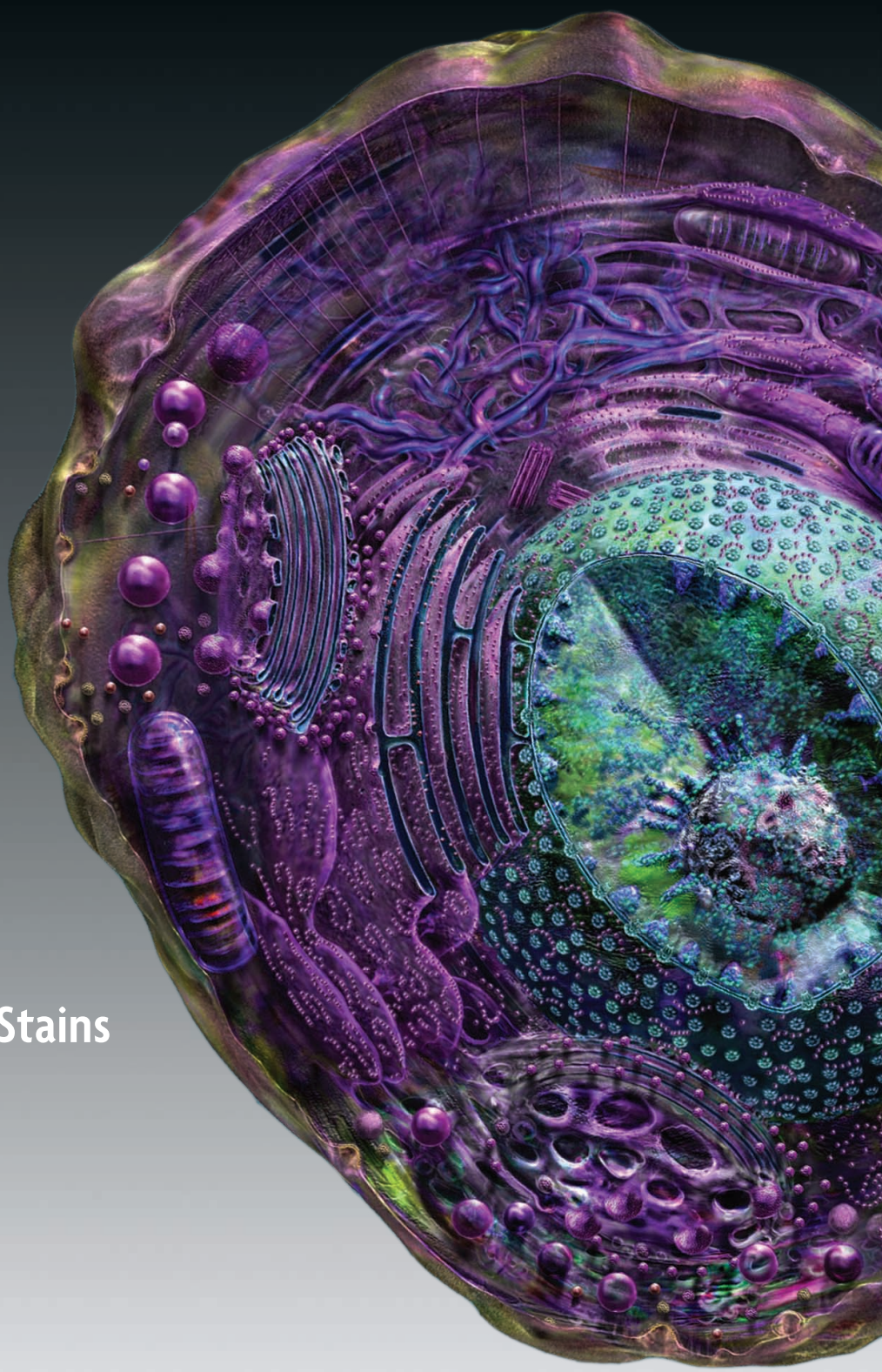
Products for
Cell Biology



In-Cell Westerns

Phospho ELISAs

Protein Transfection

SUMOylation

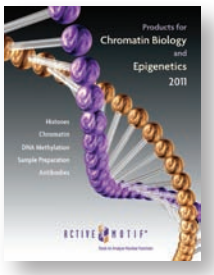
Sample Preparation

Fluorescent Assays & Stains

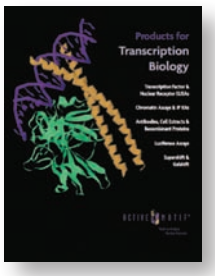
ACTIVE MOTIF®

Tools to Analyze Nuclear Function

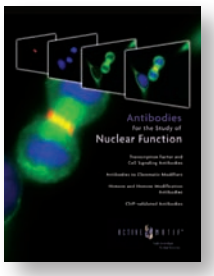
More Tools to Analyze Nuclear Function



Products for Chromatin Biology & Epigenetics



Products for Transcription Biology



Antibodies for the Study of Nuclear Function

ChIP-IT™ Express Products for Chromatin IP

Products for DNA Methylation

Products for Histone Analysis

TransAM™ Transcription Factor ELISAs

FACE™ Cell-based Phospho-specific ELISAs

Fluorescent Dyes, Conjugated Secondaries & Labeling

Get any (or all) of our product information by mail or by download

In addition to this product area profile, *Products for Cell Biology*, Active Motif has created product area profiles that describe our products in other areas of nuclear function, such as *Chromatin Biology & Epigenetics, Transcription Biology* and *Antibodies*. These profiles, more detailed brochures for most products and several informative posters can be downloaded or requested by mail at **www.activemotif.com/info**. Product manuals and technical data sheets for all products are also available.

ACTIVE MOTIF®

Tools to Analyze Nuclear Function

Products for Cell Biology

FACE™

simple, efficient analysis of protein phosphorylation

Fast Activated Cell-based ELISA (FACE™)* Kits provide a simple, efficient, cell-based method to monitor proteins activated by phosphorylation. FACE Kits enable modification-specific analysis directly within the cell, without the need for cell extractions, gel electrophoresis or membrane blotting. And, because the cells are grown, stimulated and assayed in a single 96-well plate, FACE Kits are easily automated.

Traditionally, protein phosphorylation has been studied using radioactive kinase assays and Western blotting, which can be time-consuming, complicated and expensive. FACE assays are non-radioactive and generate data that is more quantitative, specific and reproducible than these other methods. This makes FACE Kits the simplest, most cost-effective phospho-specific assays available.

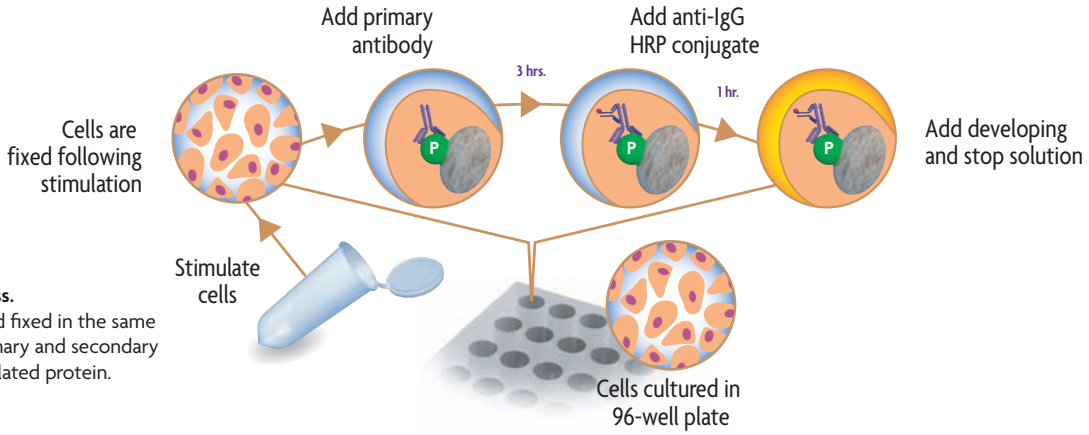
* Developed in collaboration with Dr. M. Peppelenbosch and Dr. H. Versteeg.

The FACE advantage

FACE Kits are easy to use and require less than 2 hours of hands-on time. 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 activated protein of interest. Subsequent incubation with secondary HRP-conjugated antibody and developing solution provides a colorimetric or chemiluminescent readout that is quantitative and reproducible. The number of cells in each well can be determined easily with the provided Crystal Violet solution. FACE Kits also contain a primary antibody specific for the total protein, so you can monitor both total and activated protein levels in the same experiment. FACE eliminates cellular extractions, radioactive kinase assays, time-consuming Westerns and inefficient epitope interactions that occur on membranes. FACE is a highly sensitive, high-throughput assay designed for detecting activated proteins within mammalian cells. FACE Kits are currently available for monitoring a large number of phosphorylated and total kinases, including p38, JNK, AKT, ERK1/2 and EGFR to name a few; to see a complete list of the over 20 targets available, please visit us at www.activemotif.com/face.

WHY USE FACE?

- Simple, non-radioactive method that is easily quantified
- Fixing preserves activation-specific protein modifications
- Cell-based assay eliminates cell extracts, gels and blotting
- Minimal hands-on time
- Antibodies and plates to compare both phospho and total protein levels
- Study any phospho-protein of interest using FACE Maker (see page 4)
- Chemiluminescent sensitivity to detect extremely small changes in phospho-protein levels
- Optimized module for working with suspension cells (page 4)
- Compatible with high-throughput automation



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.

Improved accuracy

Preparing cellular extracts for Western blotting is time-consuming, and additional protein modifications can occur during the extraction process that may alter the final results. To eliminate these problems, FACE Kits use a special fixation step that "freezes" the cellular state of the cell and prevents further protein modifications. This enables the detection of the exact protein state within the cell at a chosen time point, which provides you with more accurate results.

Unmatched specificity

To be certain that only the protein of interest is detected, all FACE Kit antibodies are stringently tested for cross-reactivity by Western blot analysis. In particular, the phospho-specific antibodies are assayed to verify that they detect only the activated form of the target protein. The phospho and total antibodies are used in tandem to make sure that the phospho-antibody does not interact with other phosphorylated proteins. This ensures that FACE Kits are highly specific and detect only the correct protein of interest at the specific phosphorylated site.

More quantitative data

Although sensitive, Western blots are more of a qualitative rather than quantitative tool. In contrast, FACE Kits provide data that can be easily quantified relative to the number of cells or to the total amount of target protein present. To illustrate, FACE p38 MAPK assays and Western blots were performed on anisomycin-treated murine macrophage 4/4 cells. FACE Kits clearly yield results that are more quantifiable than Western blot (Figure 1).

Reproducible results

FACE Kits are highly reproducible, which is extremely important when measuring small changes in the amounts of a phosphorylated protein. To demonstrate, FACE assays were performed on three different samples of macrophage 4/4 cells that had been treated in an identical manner with PMA to induce ERK phosphorylation. Levels of phosphorylated ERK were highly consistent between each sample (Figure 2). The high level of reproducibility using FACE Kits makes it possible to accurately monitor subtle differences in protein phosphorylation.

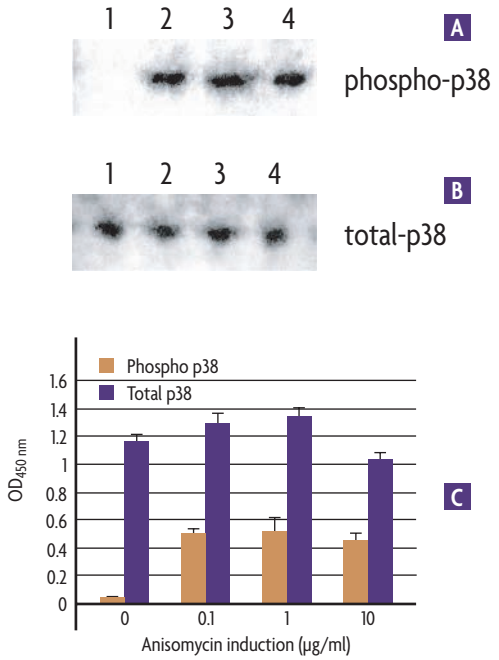


FIGURE 1:

Phospho and total p38 MAPK assays.

