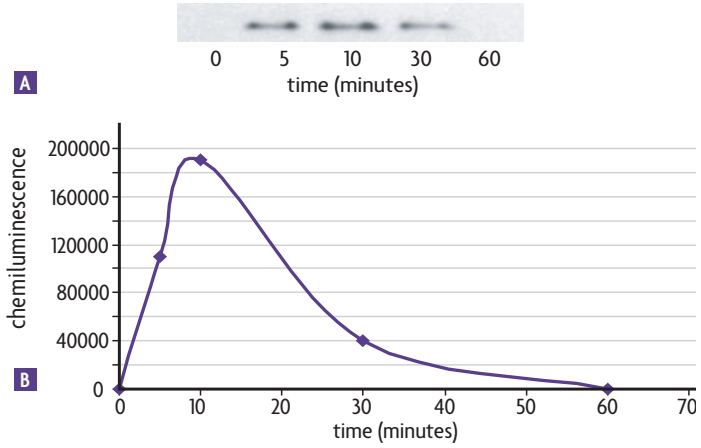

Figure 3: Induction of IκBα phosphorylation.
Jurkat cells were treated with 1 nM TNF-α and harvested at the indicated time points. Whole-cell extracts were assayed in Western blot analysis (A) using Phosphorylated-IκBα mAb (Cat. No. 40904) and the FunctionELISA IκBα Kit (B).

New: TransAM™ Flexi Kits Enable Profiling of NFκB Binding at Any DNA Sequence

Active Motif's new TransAM™ Flexi NFκB Family Kit enables you to profile the binding of the NFκB Family members p65, p50, p52, c-Rel and RelB to any DNA-binding site. Flexi Kits are the latest version of the original TransAM Kits, which are the method of choice for studying transcription factor binding activity.

Flexi or Original TransAM?

The original TransAM Kits offer a fast, non-radioactive alternative to gelshift assays and provide a 96-well plate that is pre-coated with the consensus binding site for the factor of interest. This original format is convenient for measuring binding activity at the consensus site but does not enable you to study alternative sites. That's why we've developed the new Flexi Kits; we provide pre-optimized reagents including proven antibodies, controls and reaction buffers, while you have the flexibility to immobilize any oligo in the 96-well plate.

How do TransAM Flexi Kits work?

In order to measure binding at your sites of interest, you first design biotinylated oligos or PCR products that contain the binding sites you wish to study. Each biotinylated oligo is then incubated with nuclear extract that has been treated to activate the transcription factor of interest. The extract/oligo mixture is then transferred to a 96-well, streptavidin-coated plate, where the bound, biotinylated oligonucleotide is captured. A primary antibody specific for the activated transcription factor is added, followed by HRP-conjugated secondary antibody and developing reagent. The levels of DNA-bound transcription factor are then read on a spectrophotometer, which provides a quantitative, readout of transcription factor activation (Figure 1).

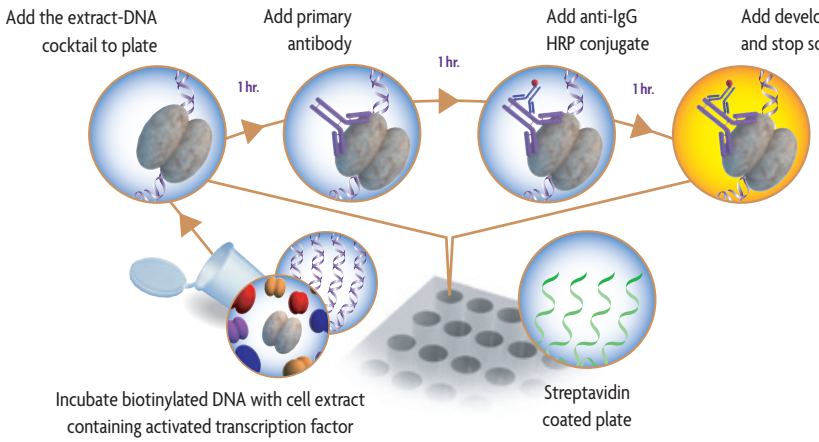

Figure 1: Flowchart of the TransAM Flexi procedure.

TransAM Flexi advantages

- Monitor binding at any sequence
- High-throughput 96-stripwell format
- Sensitive & quantitative
- Results in less than 5 hours
- Assay cell or tissue samples

Check binding affinities at multiple sites

The new Flexi Kits make it easy to quantitate the binding affinity of a given NFκB Family member at any binding site. To demonstrate, three different oligos were synthesized that included the natural binding sites of promoters regulated by NFκB: Igκ, IL-8 and uPA. The binding affinity of NFκB p65 was then tested at these sites. The results demonstrate that NFκB p65 had its weakest affinity for the oligo that contained the natural binding site of the IL-8 promoter (Figure 2).