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

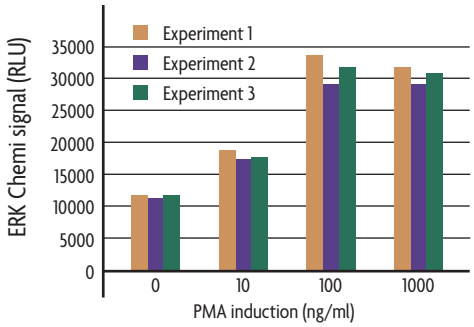


FIGURE 2:

Reproducible assay of phosphorylated ERK.

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

FACE Chemi – increased sensitivity

All of Active Motif's FACE Kits are available in a Chemi format that is ideal for those researchers who require maximum sensitivity. These ultra-sensitive kits use chemiluminescent detection on a luminometer to accurately monitor even the smallest changes in protein phosphorylation (Figure 3). Chemiluminescent detection provides more flexible measurement parameters than traditional colorimetric kits, enabling detection limits to be adjusted to maintain linearity and ensure that the detection sensitivity is appropriate to the sample type being assayed. FACE Chemi Kits require the use of a microplate luminometer or a CCD camera.

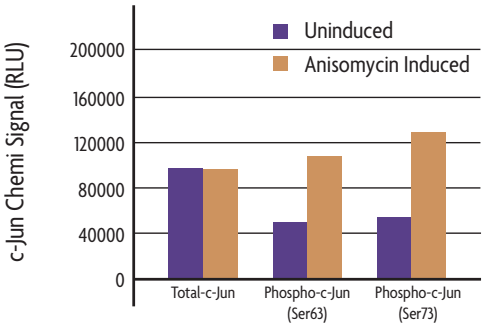


FIGURE 3:

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

FACE NFκB p65 Profiler – study multiple residues

Many proteins, such as NFκB, are phosphorylated on multiple residues and can be induced by a variety of stimuli. Being able to distinguish between different phosphorylation sites on a protein can be difficult to determine using conventional Western blot analysis. Active Motif's FACE NFκB p65 Profiler Kits make it possible to rapidly profile the levels of different phosphorylation sites in one simple experiment. Plus, like all the FACE Kits, Profiler Kits include an antibody against the total form of the protein, enabling comparisons of phosphorylated to native protein levels. The FACE NFκB p65 Profiler Kit contains antibodies specific for two phosphorylated sites: serine 468 and serine 536 (Figure 4).

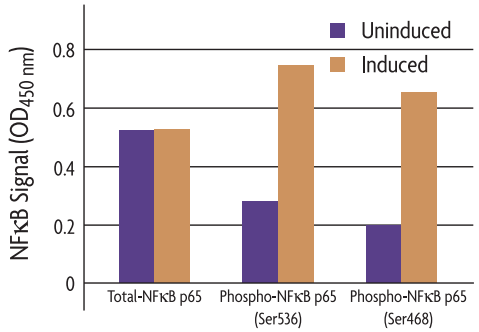


FIGURE 4:

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).

FACE Maker – study any phospho-protein

FACE Maker is an adaptable version of the FACE Kit, enabling you to study any phosphorylated protein you choose in a simple, fast and sensitive cell-based assay. Using antibodies specific to your desired target protein, modification-state specific analysis is performed directly within the cell without the need for lysates and time-consuming immunoblotting. FACE Maker Kits provide all the optimized buffers of our target-specific FACE Kits, but FACE Maker Kits do not include antibodies. So, you are able to study any phosphorylated protein you want with your own antibodies while taking advantage of the effective FACE method and optimized FACE reagents.

Suspension Cell FACE – measure more cells

The Suspension Cell FACE module was designed to be used with any colorimetric or chemiluminescent FACE Kit (Figure 5); it provides you with 96-well filter plates that make it easier to perform washing & liquid handling steps through use of an appropriate vacuum manifold. This helps eliminate the loss of cells that can occur when performing wash and fixation steps in 96-well plates. Because you're able to measure more cells per well, this makes the assay better able to discern small effects.

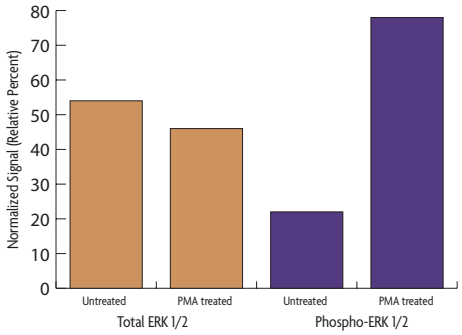


FIGURE 5:

FACE ERK1/2 with the Suspension Cell FACE module. The Suspension Cell FACE module was used with FACE ERK1/2 Chemi to assay 50,000 Jurkat cells per well. Jurkat cells were serum-starved and treated with 100 ng/ml PMA (Phorbol 12-myristate 13-acetate) for 15 minutes. A three-fold increase in phospho-ERK1/2 was detected in the assay.

For an up-to-date list of available FACE Kits, please visit us at www.activemotif.com/face.

FunctionELISA™

accurate quantification of signal pathway proteins

FunctionELISA™ Kits offer a simple, rapid method to monitor changes in signal pathway proteins. The FunctionELISA technique is an improvement over other methods used to study proteins, such as Western blotting, as it provides sensitive, quantitative protein measurement in just hours. The 96-well format requires only a small amount of extract, making it possible to perform multiple-sample, high-throughput analysis.

How do the FunctionELISA Kits work?

The FunctionELISA Kits utilize the Sandwich ELISA technique to capture and quantifiably measure the amount of a specific protein present in a sample. Sandwich ELISAs use two antibodies that recognize different epitopes on the protein of interest. The Capture Antibody is provided immobilized in the wells of an ELISA plate. When cell lysate is added, the protein of interest is bound by this antibody. The Detecting Antibody then binds to the captured protein. FunctionELISA IκBα and TRAIL Kits use a horseradish peroxidase (HRP) or alkaline phosphatase (AP) conjugated secondary antibody, respectively, that binds to the Detecting Antibody to quantify the bound protein (Figure 1).

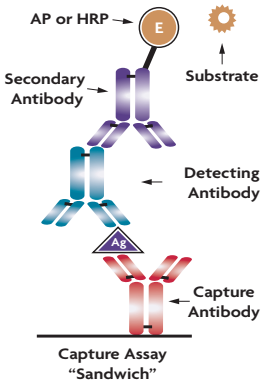


FIGURE 1:
Sandwich ELISA schematics.
Capture and Detecting Antibodies are used for sensitive, accurate quantification of the antigen of interest (Ag).

FunctionELISA IκBα

NFκB is regulated by the IκB family of inhibitory proteins. Phosphorylation of IκBα leads, ultimately, to activation of NFκB. Thus, analysis of the phosphorylation state of IκBα provides insights about NFκB and the many genes it regulates. FunctionELISA IκBα Kits can be used to study the phosphorylation state of IκBα, and to correlate this information with the activation and translocation of NFκB (Figure 2). FunctionELISA IκBα uses a Capture Antibody specific for phosphorylated IκBα and highly sensitive and accurate chemiluminescent detection. Therefore, the kit requires the use of a luminometer.

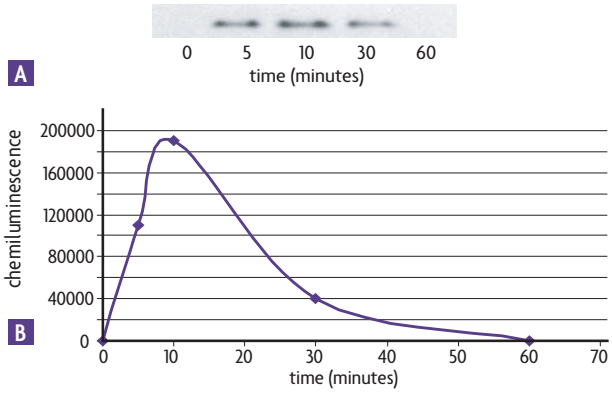


FIGURE 2:
Induction of IκBα phosphorylation.
Jurkat cells were treated with 1 nM TNF-α and harvested at the indicated time points. Whole-cell extracts were assayed in Western blot analysis (A) using Phosphorylated-IκBα mAb (Cat. No. 40904) and the FunctionELISA IκBα Kit (B).

WHY USE FUNCTIONELISA?

- Eliminates the need to run, blot and develop gels
- Each kit includes a positive control protein to generate a standard curve for sample quantification
- Ready-to-use format with Capture Antibody precoated on the plate
- Ability to assay both cell and tissue samples

FunctionELISA TRAIL

Tumor Necrosis Factor-Related Apoptosis Inducing Ligand (TRAIL) is a member of the TNF ligand family. It induces apoptosis in many cancer cells but is not toxic to most normal cells. FunctionELISA TRAIL can accurately measure picogram quantities of soluble TRAIL in lysates and fluid samples. It utilizes an AP-conjugated secondary antibody and detection is measured through the use of a spectrophotometer.

Product	Format	Cat. No.
FunctionELISA™ IκBα	1 x 96-well plate	48005
	5 x 96-well plates	48505
FunctionELISA™ TRAIL	1 x 96-well plate	48010
	5 x 96-well plates	48510

Chariot™

simple, efficient protein delivery

Chariot™* is a revolutionary transfection reagent that efficiently transports biologically active proteins, peptides and antibodies directly into cultured mammalian cells in less than two hours[1, 2]. The typical delivery efficiency is 65-95%. After delivery, living cells can be assayed to determine the effects of the introduced materials, without the need for fixation. This makes Chariot ideal for a wide variety of functional studies, including delivery of inhibitory proteins, organelle labeling, screening peptide libraries and transient complementation studies.

While there are many techniques available for introducing DNA into cells, including microinjection,[3] calcium phosphate coprecipitation,[4] cationic liposomes,[5] viral vectors[6] and electroporation,[7] these methods have several limitations. After transfection, the researcher must wait overnight to several days to detect gene expression. A greater drawback is that the cytotoxicity of these methods can make it difficult to determine if any changes observed are caused by the recombinant protein that was introduced, or by the transfection method itself.

* Covered under U.S. Patent No. 6,841,535. Purchase includes the right to use for basic research only. Other-use licenses available, please contact Tech Service.

Chariot advantages

Chariot is a peptide that forms a non-covalent complex with the macromolecule to be delivered. This complex stabilizes the protein, protecting it from degradation during delivery and preserving its function. Upon addition to cells, the complex is rapidly internalized, where it dissociates. The Chariot peptide then localizes to the cell nucleus where it is degraded, leaving the macromolecule free to proceed to its target organelle. By directly delivering protein, peptide or antibody, Chariot completely bypasses the transcription-translation process associated with recombinant gene expression, saving hours to days of time.

WHY USE CHARIOT?