Order one today

TransAM Flexi Kits make it fast and easy for you to study transcription factor binding at any DNA sequence you choose. A Kit for the NFκB Family (p50, p65, p52, c-Rel and RelB) and individual kits for NFκB p50 and p65 are now available, with more on the way. For complete details, visit us at www.activemotif.com/transam.

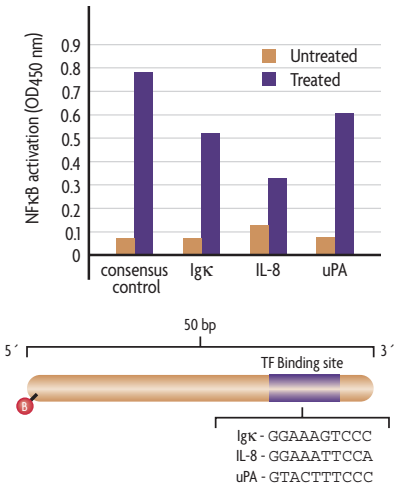

Figure 2: Measuring affinity of NFκB p65 for different binding sites. Five µg of nuclear extract from untreated and TNF-α-treated HeLa cells were used to assay the binding affinity of NFκB p65 for four different biotinylated 50-mer oligonucleotides. Each of the 3 test oligos (Igκ, IL-8 and uPA) contained the wild-type binding site of a promoter that is regulated by NFκB. The binding affinities of these natural binding sites were compared to the consensus binding site control that is provided in TransAM Flexi Kits.

Product	Format	Catalog No.
TransAM™ Flexi NFκB Family	2 x 96-well plates	43298
TransAM™ Flexi NFκB p50	1 x 96-well plate	41098
TransAM™ Flexi NFκB p65	1 x 96-well plate	40098
TransAM™ NFκB Family	2 x 96-well plates	43296
TransAM™ NFκB p50	1 x 96-well plate	41096
TransAM™ NFκB p50 Chemi	1 x 96-well plate	41097
TransAM™ NFκB p65	1 x 96-well plate	40096
TransAM™ NFκB p65 Chemi	1 x 96-well plate	40097

Chariot™ Delivers Functional Protein into Living 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 an ideal tool for a variety of functional studies.

Targeted delivery

Chariot is a peptide that forms a non-covalent complex when incubated with your purified protein, peptide or antibody for 30 minutes at room temperature. Addition of the complex to cells results in its rapid internalization. Once inside the cell, the complex dissociates and Chariot is transported to the nucleus, while the delivered protein is biologically active and free to proceed to its cellular target (Figure 1).

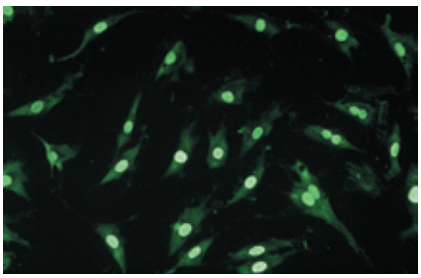

Figure 1: Targeted protein delivery.
50 ng of a 10 kDa nuclear protein labeled with Lucifer yellow at the C-terminus was complexed with Chariot and delivered into HS-68 cells. Unfixed cells were observed 90 minutes post-delivery.

Non-covalent delivery

Many protein delivery systems require that you begin by fusing a carrier protein to your macromolecule. However, this can change the folding characteristics of your protein and, ultimately, its function. Because Chariot forms a non-covalent bond with your protein, it does not affect the delivered protein's folding or function.

Deliver biologically active proteins

The ability of Chariot to deliver biologically active compound was demonstrated using

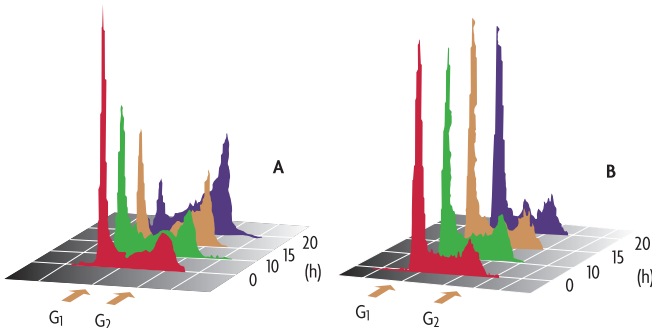

Figure 2: Chariot delivery of functional protein.
HS-68 cells arrested in G_0 phase by serum deprivation for 48 hours were released by addition of serum for 3 hours. Flow cytometry performed 0, 10, 15 and 20 hours after addition of Chariot alone and a Chariot-p27^{kip1} complex indicates that cells receiving Chariot alone progressed into G_2 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.

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}.

Advantages

- Delivers active protein directly into living 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

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 (see table below); 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 more extensive list of papers that cite the use of Chariot, simply return the enclosed reply card or download the list at www.activemotif.com/chariot.

Cell/Tissue Type	Delivered	Citation
Mouse Lung Tissue	Protein	Aoshiba, K. *et al.* (2003) *Amer. J. Resp. Cell Mol. Biol.* 28: 555-562.
Neuronal Growth Cones	Protein	Jurney, W. *et al.* (2002) *J. Neuroscience.* 22(14): 6019-6028.
Primary Mouse Hepatocytes	Protein	Bardag-Gorce, F. *et al.* (2003) *Exp. Mol. Pathology* 74:160-167.
Hodgkin's and B Cells	Smac protein and Smac peptide	Kashkar, H. *et al.* (2003) *J. Expr. Med.* 198(2): 341-347.
Human Primary T cells	Unc119 protein	Gorska, M. *et al.* (2004) *J. Expr. Med.* 199(3): 369-379.
Rat DRG Axons	Dynamitin protein	Heerssen, H. *et al.* (2004) *Nature Neuro.* 7(6): 596-604.
Chicken Embryo Retina	XAC (various mutations)	Gehler, S. *et al.* (2004) *J. of Neuro.* 24(47): 10741-10749.
Mouse Photoreceptor cells	Peptide	Koulen, P. *et al.* (2005) *Invest. Ophth. Vis. Sci.* 46: 287-291.
Mouse Adrenal Gland slices	PP-19 peptide	Chan, S. *et al.* (2005) *J. of Neurophysiology* 10.1152/jn.01213.2004
Rat Lung Tissue	PKA holoenzyme	Maron, M. *et al.* (2005) *Amer. J. Physiol. Lung Cell Mol. Physiol.* 10.1152/ajplung.00134.2005

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

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

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

Improved Gene Silencing Through Higher Specificity Binding

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 seemed to make them ideal for gene silencing. PNAs are resistant to nucleases and display high-affinity and sequence-specific binding. However, PNAs have proven to be inefficient tools for gene silencing, due mostly to their lack of cell permeability, tendency to self-aggregate and poor solubility. To capitalize on the promise of PNAs, Active Motif developed its gripNAs. gripNAs are a novel form of negatively charged PNA that overcome the limitations of classical PNAs. The inclusion of a negative charge in gripNAs has eliminated the solubility and aggregation issues associated with PNAs, while retaining their high affinity and specificity. gripNAs can be delivered efficiently using multiple approaches and have been shown to be highly effective at gene silencing (Figure 1).

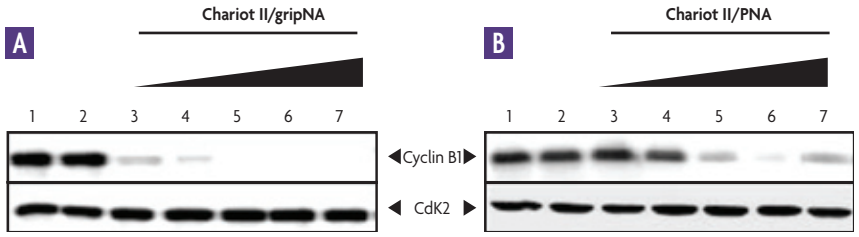

Figure 1: Inhibition of expression by gripNAs and PNAs directed against cyclin B1.
Increasing concentrations (lane 3: 50 nM, lane 4: 100 nM, lane 5: 500 nM, lane 6: 1 µM, lane 7: 2 µM) of anti-cyclin B1 gripNA (A) or PNA (B) were incubated with Chariot II at a molar ratio 1:25 at 37°C for 1 hour, then 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.[1]

Why choose gripNAs?

- Unsurpassed sequence specificity reduces unintended phenotypes
- Resistant to nucleases
- Flexible synthesis modification
- Multiple methods for gripNA delivery
- Convenient online ordering

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 even 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*, *uro*D 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.[2]

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.

* Patent pending. Purchase includes all rights for research use. Other-use licenses are available, please contact Technical Services for details.

1. Morris *et al.* (2004) *Gene Therapy* 11: 757-764.
2. Urtishak *et al.* (2003) *Developmental Dynamics* 228: 405-413.

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

Simple, Efficient Analysis of Protein Phosphorylation

As interest in kinase activation and their effect on cellular regulation increases, so does the need for fast, efficient and robust assays to measure protein phosphorylation. That is why Active Motif developed its Fast Activated Cell-based ELISA (FACE™) Kits. FACE Kits provide a simple, sensitive, cell-based method for monitoring protein phosphorylation. They enable you to perform modification-specific analysis directly within the cell, without the need for time-consuming cell extractions, gel electrophoresis or membrane blotting, all of which saves you time and money.

The FACE method

In the FACE method, cells are cultured in 96-well plates and stimulated to induce the pathway of interest. Following stimulation, the cells are rapidly fixed, which preserves activation-specific protein modifications. Each well is then incubated with a primary antibody specific for the protein modification of interest. Subsequent incubation with secondary HRP-conjugated antibody and developing solution provides a colorimetric or chemiluminescent readout that is quantitative and reproducible (Figure 1). FACE Kits also contain a primary antibody for the native non-modified protein, so you can monitor both native and activated protein levels in the same experiment (Figure 2).