- Delivers active proteins, peptides and antibodies directly into mammalian cells
- Up to 95% efficiency in less than 2 hours
- Facilitates functional studies in living cells
- Non-cytotoxic and serum independent
- Non-covalent binding preserves the function of delivered proteins and eliminates the need to create fusion proteins
- Works in a variety of cell lines, including hard-to-transfect cells and neurons, as well as *in vivo* with animal models

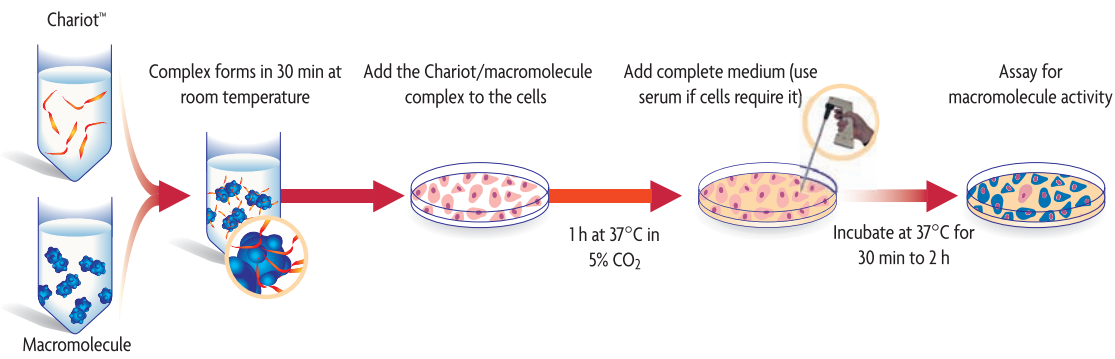
Applications of Chariot

Direct delivery of active protein makes it easy to perform studies not even possible using classical methods. Common applications for Chariot include:

- Delivery of inhibitory peptides and gripNAs™ (see page 15)
- Transient complementation studies
- Antibody studies
- Screen peptide libraries
- Protein half-life studies
- Organelle labeling

Chariot delivers results

Chariot is non-cytotoxic and efficiently delivers proteins into a wide range of cell lines, including hard-to-transfect PC-12 and primary cells. It has proven to be effective on both adherent and suspension cells, as well as *in vivo* with animal models.[8] As efficient delivery occurs at 4°C, the Chariot process is independent of the endosomal pathway,[9] which can modify macromolecules during internalization. The process is also serum-independent, so delivery can occur in the presence or absence of serum.



Flow chart of the Chariot process. Chariot is easy to use. Simply combine it with your macromolecule, wait 30 minutes, then add the complex to your cells. Activity can be assayed in as little as 90 minutes post-delivery.

Proven Chariot performance

The ability of Chariot to effectively deliver biologically active proteins, peptides and antibodies directly into a variety of different cell types has been well documented. Table 1 below lists some of the results reported to Active Motif by Chariot users. Please go to www.activemotif.com/chariot and select the tab for User Data to view the complete table.

Cell Line or Model	Cell Type	Delivery Efficiency
3T3-L1	Mouse fibroblast	80%
A549	Human lung carcinoma	95%
Arabidopsis	Primary plant protoplasts	Not provided
COS-7	Monkey kidney	80%
Embryonic cells	Primary mouse	90%
HeLa	Human cervix carcinoma	95%
HMSC	Primary human mesenchymal stem cells	80%
HS-68	Human foreskin fibroblast	95%
J774	Mouse macrophage	90%
Jurkat	Human T-cell leukemia	75%
Mouse hepatocytes	Primary liver	95%
Mouse model (*in vivo*)	Alveolar wall tissue	88%
Neural retina cells	Primary chicken	80%
NIH/3T3	Mouse embryo	80%
PC-12	Rat pheochromocytoma	80%
Sensory neurons (DRG)	Primary chick and primary rat	80%
Thyrocytes	Primary human	90%
WI-38	Human lung fibroblast	95%

TABLE 1:

Delivery efficiencies of Chariot into specific cell types.
Chariot has been used to deliver proteins, peptides and antibodies into a variety of different cell types, some of which are shown above. Data was provided to Active Motif by users of Chariot, who completed our online Chariot survey. For complete results, please visit our website.

β-Galactosidase Staining Kit

Need a control for Chariot? The β-Galactosidase Staining Kit provides an easy-to-use and efficient method to determine the percentage of cells expressing *lac*Z following transient or stable transfection. When the positive control β-galactosidase protein is used in conjunction with Chariot for protein delivery, the β-Galactosidase Staining Kit enables visualization of the efficiency of direct β-galactosidase delivery by Chariot.

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

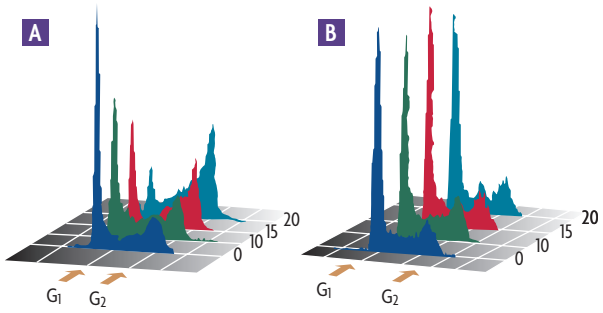


FIGURE 1:

Cell cycle arrest by delivered peptide.
$p27^{kip1}$ is a 27 kDa cyclin-dependent kinase inhibitor that causes cell-cycle arrest in G_1 phase. 100 µl of 50 nM $p27^{kip1}$ protein was complexed with 5 µl of Chariot for 30 minutes. Chariot alone, $p27^{kip1}$ alone and the Chariot-$p27^{kip1}$ complex were added to HS-68 cells. Flow cytometry was performed 0, 10, 15 and 20 hours post-delivery. Cells that received Chariot alone (A) and $p27^{kip1}$ alone (data not shown) were able to progress into G_2 phase. Over 90% of the cells that received the Chariot-$p27^{kip1}$ complex (B) remained in G_1 phase. Data generously provided by Dr. Gilles Divita, CNRS, France.

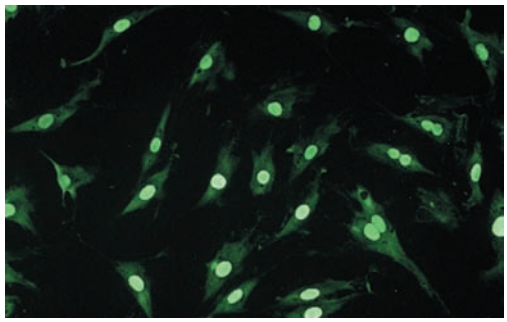


FIGURE 2:

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

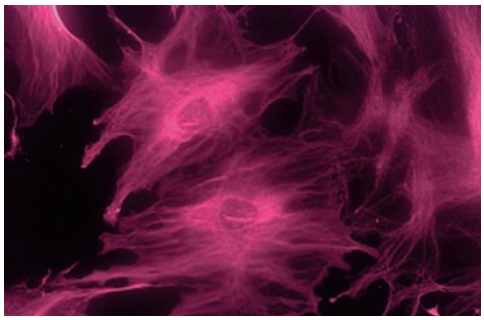


FIGURE 3:

Delivery of fluorescent antibody.
A 1:1,000 dilution of an anti-actin antibody was used to label actin filaments. Unfixed cells were observed 2 hours post-delivery.

REFERENCES

1. Morris, M. *et al.* (2001) *Nature Biotech.* **19**: 1173-1176.
2. Morris, M. *et al.* (1999) *J. Biol. Chem.* **274(135)**: 24941-2494.
3. Capecchi, M.R. (1980) *Cell* **22**: 479-488.
4. Graham, F.L. and van der Eb, A.J. (1973) *Virology* **52**: 456-467.
5. Felgner, P.L. *et al.* (1987) *Proc. Natl. Acad. Sci. USA* **84**: 7413-7417.
6. Cepko, C.L. *et al.* (1984) *Cell* **37**: 1035-1062.
7. Neumann, E. *et al.* (1982) *EMBO J.* **7**: 841-845.
8. Aoshiba, K. *et al.* (2003) *Amer. J. Resp. Cell Mol. Biol.* **28**: 555-562.
9. Schwarze, S.L. *et al.* (2000) *Trends Cell Biol.* **10**: 290-295.

LigandLink™ Universal Labeling

specific, flexible labeling of proteins in living cells

Use of green fluorescent protein (GFP) and its derivatives has become commonplace in modern cell biology. However, although they are widely used, biologically fluorescent proteins (FPs) are limited by their inherent properties. For example, FPs have a relatively low quantum yield, and it is difficult to engineer their spectral properties to suit specific applications.

Fluorescent dyes are a potential alternative to biological FPs as they are available in a broad variety of formats and can be engineered easily to ensure desirable spectral properties. However, because fluorescent dyes are synthetic molecules, it has not been possible to use them as a general tool for labeling specific proteins within a cell.

Active Motif's LigandLink™ Universal Labeling* technology overcomes these limitations by providing a small ligand that can carry a variety of functional tags, including fluorescent dyes. Expression of your protein of interest as a LigandLink fusion enables you to specifically label the protein *in vivo* simply by adding one of the LigandLink Labels to the medium. To change the properties of your tag, all you need to do is add a different LigandLink Label. This means that it is now possible to create a single protein fusion that can be labeled with a variety of tags, depending on the needs of your experiment.

* Patent pending.

The LigandLink method

In the LigandLink method, the gene of interest is cloned in frame with the gene for *E. coli* dihydrofolate reductase (eDHFR) in the pLL-1 vector (Figure 4). The vector is then transfected into mammalian cells and used to express the fusion protein. Twenty-four hours after transfection, the protein of interest can be labeled simply by adding the LigandLink Label of choice to the cell medium (Figure 1). Depending on the cell type and the characteristics of the label, cells can be imaged in as little as 10 minutes.

The eDHFR protein was chosen as a fusion partner because it is a relatively small, monomeric protein (18 kDa vs. 27 kDa for GFP) that has been shown to have a high affinity for the ligand trimethoprim (TMP). TMP binds with a high specificity to the *E. coli* form of DHFR (K_i = ~1 nm), and a substantially lower affinity for endogenous DHFR (K_i = ~4 µm). This is because TMP is an antibiotic that was designed to specifically inhibit the bacterial enzymes responsible for the production of folic acid while not interacting with mammalian proteins. Because there is minimal binding of LigandLink Labels to non-tagged mammalian proteins, the result is extremely low background. Moreover, TMP can be derivatized to carry a number of tags without substantially altering its affinity and specificity for eDHFR.

Thus, the LigandLink Universal Labeling technology uses eDHFR and a number of TMP derivatives as a ligand-receptor pair to provide a variety of functionalities.[1] Cell-permeable LigandLink Labels are available with red and green fluorescent dyes (Figures 2 & 3).

WHY USE LIGANDLINK?

- Label your protein in living cells – LigandLink Labels are cell permeable
- Spend less time cloning – rather than using multiple vectors, clone only once; obtain different functionalities using different LigandLink Labels
- Small label – LigandLink Labels are unlikely to alter the characteristics of your protein of interest

LIGANDLINK APPLICATIONS

- Protein localization & trafficking
- Protein:protein interactions
- Protein capture

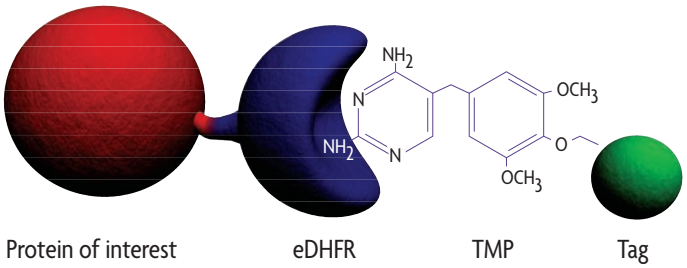


FIGURE 1:
Specific protein labeling using LigandLink.
The gene of interest is cloned into pLL-1 in frame with the vector's *E. coli* dihydrofolate reductase (eDHFR) gene. After transfection into cells, the protein of interest is expressed as a fusion to eDHFR. Addition of cell-permeable LigandLink Label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

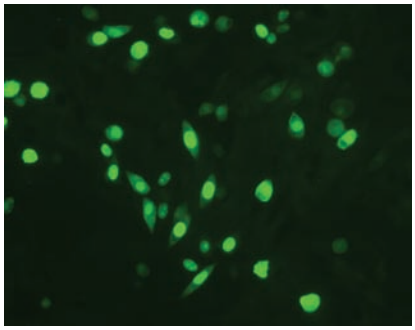


FIGURE 2:
Labeling of nuclear-localized eDHFR by LigandLink Fluorescein.
A nuclear localization sequence was cloned into pLL-1 and transfected into CHO cells. Twenty-four hours post-transfection, 5 µM LigandLink Fluorescein was added to the cells; 10 minutes later, the above image was taken.