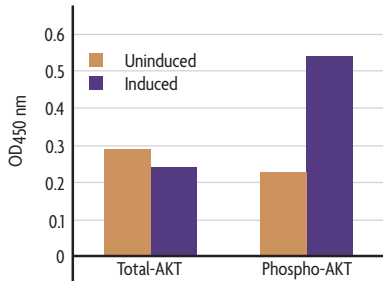

Figure 2: Measurement of phosphorylated and total AKT.
NIH/3T3 cells were cultured in 96-well plates and serum-starved for 16 hours. Cells were then treated with 50 ng/ml PDGF for 5 minutes and fixed. Total and phospho-AKT were each assayed in triplicate using the phospho and total AKT antibodies from the FACE AKT Kit.

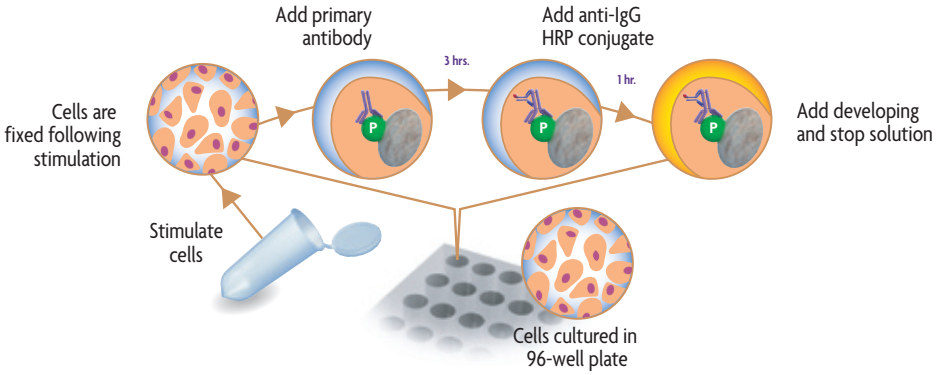

Figure 1: Flow Chart of the FACE Process.
Cells are grown, stimulated and fixed in the same 96-well plate. Addition of primary and secondary antibodies detects phosphorylated protein.

FACE advantages

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

A variety of kits for all your needs

Many cellular kinase cascades contain critical control points, where multiple downstream components can be phosphorylated by a single kinase. The phosphorylation of one or other substrate can cause significant shifts in the cellular response, which means that monitoring phosphorylation events across multiple proteins can become commonplace. However, trying to monitor this type of "signaling switch" using Western blotting or classical radioactive kinase assays is a labor intensive and expensive undertaking, especially if there are multiple treatments to be measured. To overcome this problem, using FACE can be as simple as an extra pipetting step. This is because in the FACE method, cells are cultured and analyzed within the same well and therefore do not require the use of multiple culture plates, extract preparations, gels, membranes, *etc*. And, because you can easily increase sample throughput, your results will be statistically more relevant.

Prevents unwanted sample modifications

When analyzing protein phosphorylation via Western blotting or ELISA, it is necessary to prepare cellular extracts. However, additional protein modifications can occur during the extraction process, which may alter your results. To eliminate this problem, FACE Kits use a fixation step that "freezes" the cellular state and prevents further protein modifications. This enables detection of the exact protein state in the cell at a chosen time point, which provides you with more accurate results. So, for a better phospho-specific assay, try FACE.

FACE™ Product Line			
FACE™ AKT	FACE™ ATF-2	FACE™ Bad	FACE™ c-Jun (S63)
FACE™ c-Jun (S73)	FACE™ c-Src	FACE™ EGFR (Y992)	FACE™ EGFR (Y1173)
FACE™ ErbB-2 (Y877)	FACE™ ErbB-2 (Y1248)	FACE™ ERK1/2	FACE™ FAK
FACE™ FKHR (FOXO1)	FACE™ GSK3β	FACE™ JAK1	FACE™ JNK
FACE™ MEK1/2	FACE™ NFκB Profiler	FACE™ p38	FACE™ PI3 Kinase p85
FACE™ STAT2	FACE™ STAT4	FACE™ STAT6	

New: Complete ChIP Kit with Enzymatic Shearing

Active Motif is excited to announce the release of a complete chromatin immunoprecipitation (ChIP) kit that includes all of the reagents for performing ChIP with enzymatic shearing. The new ChIP-IT™ Enzymatic Kits combine Active Motif's innovative Enzymatic Shearing Kit with our popular complete ChIP-IT Kit. Together, this combination provides you with the simplest solution for performing ChIP experiments.

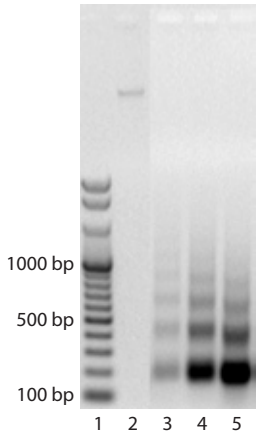

Figure 1: Analysis of DNA sheared using the Enzymatic Shearing Kit. HeLa cells were fixed for 10 minutes with 1% formaldehyde and then chromatin was prepared using the Enzymatic Shearing Kit protocol. Chromatin was sheared with the enzymatic shearing cocktail for 5, 10 & 15 minutes and the reaction was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

Lane 1: 100 to 1000 bp ladder.
Lane 2: Unsheared HeLa DNA.
Lane 3: HeLa DNA treated for 5 minutes.
Lane 4: HeLa DNA treated for 10 minutes (optimized).
Lane 5: HeLa DNA treated for 15 minutes.

Enzymatic vs. Sonication Shearing

The starting point for any ChIP experiment is the preparation of chromatin. This critical step must be optimized to ensure the chromatin is sheared to 200-1000 bp fragments. Typically, chromatin shearing is performed by using sonication. Although sonication is an effective method for shearing DNA, it can also be time consuming and difficult to optimize due to complications arising from emulsification and overheating during the sonication process. Also, because the quality of your sheared sample depends greatly upon the quality of your sonicator, it may be necessary to purchase an expensive, "high-end" sonicator to get reproducible shearing. In contrast, the ChIP-IT Enzymatic and Enzymatic Shearing Kits utilize a proprietary enzymatic shearing cocktail that quickly and easily shears DNA into 200-1000 bp fragments (Figure 1). And, because enzymatic shearing is dependent only on time and temperature, all of the problems associated with sonication are eliminated, which greatly improves downstream ChIP results.

How do I order one

The Enzymatic Shearing Kit is sold separately and also as part of the new ChIP-IT Enzymatic Kits. The ChIP-IT Enzymatic Kits are available with and without positive control antibodies and primers. Want to learn more? Give us a call or download a manual at www.activemotif.com/chip.

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

ChIP Product Line

Complete ChIP-IT Kits

Active Motif's ChIP-IT Kits are the most effective products available for performing ChIP. The kits contain nearly everything you need to perform ChIP including a comprehensive protocol and optimized buffers, inhibitor cocktails, DNA purification columns as well as positive control primers and antibodies, all of which have been validated in actual ChIP experiments. The ChIP-IT Kits are available with either sonication and enzymatic shearing.

Pre-validated ChIP antibodies

Finding a ChIP validated antibody can be difficult. Active Motif is working diligently to validate our extensive antibody list for use in ChIP. Below is a list of antibodies that have been positively tested in ChIP to date, which are ideal when used with our ChIP-IT w/o controls Kits. Be sure to keep up to date on new ChIP-validated antibodies by visiting our website.

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

Sensitive Quantification of Activated Ras GTPase

Active Motif's new ELISA-based kit for studying activated Ras GTPase is a marked improvement over other commercially available kits. All other products utilize immunoprecipitation of Ras followed by Western blotting. In contrast, Active Motif's Ras GTPase Chemi ELISA Kit uses a combination of Raf-RBD protein and antibodies in a 96-well format to provide a faster and more sensitive alternative. This enables you to use less of your precious sample and still detect low-level events. In addition, because ELISAs provide more quantitative results than Westerns, your data is more meaningful. And, as the Ras ELISA Kit uses a 96-well plate made up of 12-well strips, you can run anywhere from 1 to 96 samples at a time, which lets you accommodate whatever throughput your experiment requires.

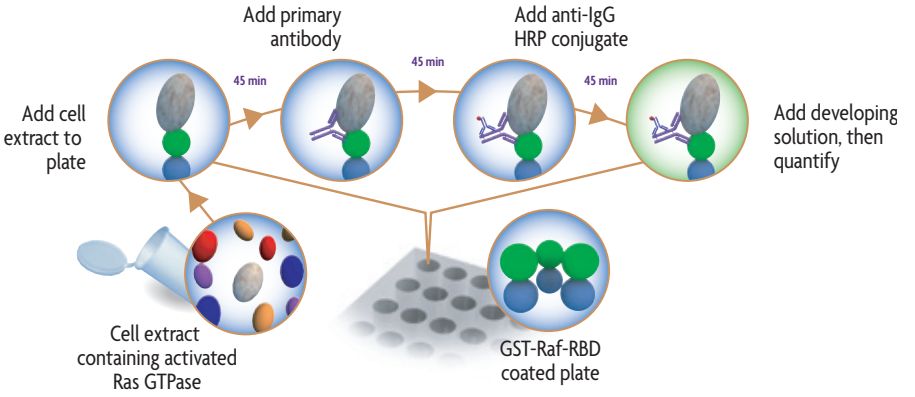

Figure 1: Flowchart of the Ras GTPase Chemi Kit.
Cell extract is added to a glutathione-coated plate that contains immobilized GST-Raf-RBD protein. Activated Ras in the extract binds to the Raf-RBD protein. Addition of primary & secondary antibodies and developing solution followed by reading on a luminometer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

Ras GTPase is a much-studied regulator

Small GTPases, also called GTP-binding proteins, are a family of enzymes that serve as molecular switches in regulating a number of signal transduction pathways including growth, apoptosis and differentiation. Ras is of particular interest to researchers because aberrant regulation by Ras has been implicated in a number of disease states. Mutant, constitutively active forms of Ras are estimated to be present in ~30% of all human cancers.