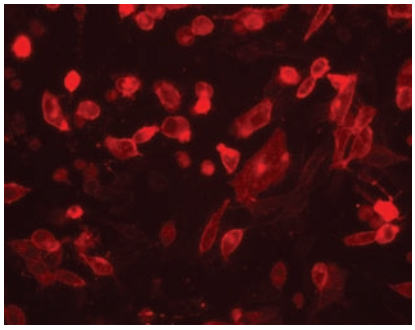


FIGURE 3:
Membrane-localized eDHFR labeling by LigandLink Hexachlorofluorescein.
The myristoylation/palmitoylation sequence from Lyn was cloned into pLL-1 and transfected into CHO cells. Twenty-four hours post-transfection, 10 nM LigandLink Hexachlorofluorescein was added to the cells; 2 hours later,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

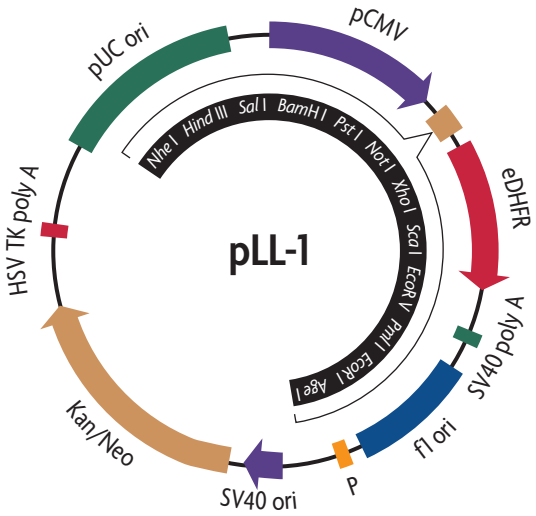


FIGURE 4:
The LigandLink pLL-1 vector.

User-friendly vector

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

Pre-made translocation vectors

Signal transduction pathways typically involve the movement of proteins throughout the cell in response to activation by some particular stimulus. The LigandLink Universal Labeling technology is ideal for studying such translocation events because it makes it simple to label the proteins *in vivo*. To make it even easier, we have already cloned a number of transcription factors into the pLL-1 vector, such as NFκB p65, which are ready to transfect into the mammalian cell line of your choice.

Ideal for multi-image capture experiments

Analysis of co-repressor and co-activator interactions is an important part of understanding protein function. However, analyzing interactions across multiple proteins can be time consuming and expensive. This is because each interacting partner must either be cloned into a different FP fusion vector, or primary antibodies must be labeled with multiple dyes. To overcome this problem, LigandLink Label comes in multiple formats that can be easily interchanged, depending on the FP partner of the interacting protein of interest. This saves you time and money.

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

REFERENCE
1. Miller, L.W. *et al* (2005) *Nature Methods* **2(4)**: 255-257.

To learn more about LigandLink and the available labels, please visit us at www.activemotif.com/ligandlink.

DUB-Detector™

simple fluorescent assay to detect deubiquitinating enzyme activity

Ubiquitination is a reversible, post-translational modification in which one or more ubiquitin molecule is added to proteins by the sequential action of three enzymes: E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme and E3 ubiquitin ligase. The group of enzymes responsible for the removal of ubiquitin from proteins are known as deubiquitination enzymes (DUBs), of which the human genome encodes nearly 100, making them the largest family of enzymes in the ubiquitin system. DUBs are responsible for ubiquitin precursor processing, ubiquitin recycling, trimming of ubiquitin chains, as well as other diverse roles in cell growth and differentiation, development, DNA damage, disease pathways, transcriptional regulation and chromatin remodeling.

Active Motif's DUB-Detector™ Deubiquitination Assay provides a simple fluorescent solution for screening the cysteine protease class of DUBs from cell extracts or purified recombinant proteins. The assay can be used for either endpoint or kinetic analysis of enzyme activity, or to screen for DUB inhibitors.

How does DUB-Detector work?

Despite the differences in their cellular roles and molecular sizes, the deubiquitinating enzymes all appear to hydrolyze their substrates through a common mechanism. Active Motif's DUB-Detector Assay uses a universal ubiquitin substrate to detect enzymatic activity or screen for potential inhibitors. The fluorescent substrate is based on a C-terminal derivative of ubiquitin which is hydrolyzed by the cysteine protease class of enzymes to release a fluorescent signal proportional to the amount of enzyme activity. The fluorescence can be detected using a microplate reader with excitation of 485 nm and emission of 535 nm. For added convenience, the assay also includes HeLa nuclear extract and an inhibitor to all classes of deubiquitinating enzymes as positive and negative controls (Figure 1).

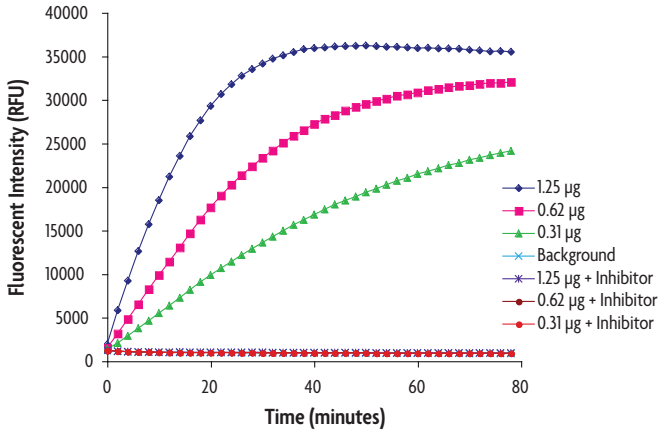


FIGURE 1:

Kinetic analysis of DUB activity in HeLa nuclear extract.
HeLa nuclear extracts were assayed at 0.313, 0.625 and 1.25 µg per reaction in the presence or absence of 1 µM inhibitor for 20 minutes. Following the incubation, Fluorescent Substrate (100 nM) was added to each well and the fluorescent intensity was measured every two minutes.

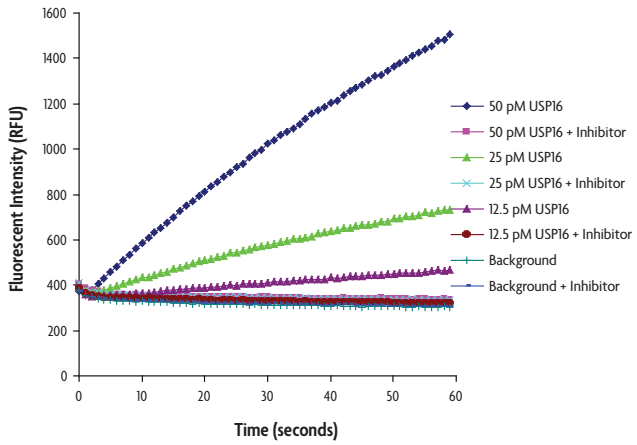


FIGURE 2:

Recombinant USP16 deubiquitination activity.
The DUB-Detector assay was used to evaluate the activity of recombinant USP16 at 12.5, 25 and 50 pM per reaction in the presence or absence of 100 nM inhibitor for 20 minutes. Following the incubation, Fluorescent Substrate (100 nM) was added to each well and fluorescent intensity was immediately measured every minute with a total reaction time of 60 minutes. Data shown are the results from duplicate wells.

WHY USE DUB-DETECTOR?

- Complete assay with optimized buffers for enhanced enzymatic activity
- Includes both positive control extract and a universal DUB inhibitor
- Fast procedure can be completed in less than 1 hour
- Great for either endpoint or kinetic analysis
- Fluorescence can be detected with an excitation wavelength of 485 nm and an emission wavelength of 535 nm

Product	Format	Cat. No.
DUB-Detector™ Kit	1 x 96 rxns	40110

SUMOlink™

simple, effective *in vitro* SUMOylation of proteins with proven controls

Post-translational modifications can alter protein function by modifying activity or intracellular localization. Small ubiquitin-related modifiers (SUMOs) are ubiquitin-like polypeptides that covalently bind to specific lysine residues on the target protein via isopeptide bonds. Mammalians express three major SUMO paralogs: SUMO-1, SUMO-2 and SUMO-3. SUMO-2 and SUMO-3 are approximately 96% identical to each other and have yet to be functionally differentiated, so they are often referred to as SUMO-2/3. There is only 45% identity shared between SUMO-1 and SUMO-2/3, which may explain the increasing evidence that these SUMO modifications have distinct cellular functions. The pathway of SUMO conjugation affects many biological processes including chromosomal organization, gene transcription, DNA replication and repair, nucleocytoplasmic transport and signal transduction pathways.

Active Motif's SUMOlink™ Kits provide a fast, simple method for generating SUMOylated proteins *in vitro* using the E3-independent SUMOylation enzyme cascade. SUMOlink Kits provide all the reagents required for performing 20 wild-type and 20 mutated *in vitro* SUMOylation reactions. The Kits also include positive control p53 protein and antibody to help ensure success. Applications of the kit include the investigation of SUMOylation on enzyme function, understanding the role of SUMOylation in the regulation of cellular processes and the identification of novel proteins as targets for SUMO.

The SUMOlink advantage

Active Motif's SUMOlink Kit is fast and easy to use. Simply combine your protein of interest with the provided E1 and E2 enzymes, SUMO-1 or SUMO-2 & SUMO-3 protein(s) and buffers. After a 3-hour incubation, the reaction is stopped by adding SDS-PAGE Loading Buffer. SUMOylation can then be analyzed by Western blot with the provided SUMO-1 or SUMO-2/3 antibodies to determine the extent to which your target protein has been SUMOylated. p53 protein and antibody are also included in the kits for use as a positive control (Figure 1).

WHY USE SUMOLINK?

- Simple, effective method for SUMO conjugation and detection of SUMOylated proteins
- Study either SUMO-1 or SUMO-2 & SUMO-3 activity
- Positive control p53 protein and antibody provided to ensure success
- Wild-type and mutated SUMO proteins are provided
- Versatile – study SUMOylation of cell or tissue extracts, or recombinant protein

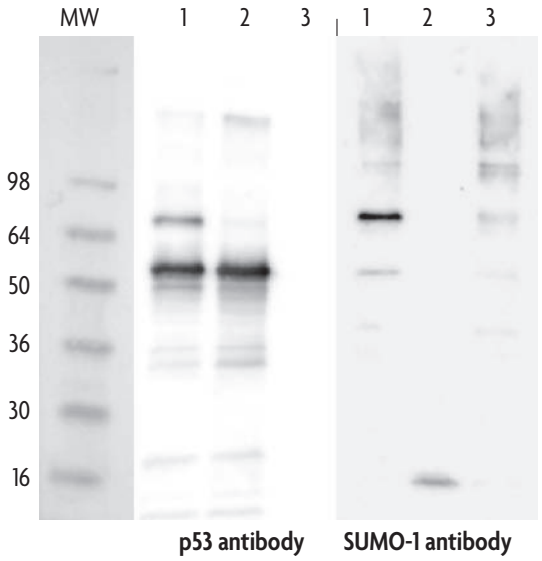


FIGURE 1:

Specific labeling of p53 with SUMO-1 by SUMOlink.
Western blot analysis of *in vitro* SUMOylation of p53 protein with wild-type and mutated isoforms of SUMO-1: The two Western blots were incubated with p53 antibody (1:5,000 dilution) and SUMO-1 antibody (1 :4,000 dilution). The p53 protein is SUMOylated only by the wild-type SUMO-1 protein.

Lanes 1: Wild-type SUMO-1 protein conjugation reaction.
Lanes 2: Mutated SUMO-1 protein conjugation reaction.
Lanes 3: No p53 control protein used in a wild-type SUMO-1 protein conjugation reaction.

Product	Format	Cat. No.
SUMOlink™ SUMO-1 Kit	20 rxns	40120
SUMOlink™ SUMO-2/3 Kit	20 rxns	40220

To learn more about SUMOylation or to see additional data, please visit us at www.activemotif.com/sumolink.

DNA Repair Protein Kits

quantitative ELISAs for measuring DNA repair protein activity

Active Motif's DNA Repair Protein Kits are DNA-binding ELISA assays that facilitate the detection of DNA repair protein activity in both mammalian tissue and cell culture extracts. These kits combine a fast, user-friendly format with a sensitive, specific assay that is 10-fold more sensitive than traditional gelshift while eliminating the use of radioactivity. Quantitative results are achieved in approximately 3.5 hours.

DNA repair proteins are critical for maintaining genome integrity, and deficiencies in their activity are linked to the development of many diseases, including cancer. Traditionally, techniques such as gelshift and immunoblotting have been used to study DNA damage and repair protein interactions. Although these methods are sensitive, they are typically more qualitative rather than quantitative tools (Figure 1).

The DNA Repair Protein Kit advantage

Active Motif's DNA Repair Protein Kits provide a quick and easy method for sensitive DNA repair protein detection. Each kit contains a 96-well plate with multiple copies of an immobilized oligonucleotide containing a DNA lesion. Cell extract is added to each well and the repair protein of interest binds specifically to the oligonucleotide on the plate. Each well is then incubated with a primary antibody directed against the repair protein being studied. Addition of a secondary HRP-conjugated antibody and developing solution provides an easily quantified colorimetric readout.

Why use the DNA Repair Protein Kits?

- Sensitive, colorimetric readout easily quantified by spectrophotometry
- ELISA format eliminates gels, blotting and radioactivity
- From cell extract to completed assay in less than 3.5 hours
- Up to 10-fold greater sensitivity than gelshift assays
- Compatible with high-throughput automation
- Ability to assay both cultured cells and tissue samples

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

Please visit us at www.activemotif.com/dnarepair to learn more.

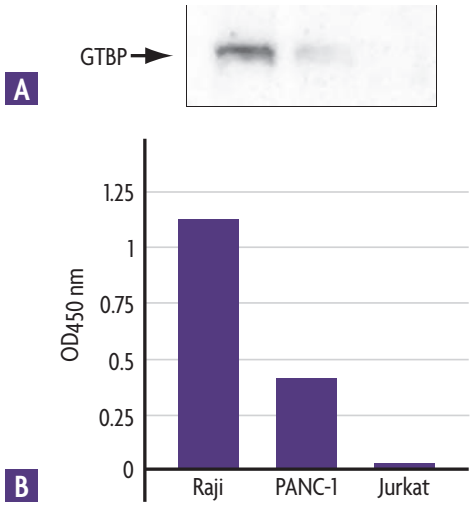


FIGURE 1:
Measurement of GTBP activity across multiple cell lines.
Jurkat, PANC-1 and Raji nuclear extracts were assessed for GTBP activity using Western blot (A) and the GTBP DNA Repair Protein Kit (B). The DNA Repair Protein Kit is clearly more quantitative and sensitive.

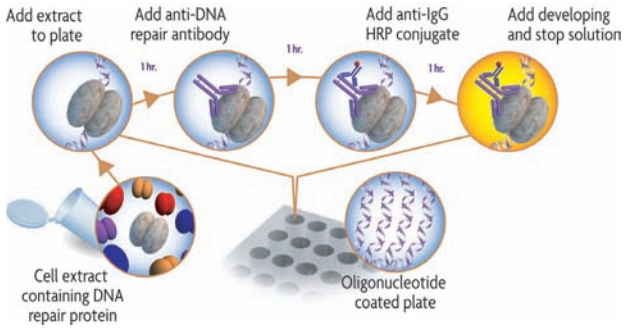


FIGURE 2:
Flow chart of the DNA Repair process.
DNA repair proteins bind to oligonucleotides with specific DNA lesions that are immobilized in the well. Incubation with primary and secondary antibodies measures DNA repair protein activity.

Ras GTPase Chemi ELISA Kits

fast, sensitive analysis of activated GTPases

Small GTPases, also known as GTP-binding proteins, are a family of enzymes that serve as molecular switches to regulate many important signal transduction pathways by alternating between active (GTP-bound) and inactive (GDP-bound) states. Because GTPases are involved in cell growth, apoptosis and differentiation, understanding the mechanisms that regulate their activation is the subject of intense investigation. However, the more traditional methods used to monitor GTPase activation, such as Western blot or pull-down assays, are tedious, time consuming and not suitable for high-throughput analysis.

In contrast, Active Motif's Ras GTPase Chemi ELISA Kit is the first ELISA-based kit designed to detect and quantify activated Ras. Because the assay is an ELISA, sensitivity is improved over pull-down/Western methods, enabling detection of low levels of protein. In addition, ELISAs are more quantitative than Westerns, and eliminate the need to run and develop gels. And, as the assay uses a 96-well plate made up of 12-well strips, it is convenient to run anywhere from 1 to 96 samples.

The Ras GTPase Chemi ELISA advantage

In the Ras GTPase Chemi ELISA Kit method, a Raf-RBD binding domain protein that has been fused to GST is added to a 96-well, glutathione-coated assay plate. Addition of sample to the plate results in the binding of activated Ras to the Raf-RBD binding domain protein on the plate, while inactive Ras does not bind. Next, a primary antibody specific for H-Ras in mouse and H- & K-Ras in human samples is added. Addition of an HRP-conjugated secondary antibody and developing reagent provides a sensitive readout that can be measured with a luminometer (Figure 1).

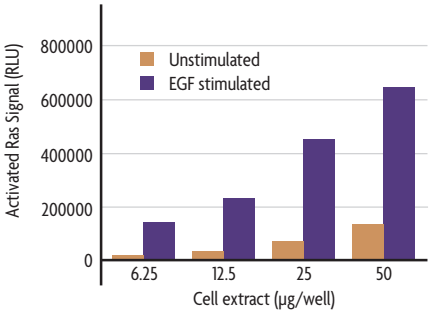


FIGURE 1:
Quantification and comparison of Ras activation in HeLa cell extracts. Increasing amounts of whole-cell extracts from HeLa cells that were either unstimulated or had been stimulated with 5 ng/ml of EGF for 2 minutes were assayed for Ras activation using the Ras GTPase Chemi ELISA Kit.

Why use Ras GTPase Chemi ELISAs?

- More sensitive – uses 20-fold less sample than pull-down/Westerns
- Better results – quantitative readout lets you easily compare samples
- Less effort – no need to run gels or develop Westerns
- Save time – obtain results in less than 5 hours
- Versatile – study cell or tissue extracts, or recombinant protein

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

For complete details on Active Motif's Ras GTPase Kit, please visit us at www.activemotif.com/gtpase.

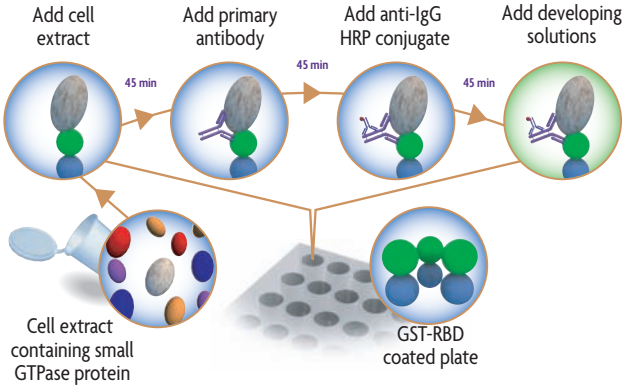


FIGURE 2:
Flow chart of the GTPase ELISA process.

Nitric Oxide Quantitation Kit

faster, more accurate measurement of nitric oxide production

The Nitric Oxide Quantitation Kit is faster and easier-to-use than existing methods for measuring the production of NO in biological samples. The kit employs an innovative cofactor technology that reduces the time and number of steps required, while increasing the dynamic range of NO measurement. The NO Quantitation Kit can be used with a wide variety of samples, including cell or tissue extract, urine, plasma, serum and saliva.

Because NO has an extremely short half-life (< 10 seconds), it is difficult to detect and study directly. However, because NO naturally metabolizes to nitrite and nitrate, quantitation of these stable anions is often used to indirectly determine the amount of NO originally present in a sample.

Typically, NO assays are performed using either a two-step assay or a three-step lactate dehydrogenase (LDH) assay. In both methods, the first step is the reduction of nitrate into nitrite by nitrate reductase. Griess Reagent is then used to convert the nitrite into a purple-colored azo compound, which is quantitated by spectrophotometer at A_{540} (Figure 1). However, excess NADPH, an essential cofactor in nitrate reduction, interferes with the Griess reaction, limiting the sensitivity of the two-step assay. One method to remedy this is to add a third step, whereby lactate dehydrogenase (LDH) eliminates excess NADPH prior to the Griess reaction. While this additional step increases assay sensitivity, it also increases the amount of time required and the level of complication.

NO Quantitation Kit advantages

Active Motif's Nitric Oxide Quantitation Kit offers advantages in time, ease-of-use and accuracy over the methods described above. It employs an innovative cofactor technology that accelerates the conversion of nitrate to nitrite while simultaneously degrading NADPH. Thus, the time required for the reductase reaction is reduced to only 30 minutes, and sensitive colorimetric quantification can be performed without the need for LDH treatment. Moreover, the Active Motif method is linear over a broader dynamic range, which increases the range of sample concentrations that can be measured accurately (Figure 2). This saves time and money as fewer samples will need to be diluted and re-assayed to obtain accurate results. To make it easier to process large numbers of samples, the NO Quantitation Kit is provided in a convenient 96-well format that facilitates high-throughput automation.

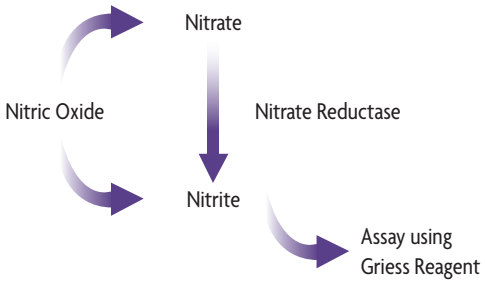


FIGURE 1:
Measurement of nitric oxide.
Nitric oxide metabolizes rapidly into nitrate and nitrite, which are stable. In NO assays, the nitrate is converted to nitrite, then assayed with Griess Reagent.

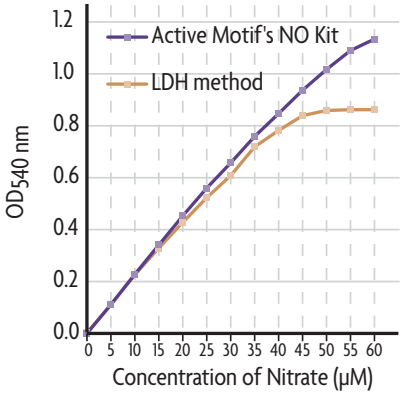


FIGURE 2:
Dynamic range of nitrate standard curves.
Nitrate standard curves produced using the Nitric Oxide Quantitation Kit and a conventional Three-Step LDH-method kit.

WHY USE THE NO QUANTITATION KIT?

- Cofactor technology accelerates nitrate reduction
- Assay enables high sensitivity in fewer steps
- Linear results over a wider range of concentrations
- 96-well format enables high-throughput automation

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

For more information on Active Motif's Nitric Oxide Quantitation Kit, please visit www.activemotif.com/no.

gripNAs™

stronger sequence specificity for superior silencing

Active Motif's custom gripNAs™* synthesis service provides a better alternative to more traditional gene inhibition techniques. gripNA oligonucleotides are a novel form of negatively charged Peptide Nucleic Acids,[1-3] which have a number of properties that make them ideal for use in gene silencing. These unique molecules display superior sequence specificity compared to conventional gene silencing reagents. Plus, gripNAs are resistant to nuclease degradation and delivery is simple with Chariot™ II, a unique formulation of Active Motif's protein delivery reagent (see page 6).