The Ras GTPase Chemi ELISA method

Because activated Ras binds specifically to the Ras-binding domain (RBD) of the Raf effector protein, Raf-RBD is used as a probe to isolate activated Ras. The Ras ELISA Kit contains a Raf-RBD protein fused to GST and a 96-well, glutathione-coated assay plate. GST-Raf-RBD is first incubated on the plate for one hour to immobilize the capture protein. Addition of sample to the plate results in the binding of activated Ras to the Raf-RBD. A primary antibody specific for Ras is then added, followed by an HRP-conjugated secondary antibody and developing reagent (Figure 1). The plate is then read on a luminometer, which provides a sensitive, quantitative chemiluminescent readout of activated Ras (Figure 2).

Ras ELISA advantages

- **More sensitive** – assay uses only 25 µg of extract, which is 20-fold less than pull-down/Western methods
- **Better results** – quantitative readouts make it easier to compare results
- **Less effort** – no need to run gels or develop Western blots
- **Save time** – results in less than 5 hours
- **Versatile** – assay activated extracts from cells or tissue samples, or study recombinant Ras protein

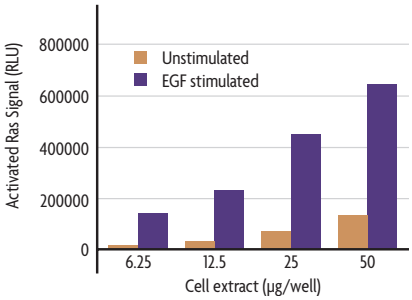

Figure 2: Quantification of activated Ras in stimulated HeLa cells.
Increasing amounts of whole-cell extract from HeLa cells that had been stimulated with 5 ng/ml of EGF for 2 minutes were assayed using the Ras GTPase Chemi ELISA Kit.

Try sensitive, quantitative ELISAs

The Ras GTPase Chemi ELISA Kit makes it simple to quantify activated Ras. It is ideal for studying signaling pathways that activate Ras. Please give us a call, return the enclosed reply card or log on to www.activemotif.com/gtpase for complete information. To get to the only ELISA-based Ras activation assay, order the Ras GTPase Chemi ELISA Kit.

Product	Format	Catalog No.
Ras GTPase Chemi ELISA Kit	1 x 96-well plate	52097

A Complete Solution for Studying Nuclear Receptor Protein Activity

Active Motif offers a variety of nuclear receptor analysis tools that make studying nuclear receptor proteins both faster and more accurate than using traditional methods. Active Motif has products for monitoring DNA-binding activity, activation state, protein level or agonist/antagonist effects.

Monitor ligand activation with NR Peptide
Studying the agonist/antagonist effects of potential drug targets is an important element of nuclear receptor-targeted drug discovery. Active Motif's NR Peptide ELISAs are specifically designed to capture ligand-activated nuclear receptor and can be used with both cell extracts and proteins. Each NR Peptide ELISA Kit provides a 96-well plate that is coated with a Capture Peptide that includes the consensus-binding motif of the nuclear receptor's co-activator. Addition of sample results in binding of ligand-activated nuclear receptor to the Capture Peptide. Each well is then incubated with a primary antibody specific for the nuclear receptor of interest, followed by an HRP-conjugated secondary antibody and developing solution to provide an easily quantified readout. This enables you to quickly and quantitatively measure the agonist/antagonist effects of target compounds on the binding of ligand-activated nuclear receptors (Figure 1).

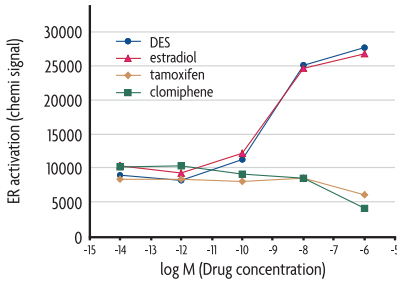

Figure 1: ERα agonism/antagonism dose-response curves. Nuclear extracts from the breast cancer cell line MCF-7 are incubated in wells of the NR Peptide ELISA ER plate in the presence of 100-fold serial dilutions (from 10^{-6} to 10^{-14} M) of the agonist compounds diethylstilbestrol (DES) and estradiol, and the antagonist compounds tamoxifen and clomiphene. Only ligand-activated ER protein can bind to the Capture Peptide immobilized in the plate. Bound ER is specifically detected with ERα antibody and quantified using a secondary antibody and Detection Solution.