While gripNAs hybridize tightly to sequences with exact complementarity, their binding affinity is significantly reduced (or completely eliminated) if there is even a single base-pair mismatch (Table 1). This strong sequence discrimination minimizes non-specific gene interactions, which can greatly improve the quality of your gene silencing experiments by eliminating the occurrence of unintended phenotypes.[4]

* Covered under U.S. Patent No. 6,962,906. Purchase includes the right to use for basic research only. Other-use licenses available, please contact Tech Services.

gripNAs advantages

The large number of publications utilizing gene silencing to determine protein function illustrates the importance of this strategy in modern cell biology. Inhibition of gene expression has primarily been performed using antisense DNA oligos, small interfering RNAs (siRNAs) or morpholinos. However, despite their potential, the control of gene expression using such methods is often confounded by non-specific interactions with other genes,[5] which leads to the occurrence of unintended, mutant phenotypes. gripNAs have been shown to be highly effective in gene silencing experiments in mammalian cells, zebrafish, *Xenopus* and in *in vitro* translation systems.

Delivery of inhibitory molecules into the cell is also a key limitation for any gene silencing experiment. Classical delivery mechanisms such as microinjection and DNA/RNA transfection can be time consuming and inefficient. However, their biggest limitation is that they induce cytotoxic responses within the target cells. Delivery of gripNA oligonucleotides using Chariot II is simple and gives high transfection efficiencies with minimal cytotoxicity. These characteristics make gripNAs the tool of choice for your gene silencing studies.

WHY USE GRIPNAS?

- Unsurpassed sequence specificity eliminates non-specific gene interactions
- Resistant to nuclease degradation
- Easy delivery with Chariot II (see page 6)
- Flexible synthesis modifications
- Highly soluble

Complete information online

More complete information on gripNAs, including experimental data, structure information of the PNAs and ordering options for gripNAs, is available online at www.activemotif.com/gripnas.

REFERENCES

1. Efimov, V.A. *et al.* (1998) *NAR* **26**: 566-575.
2. van der Laan *et al.* (1996) *Tetrahedron Lett.* **37**: 7857-7860.
3. Efimov, V.A. *et al.* (1999) *NAR* **27**: 4416-4426.
4. Urtishak, K.A. *et al.* (2003) *Developmental Dynamics* **228**: 405-413.
5. Stein, C.A. (1999) *Nature Biotech* **17**: 209.

	DNA/DNA		gripNA/DNA		
	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m (°C)	DNA oligonucleotide sequence
No mismatches	63.9	–	58.6	–	5´-CAC-TGA-CTT-GAG-ACC-A-3´
Mismatch A	57.0	6.9	41.3	17.3	5´-CAC-TGA-**G**TT-GAG-ACC-A-3´
Mismatch B	49.7	14.2	No T_m	–	5´-CAC-TGA-**G**T**G**-GAG-ACC-A-3´
Mismatch C	61.8	2.1	54.6	4.0	5´-CAC-TGA-CTT-GAG-AC**G**-A-3´
Mismatch D	56.7	7.2	No T_m	–	5´-C**GG**-TGA-CTT-GAG-ACC-A-3´
Mismatch E	53.6	10.3	No T_m	–	5´-CAC-TGA-C**G**T-GAG-ACC-A-3´
Mismatch F	56.2	7.7	No T_m	–	5´-CAC-TGA-CT**G**-GAG-ACC-A-3´
Mismatch G	54.2	9.7	42.4	16.2	5´-CAC-TGA-C**A**T-GAG-ACC-A-3´

TABLE 1:

Stringent binding specificity of gripNAs.
Identical 16-mer gripNA and DNA probes with the sequence 5´-TGG-TCT-CAA-GTC-AGT-G-3´ were synthesized. These were annealed to a complementary DNA oligo (5´-CAC-TGA-CTT-GAG-ACC-A-3´) in Hybridization Buffer, heated to 90°C for 3 minutes, then cooled gradually to room temperature. The samples were then heated at a rate of 1°C per minute from 20°C to 100°C using a thermal control unit linked to a spectrophotometer. Changes in A_{260} were recorded and a melting temperature (T_m) was calculated for each DNA/DNA and gripNA/DNA duplex. The experiment was then repeated by hybridizing the gripNA and DNA probes to a series of DNA oligos with one- or two-base mismatches (shown above as bold and underlined). T_m values were measured for each DNA/DNA and gripNA/DNA duplex and a ΔT_m was calculated by subtracting the difference in the melting temperatures of the complementary and mismatched probes. Samples that are unable to form a stable duplex generate a "No T_m" value in this assay, so have no ΔT_m value.

mTRAP™

isolate more mRNA with gripNAs

mTRAP™ Kits isolate high yields of quality mRNA from mammalian cells, tissue and total RNA. mTRAP Maxi and Midi 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.

Peptide Nucleic Acids (PNAs) are DNA analogs in which the nucleosides are attached to an N-(2-aminoethyl)glycine backbone instead of to deoxyribose, as in DNA.[1-4] However, poor water solubility and a tendency to self-aggregate have limited the utility of traditional PNAs. Active Motif has overcome these shortcomings by developing negatively charged gripNAs.[5-7] This class of PNAs has a number of properties that make them ideal for mRNA isolation.

* Covered under U.S. Patent No. 6,962,906. Purchase includes the right to use for basic research only. Other-use licenses available, please contact Tech Services.

The mTRAP advantage

gripNAs bind to nucleic acids with high affinity and specificity (see page 15). 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.

Another benefit of gripNAs is that they have a higher affinity for DNA than for RNA, making possible selective elution of the mRNA. While non-complementary nucleic acids are removed during the wash steps, DNA with homology to the Poly T gripNA Probe remains bound, even during the 75°C mRNA elution step. mTRAP Kits utilize this high-temperature elution step to remove all mRNA from the probe, which greatly increases the yield. For absolute certainty that no genomic DNA is co-isolated, an optional DNase step can be performed during isolation because gripNAs, unlike oligo dT, are resistant to enzymatic degradation.

Complete product details are available online at www.activemotif.com/mtrap.

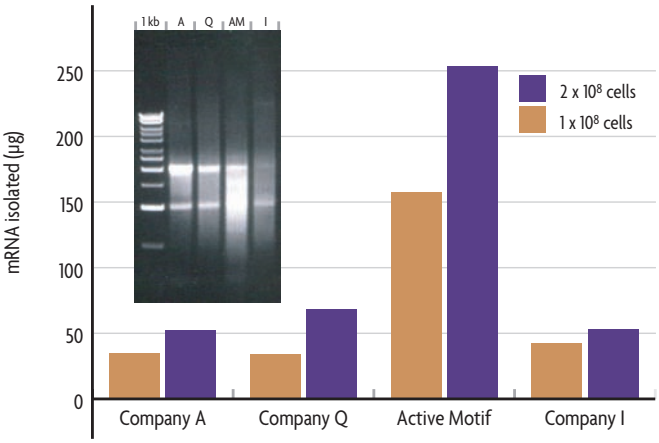


FIGURE 1:

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. 2 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.

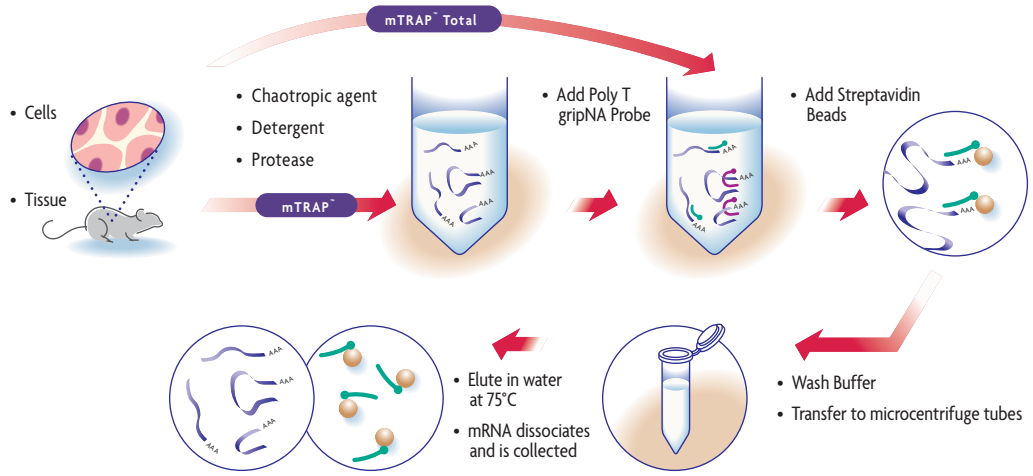
Lane 1: 1 kb DNA Ladder
Lane 2: mRNA isolated using Company A's kit
Lane 3: mRNA isolated using Company Q's kit
Lane 4: mRNA isolated using mTRAP Maxi Kit
Lane 5: mRNA isolated using Company I's kit

Why mTRAP and negatively charged gripNAs?

- Isolates substantially more mRNA than oligo dT methods
- Reduces ribosomal RNA and genomic DNA contamination
- Captures mRNA with secondary structure and short tails
- Purifies a more representative mRNA population
- Includes TouchDown™ for precipitating small quantities of nucleic acids

REFERENCES

1. Nielsen, P.E. *et al.* (1991) *Science* **254**: 1497-1500.
2. Egholm, M. *et al.* (1992) *J. Am. Chem. Soc.* **114**: 1895-1897.
3. Harvey, J.C. *et al.* (1992) *Science* **258**: 1481-1485.
4. Buchardt, O. *et al.* (1993) *Trends Biotechnol.* **11**: 384-386.
5. Efimov, V.A. *et al.* (1998) *NAR* **26**: 566-575.
6. van der Laan *et al.* (1996) *Tetrahedron Lett.* **37**: 7857-7860.
7. Efimov, V.A. *et al.* (1999) *NAR* **27**: 4416-4426.



Flow chart of the mTRAP procedure.

mTRAP Maxi and mTRAP Midi

The mTRAP Maxi Kit provides all reagents and plasticware required for 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 all reagents and plasticware required for 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-related Products

Poly T gripNA™ Probe has been optimized to ensure efficient recovery of mRNA from cell lysates and tissue homogenates. It is available in amounts comparable to those provided in the mTRAP Maxi and mTRAP Midi Kits and is stable for 6 months at -20°C.

Streptavidin Beads are stable, uniform, non-porous spheres bound with highly active streptavidin. They are RNase- and DNase-free for direct addition to cell or tissue lysates. The one-micron diameter beads can bind 10 pmol biotinylated gripNA-analog probe per 1 µl of beads and are supplied at ~1% solids in 50 mM Tris, pH 8, 150 mM NaCl, 0.05% sodium azide and 0.1% stabilizing detergent.

TouchDown™ Precipitation Reagent enables precipitation of any nucleic acid without the addition of salt, mussel glycogen or other carrier. Precipitation can be carried out at +4°C, eliminating the need to freeze samples at -70°C. TouchDown Precipitation Reagent is ideal for precipitating small quantities of DNA or mRNA. TouchDown is supplied as a powder that is resuspended in 20 ml ethanol. Use 3-5 volumes per precipitation. Enough reagent is provided for 40 x 500 µl precipitations.

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.

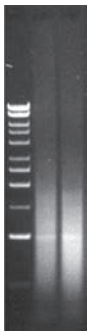


FIGURE 2:
Pure mRNA from total RNA in one passage.
mRNA was isolated from 500 µg of total RNA in duplicate using mTRAP Total, quantified by spectrophotometry and run on a 0.8% agarose gel.