Assess DNA-binding activity of NRs
Inappropriate nuclear receptor signaling is associated with numerous diseases including cancer, asthma and arthritis, which makes NRs promising drug targets. Because the end point of nuclear receptor activation is DNA binding, monitoring changes in the DNA-binding activity of a target nuclear receptor can serve as an ideal biomarker. Classical methods such as gelshifts and reporter gene assays are not well suited to this application. Active Motif's TransAM™ Kits provide an innovative alternative by using a combination of DNA binding and antibody detection to give a specific, quantitative readout of DNA-binding activity from all sample types.

Quantify total NR with Sandwich ELISAs
In order to fully examine the activation of a given nuclear receptor, it is important to be able to quantify the total levels of a given nuclear receptor within a sample. The new NR Sandwich ELISAs offer a simple, rapid method to quantify the total amount of nuclear receptor protein present in both cell and tissue samples. NR Sandwich Kits utilize the Sandwich ELISA-based method that is an improvement over other methods used to study proteins, such as Western blotting. The 96-well format is convenient and sensitive, with only a minimal amount of material required to give quantitative readout of nuclear receptor levels (Figure 2).

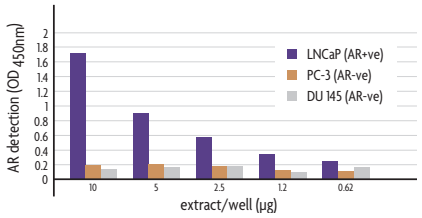

Figure 2: Monitoring expression levels of AR using NR Sandwich AR. Different amounts of nuclear extracts from three human prostate cancer cell lines, LNCaP, PC-3 and DU 145, were analyzed for levels of AR protein using the NR Sandwich AR Kit.

A collection of NR antibodies & proteins
Active Motif offers over 200 highly characterized antibodies directed against transcription factors and nuclear receptors, including antibodies for GR, PPARα and γ, PXR, RAR-β, -β2 and -γ, RXR-α and -β, and VDR. In addition, Active Motif offers a number of recombinant nuclear receptor proteins that are ideal for use as positive controls, in *in vitro* screening studies and in many other applications.

Get the tools you need for NR research
To get complete information on all of Active Motif's tools for studying nuclear receptors, please give us a call, visit our website or send in the enclosed reply card.

Product	Format	Catalog No.
NR Peptide ERα	1 x 96-well plate 5 x 96-well plates	49096 49596
NR Peptide ERα Chemi	1 x 96-well plate 5 x 96-well plates	49097 49597
NR Sandwich AR	1 x 96-well plate 5 x 96-well plates	49196 49696
NR Sandwich ERα	1 x 96-well plate 5 x 96-well plates	49296 49796
NR Sandwich PR	1 x 96-well plate 5 x 96-well plates	49396 49896
TransAM™ ER	1 x 96-well plate 5 x 96-well plates	41396 41996
TransAM™ GR	1 x 96-well plate 5 x 96-well plates	45496 45996
TransAM™ PPARγ	1 x 96-well plate 5 x 96-well plates	40196 40696

Faster, More Accurate Measurement of Nitric Oxide Production

Active Motif's Nitric Oxide Quantitation Kit is a faster, more sensitive method for measuring the production of nitric oxide (NO) in your samples. The kit employs an innovative cofactor technology that reduces the time and number of steps needed to measure NO levels. Plus, the Nitric Oxide Quantitation Kit has a wider dynamic range of NO measurement than other methods, which makes your results more accurate. And, the kit can be used with a large variety of sample types, including plasma, serum, saliva, urine, cell lysate, tissue homogenate and tissue culture medium.

Nitric oxide is a key signaling molecule that, either directly or indirectly, stimulates host defenses in the immune system, maintains blood pressure in the cardiovascular system and modulates neural transmission in the brain. Consequently, NO has become the subject of extensive research. However, because NO has an extremely short half-life (< 10 seconds), it is extremely difficult to detect and study unless you have expensive, specialized equipment.

NO Quantitation Kit advantages

- Increased sensitivity
- Fast and easy to use
- Wider dynamic range than LDH assays
- Works with a variety of sample types

Indirect detection of NO

Because NO is rapidly metabolized to nitrite and nitrate, quantitation of these stable anions can be used to indirectly measure the amount of NO originally present in a sample. The best index of total NO is the sum of the nitrite and nitrate in the sample, which are commonly quantified in a Two-Step assay. The first step is conversion of nitrate to nitrite using an NADPH-dependent nitrate reductase. The nitrite is then converted into a purple-colored azo compound by adding Griess Reagent. Quantitation of this azo compound by spectrophotometry provides an accurate measurement of the original NO concentration (Figure 1). However, the sensitivity of conventional Two-Step assays have been limited because NADPH, which is an essential cofactor in step one, interferes with Griess Reagent in step two. The sensitivity of Two-Step assays can be increased by the addition of a third step, in which lactate dehydrogenase (LDH) is used to eliminate excess NADPH prior to the Griess reaction. However, while inclusion of this additional step increases assay sensitivity, it also increases the time required to perform each assay.