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

Product	Format	Cat. No.
mTRAP™ Maxi	6 rxns 5 x 6 rxns	23006 23506
mTRAP™ Midi	24 rxns 5 x 24 rxns	23024 23524
mTRAP™ Total	12 rxns 5 x 12 rxns	23012 23512
Poly T gripNA™ Probe (from mTRAP™ Maxi)	6 rxns	29007
Poly T gripNA™ Probe (from mTRAP™ Midi)	24 rxns	29008
Streptavidin Beads	1.6 ml 3 ml	29010 29009
Touchdown™ Precipitation Reagent	400 mg	80280
mTRAP™ Lysis Buffer	100 ml	29011
Protease	20 mg	29012

Nuclear Extract Kit

high yields of specifically segregated extracts

The Nuclear Extract Kit is ideal for the preparation of nuclear, whole-cell and cytoplasmic extracts from mammalian cells and tissues. The resultant high-quality extracts may be used with Active Motif's TransAM™ and DNMT Activity / Inhibition Kits or in gelshift assays, Western blots, DNA footprinting or as a starting point for transcription factor purification.

The Nuclear Extract Kit eliminates the need to optimize reagents and ensures consistently high yields. The detailed protocol helps ensure that your extract is not contaminated with proteins from other cellular compartments (Figure 1).

The Nuclear Extract Kit advantage

In the Nuclear Extract Kit, cells are collected in ice-cold PBS in the presence of phosphatase inhibitors to limit further protein modifications. Next, the cells are resuspended in hypotonic buffer to swell the cell membrane. Addition of detergent causes leakage of the cytoplasmic proteins into the supernatant. After collection of the cytoplasmic fraction, the nuclei are lysed and the nuclear proteins are solubilized in lysis buffer in the presence of the protease inhibitors. Whole-cell extracts can also be prepared by collecting the cells in the PBS/phosphatase inhibitors solution and lysing in lysis buffer. Solubilized proteins are separated from the cell debris by centrifugation. The concentration of protein in the cell extract can then measured by Active Motif's ProStain™ Protein Quantification Kit (see page 20) or with a Bradford-based assay.

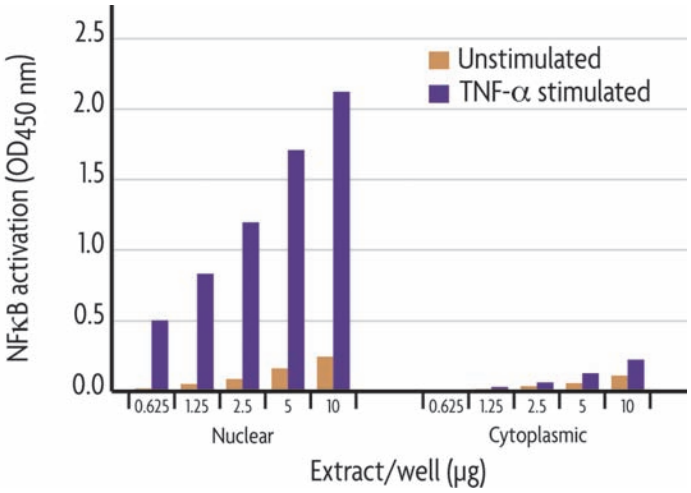


FIGURE 1:
Specific extraction of nuclear and cytoplasmic extracts.
Nuclear and cytoplasmic extracts were prepared using the Nuclear Extract Kit from HeLa samples unstimulated or stimulated with TNF-α for 30 minutes and assayed using the TransAM™ NFκB p50 Kit. Because activated NFκB translocates to the nucleus, only nuclear extract from stimulated cells should contain activated NFκB.

WHY USE THE NUCLEAR EXTRACT KIT?

- Specific segregation reduces the risk of inconsistent results from cross-contamination
- Optimized reagents and protocol ensure reproducible results
- Includes phosphatase and protease inhibitors to preserve protein modifications and prevent degradation
- Prepare nuclear, cytoplasmic or whole-cell extracts with one kit
- Ability to prepare extracts from both cultured cells and tissue samples

Product	Format	Cat. No.
Nuclear Extract Kit	100 rxns	40010
	400 rxns	40410
ProStain™ Protein Quantification Kit	1000 rxns	15001

Mitochondrial Fractionation Kit

isolate highly enriched mitochondrial and cytosolic fractions

The Mitochondrial Fractionation Kit isolates highly enriched mitochondrial and cytosolic fractions from mammalian cell lines, simplifying the study of protein translocation events that occur during apoptosis and in many signal transduction pathways. The procedure is simple to perform and does not require ultracentrifugation or the use of any toxic chemicals. Isolated fractions can be used to detect the onset of apoptosis in Western blots or in other assays.

WHY USE THE MITOCHONDRIAL FRACTIONATION KIT?

- Eliminates cross-contamination of fractions
- Quality-controlled reagents ensure reproducibility
- No need to optimize your own reagents or protocol

The Mitochondrial Fractionation Kit advantage

The Mitochondrial Fractionation Kit's high-quality reagents and optimized protocol eliminate cross-contamination and produce high yields of properly segregated mitochondrial and cytosolic fractions (Figure 1). This helps ensure that you get accurate, reproducible results.

Product	Format	Cat. No.
Mitochondrial Fractionation Kit	100 rxns	40015

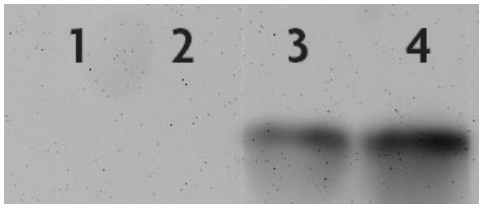


FIGURE 1:
Location of cytochrome c in HeLa cells.
The Mitochondrial Fractionation Kit was used to isolate cytosolic (Lanes 1 & 2) and mitochondrial (Lanes 3 & 4) fractions from HeLa cells. Twenty µg of each fraction was assayed by Western blot using the cytochrome c oxidase IV (COX IV) antibody at a 1:500 dilution. In non-apoptotic cells, cytochrome c is found in the mitochondria only. The Western blot results reveal that the Mitochondrial Fractionation Kit is specific for isolating segregated mitochondrial and cytosolic fractions and that there is no leakage between the two cellular compartments.

Chromeo™ Live Cell Mitochondrial Staining Kit

Mitochondria play an important role within living cells. They are involved in the generation of cellular energy, the regulation of cell growth and differentiation, and also play a critical role in the induction of the apoptotic process and various signaling cascades. Diseases of the mitochondria result in underproduction of energy within the cell, causing cell injury and even cell death.

Active Motif's Chromeo™ Live Cell Mitochondrial Staining Kit provides a non-toxic, water-soluble, membrane-permeable dye that can be used to stain the mitochondria of live or fixed cells. Because of the dye's excellent retention within the cell and low toxicity, the stain is an ideal tool for long-term labeling of cells and cellular tracking.

Chromeo Live Cell Mitochondrial Stain advantages

The Chromeo™ Mitochondrial Cell Stain is a unique dye that does not fluoresce until it has covalently reacted with an amino group in the cell, eliminating background fluorescence. The stain can be excited between 470 nm and 550 nm, enabling the use of common lasers and light sources. Additionally, the provided Hoechst Nuclear Stain can serve as a nuclear counter-stain.

Product	Format	Cat. No.
Chromeo™ Live Cell Mitochondrial Staining Kit	1 kit	15005

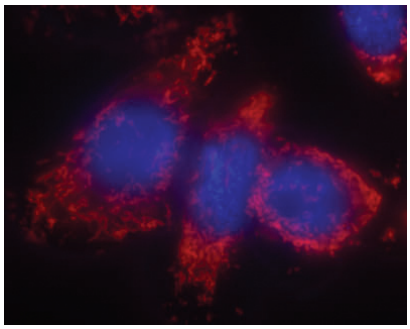


FIGURE 1:
Staining of living HeLa cells with Chromeo Live Cell Mitochondrial Stain Kit.
The mitochondria (in red) and the nuclei (in blue) of live HeLa cells were stained using the Mitochondrial Stain and Hoechst Nuclear Stain, respectively.

ProStain™ Protein Quantification Kit

simple, sensitive fluorescent quantification of cell extracts

ProStain™ simplifies the determination of cell extract protein concentrations by providing highly sensitive detection reagents in a convenient and easy-to-use format. The fluorescent dye in ProStain is not effected by many commonly used buffer components such as detergent and salt (Figure 1), which can skew the results of other protein quantification systems. And, the spectral properties of ProStain change greatly after it is bound, effectively eliminating any background from unbound dye (Table 1). These characteristics make ProStain a significant improvement over other detection systems such as the Bradford or BCA.

Determining the concentration of cell extracts is an essential step in many cell biology procedures, such as Western blot and ELISA. Not surprisingly, there are numerous methods used. Photometric methods have traditionally been based either on intrinsic absorbance at 280 nm or on reagent-based assays such as Bradford. However, these classical approaches tend to be time consuming, have limited sensitivity and may be influenced by the presence of contaminating substances.

Fluorescent-based detection methods, while generally more sensitive, have also tended to suffer from limitations such as the requirement for toxic co-factors, high rates of hydrolysis or unsuitable spectral properties. In comparison, Active Motif's ProStain Kit offers significant improvements in sensitivity, assay robustness and convenience.

The ProStain advantage

A disadvantage of many protein quantification methods, such as the Bradford assay, is that the absorbance spectra of the free and conjugated forms of the dye partially overlap. This causes non-linear protein measurement, as the free dye is excited by the same wavelength of light used to excite the bound dye. In contrast, the free versus conjugated absorbance maxima of ProStain dye are separated by > 100 nm (Table 1 and Figure 2). Nearly all free ProStain dye is hydrolyzed during the conjugation reaction, which improves the linearity and accuracy of the assay because excitation of the sample at 488 nm can only excite dye that is conjugated to protein. The small amount of free dye that remains is not excited at this wavelength, so background is completely eliminated. Finally, ProStain conjugation is fast and easy; simply resuspend the dye, add it to the wells of a microplate, then add a serial dilution of the BSA standard, along with your sample. After just 30 minutes, the fluorescence is read and the protein concentrations are easily quantified.

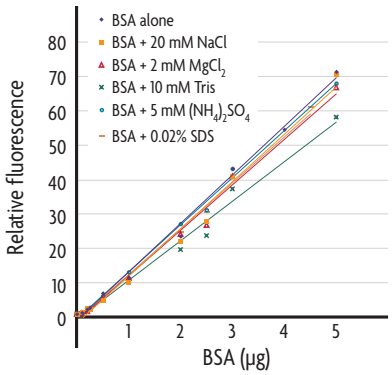


FIGURE 1:
Linear quantification in the presence of common buffer components.
Increasing amounts of BSA protein were quantified using the ProStain Protein Quantification Kit in the presence of a variety of buffer components.

Dye State	Absorption	Emission	ε L/(mol-cm)	Quantum Yield
Free	612 nm	665 nm	60,000	< 1%
Conjugated	503 nm	600 nm	24,000	~50%

TABLE 1:
Properties of ProStain dye.
The background of ProStain is extremely low because its absorption max shifts by over 100 nm after the dye is bound, and because bound dye has a 50-fold greater quantum yield than free dye.

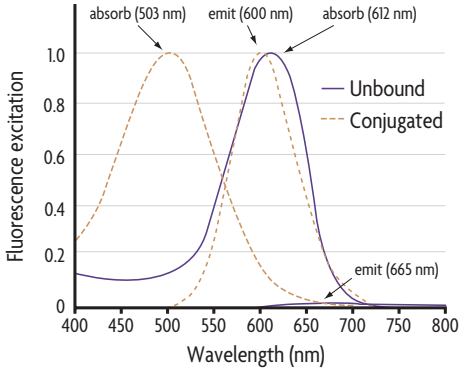


FIGURE 2:
Absorption and emission spectra of free vs. bound dye.
Normalized absorption and emission spectra of free (solid, purple lines) and conjugated dye (dotted, copper lines) in phosphate buffer of pH 7.2. As free dye and bound dye absorb at different wavelengths (612 nm vs. 503 nm) and the quantum yield of bound dye is 50-fold greater than that of free dye, background from free dye is effectively eliminated.