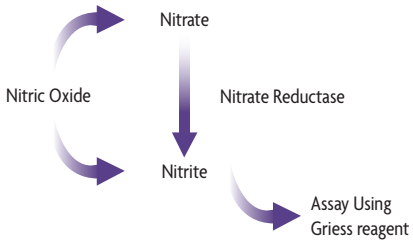

Figure 1: Measurement of nitric oxide by Griess Reagent.
Nitric oxide is converted to nitrite, then assayed using Griess Reagent.

Save time and money

Active Motif's Nitric Oxide Quantitation Kit offers a faster, easier-to-use alternative to conventional Two- and Three-Step NO assays. The kit contains a unique formulation of cofactors that accelerate the conversion of nitrate to nitrite, while simultaneously degrading NADPH. The result is that the time required for the reductase step is decreased to only 30 minutes and there's no need for a time-consuming third step, saving you both time and effort.

Improved accuracy for better results

In addition to its increased speed and user friendliness, the Nitric Oxide Quantitation Kit provides more accurate measurement over a wider range of sample concentration than possible with other currently used methods. The improved linearity over a broader dynamic range (Figure 2) means that your results will be more precise with less optimization and repetition than is possible with any other kit.

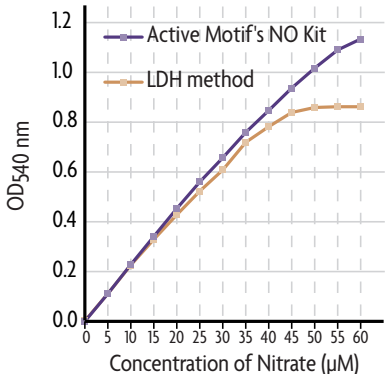

Figure 2: Dynamic range of nitrate standard curves.
Nitrate standard curves produced using the Nitric Oxide Quantitation Kit and a conventional Three-Step LDH-method kit.

Get better results today

The Nitric Oxide Quantitation Kit makes NO measurement faster, more sensitive and easier to perform than other, more conventional methods. In addition, its 96-well format enables straightforward high-throughput automation. For a better method of measuring NO production, try the Nitric Oxide Quantitation Kit today!

Product	Format	Catalog No.
Nitric Oxide Quantitation Kit	2 x 96 rxns	40020

mTRAP™ Kits Isolate 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.

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

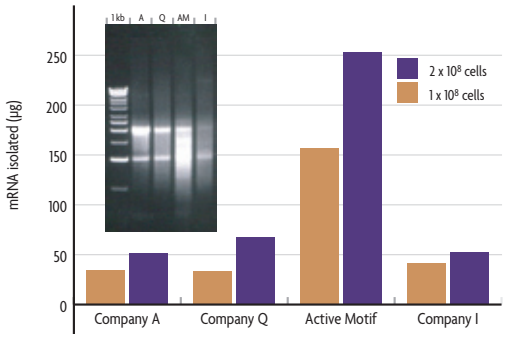

Figure 1: Higher yields of pure mRNA.
mRNA was isolated from 1 and 2 x 10^8 HeLa cells using mTRAP Maxi and three other suppliers' kits. Eluted mRNA was quantified by spectrophotometer and plotted. Two µg of mRNA from each 1 x 10^8 sample was run on a 0.8% agarose gel. mTRAP-isolated mRNA shows no genomic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

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.

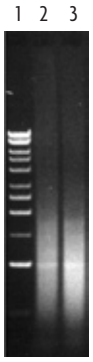

Figure 2: Pure mRNA from total RNA in one passage.
mRNA was isolated from 500 µg of total RNA in duplicate using mTRAP Total, quantified by spectrophotometry and run on a 0.8% agarose gel.

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

Active Motif Offices

U.S.

1914 Palomar Oaks Way, Suite 150
Carlsbad, CA 92008
Toll Free: 877 222 9543
Direct: 760 431 1263
Fax: 760 431 1351
Email: sales@activemotif.com

EUROPE

104 Avenue Franklin Roosevelt–Box 25
B-1330 Rixensart, Belgium
Germany Free Phone: 0800 181 99 10
France Free Phone: 0800 90 99 79
United Kingdom Free Phone: 0800 169 31 47
Other countries, Direct: +32 (0)2 653 0001
Fax: +32 (0)2 653 0050
Email: eurotech@activemotif.com

JAPAN

Azuma Bldg, 7th Floor
2-21 Ageba-Cho, Shinjuku-Ku
Tokyo, 162-0824, Japan
Direct: +81 (0)3 5225 3638
Fax: +81 (0)3 5261 8733
Email: japantech@activemotif.com

Distribution Network

Active Motif is represented in the countries listed to the right through our network of distributors. For additional contact and ordering information, please visit the distributor page on our website: www.activemotif.com/distributors.

Australia	Austria	Canada	China	Denmark	Finland
Hungary	India	Israel	Italy	Japan	Korea
New Zealand	Portugal	Spain	Sweden	Taiwan	Turkey