Product	Format	Cat. No.
ProStain™ Protein Quantification Kit	1000 rxns	15001

Albumin Blue Fluorescent Assay Kit

sensitive, quantitative determination of albumin in biological samples

Active Motif's Albumin Blue Fluorescent Assay Kit is a quantitative assay designed to measure albumin levels in biological samples including serum and urine. The kit combines unmatched sensitivity and high specificity with a fast, simple protocol to provide an effective assay for studying albumin.

Albumin, produced only in the liver, is the major plasma protein that circulates in the bloodstream. Albumin plays a significant role in the maintenance of colloid osmotic pressure and the binding of long-chain fatty acids, bile acids, bilirubin, haematin, calcium and magnesium. It also acts as a carrier for nutritional factors and drugs. Because serum albumin is a reliable prognostic indicator for morbidity and mortality, liver disease, kidney disease and malnutrition, efficient study methods are in high demand. The Albumin Blue Fluorescent Assay Kit from Active Motif provides a simple tool for highly selective and sensitive albumin detection, and comes complete with everything required for performing the assay.

The Albumin Blue advantage

Currently, assays suitable for determining low concentrations of albumin (< 100 mg/L) are nonspecific, and merely quantify total protein content. In contrast, Active Motif's Albumin Assay Kit is sensitive, easy to use and specific for albumin. Simply add the Diluent Buffer and Dye Reagent to the standards and samples, then read the fluorescence. The intensity of the fluorescent signal is directly proportional to the albumin concentration of the sample. And, as albumin-bound dye has a greatly increased excitation, the background caused by the emission of any free dye is minimal (Figure 1).

Each kit contains enough dye for 250 reactions in 96-well plates, but the assay can also be easily adapted for use in smaller or larger formats, such as 384-well plates or cuvettes. The kit also includes human serum albumin (HSA) that can be used to make standard curves for use in quantifying the amount of albumin in your samples (Figure 2). Due to the high amino acid homology and tertiary structural similarities of albumin across species, the Albumin Blue Reagent can be used for detection of albumin from various species.

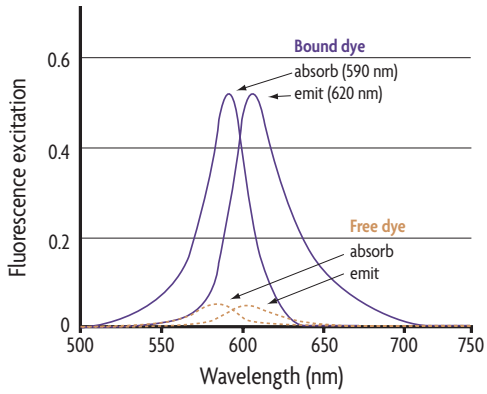


FIGURE 1:

Absorption and emission spectra of dye that is free or bound to albumin. Normalized absorption and emission spectra of free (copper curves) and conjugated dye (purple curves) in the Albumin Blue Assay.

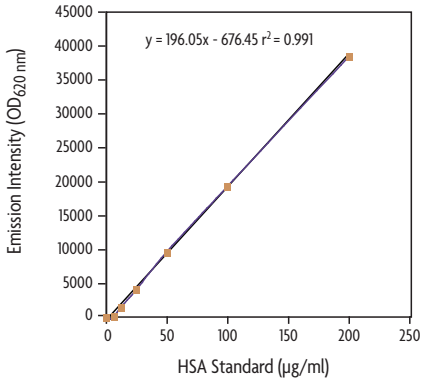


FIGURE 2:

Human Serum Albumin Standard Curve.
A HSA standard curve generated using the Albumin Blue Fluorescent Assay Kit.

WHY USE ALBUMIN BLUE?

- Quick and easy to use
- Unmatched precision
- Sensitive detection limit (< 0.5 mg/L in urine and serum samples)
- Highly selective – no interference from other proteins or lipids
- High-throughput compatible

Product	Format	Cat. No.
Albumin Blue Fluorescent Assay Kit	1 kit	15002

For more information about the Albumin Blue Fluorescent Assay, please visit us at www.activemotif.com/albumin.

LavaCell™

rapidly stain cellular membranes in live or fixed cells

Active Motif's LavaCell™ is a naturally occurring compound from the fungus *Epicoccum nigrum* with a molecular weight of 410 Daltons. It is a small, non-toxic molecule that readily diffuses into live or fixed cells and can be used to fluorescently stain cell membranes and lipophilic organelles within minutes. Cells require no pre-treatment or permeabilization for staining, and LavaCell does not stain nucleic acids or adversely affect cell growth. Once inside the cell, LavaCell binds to primary amine groups instantly to produce bright red fluorescent compounds.

Unbound LavaCell has a weak green fluorescence in water (520 nm), but following the reaction with primary amine groups on proteins, the fluorescence shifts to red (610 nm). LavaCell can be excited at 405, 488 and 532 nm, enabling the use of common filters or lasers. Because unbound LavaCell fluoresces at a different wavelength than the excitation wavelength, there is little background and no need to wash after protein binding. The staining does not self-quench, and can be multiplexed with a range of yellow-, green- and blue-emitting compounds due to LavaCell's long Stokes shift of at least 100 nm.

The LavaCell advantage

The LavaCell stain is a unique, non-toxic, cell-permeable dye that is ideal for studying cell proliferation, cell counting, chemotaxis, cell morphology or any application that requires cellular imaging. LavaCell is simple to use; just add it to your cell culture, wait 30 minutes, then analyze. The dye readily diffuses into live or fixed cells to produce bright fluorescence on the plasma and internal membranes, such as those of the nucleus, Golgi and ER. Because the emission of unbound LavaCell is 520 nm while that of bound LavaCell is 610 nm, and because LavaCell does not effect cell growth, there is no need to wash away unbound dye.

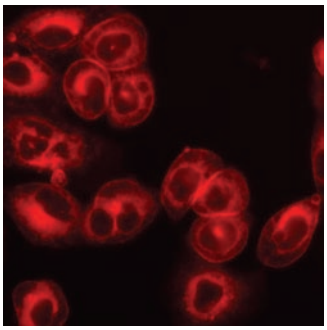


FIGURE 1:
LavaCell stains the plasma membrane and internal membranes of CHO cells.

WHY STAIN WITH LAVACELL?

- Water soluble fluorescent cell stain
- Fluoresces only on interaction with proteins; no need to wash away unbound stain
- Readily diffuses into live or fixed cells
- No effect on cell growth or viability
- 405, 488 and 532 nm excitable
- Non-fluorescent prior to cellular internalization
- Ideal for multiplexing with other fluorophores due to its long Stokes shift

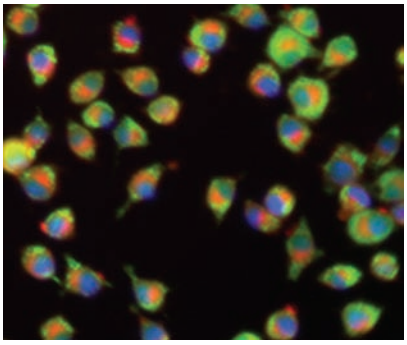


FIGURE 2:
Live mouse macrophage cells stained with LavaCell, calcein AM and Hoechst.

Product	Format	Cat. No.
LavaCell™	200 µg	15004

To learn more, please visit us at www.activemotif.com/lavacell.

ToxCount™

convenient, accurate cell viability assay

Active Motif's ToxCount™ Cell Viability Assay is a simple two-color fluorescence cell viability assay that is easy to use, and requires only 30 minutes of hands-on time. The ToxCount assay is based on the simultaneous determination of live and dead cells using two probes, calcein AM and ethidium homodimer (EthD-1). These probes recognize parameters of cell viability including intracellular esterase activity and plasma membrane integrity, respectively.

Calcein AM is a non-fluorescent cell permeable dye that converts to green fluorescent calcein in live cells after acetoxymethyl ester hydrolysis by intracellular esterases. Ethidium homodimer (EthD-1) is a red fluorescent nuclear and chromosome counterstain. EthD-1 enters cells with damaged membranes and binds to nucleic acids, where it emits a bright red fluorescent signal. Because both dyes used in ToxCount are not fluorescent before interacting with cells, background fluorescence levels are very low. ToxCount can be used on multiple fluorescence detection platforms, including multi-well plate scanning, fluorescence microscopy or flow cytometry. The assay is suitable for use with many adherent cell lines, primary cell types and small organisms. It is less expensive, safer and faster than traditional cell viability assays such as ^{51}Cr-release, LDH release and trypan blue exclusion.

The ToxCount advantage

Traditional methods for measuring cell viability can frequently be time-consuming and require the use of radioactive or toxic reagents. In contrast, the ToxCount method is safe and easy to perform. Simply grow cells directly on a microtitre plate and treat with the drug of interest. After a 30-minute incubation, cells are ready for imaging and analysis (Figure 1). As the dyes are non-fluorescent in solution, they can be used in homogenous protocols, making ToxCount ideal for the rapid screening of drug and small molecule libraries for cytotoxic effects.

WHY USE TOXCOUNT?

- Dependable, reproducible assay
- Quick and easy protocol
- Non-toxic reagents
- High-throughput compatible

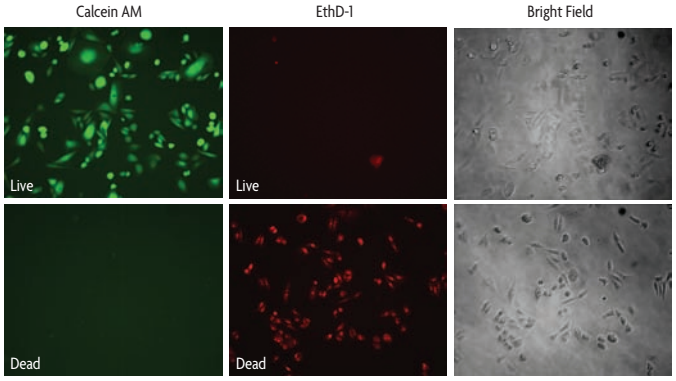


FIGURE 1:
ToxCount staining using a fluorescence microscope.
CHO cells were treated with calcein AM (0.5 mM) and EthD-1 (0.5 mM), then imaged using a fluorescence microscope. Untreated, live cells are shown in the upper images while dead cells that had been treated with 70% methanol are shown in the bottom series of images. Images were acquired for 1 second in the case of calcein AM and for 2 seconds in the case of EthD-1.

Product	Format	Cat. No.
ToxCount™	20 x 96 rxns	18010

For more information about Active Motif's ToxCount Assay, please visit us at www.activemotif.com/toxcount.

RapidTrans™

efficient, affordable competent *E. coli* in a convenient format

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. Unlike other 96-well formats that require thawing all 96 tubes 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. For your convenience, the cells are provided with SOC media, supercoiled pUC19 DNA and sterile reservoirs for use with multi-channel pipettors.

The RapidTrans Kit advantage

- Consistent high-quality, high-efficiency *E. coli*
- Freedom to use anywhere from 1-96 reactions eliminates wasted reactions
- Efficiency of > 1 x 10^8 cfu/µg
- Economical pricing
- Adaptable to high-throughput use

Strain and genotype information

TAM1 *E. coli* have been genetically modified for use in cloning, plasmid preparation and library construction. The transformation efficiency is > 1 x 10^8 cfu/µg supercoiled pUC19 DNA.

TAM1: *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *ara*D139 Δ(*ara-leu*)7697 *gal*U *gal*K *rps*L *end*A1 *nup*G

Product	Format	Cat. No.
RapidTrans™ TAM1 Competent *E. coli*	1 x 96 rxns	11096
	5 x 96 rxns	11596

Please visit us at www.activemotif.com/rapidtrans to learn more.

Fluorescent Dyes & Conjugated Secondary Antibodies

easy-to-use, effective fluorescent labels for bioanalysis

The Chromeo™ product line is a complete toolset for fluorescence applications like fluorescence microscopy or fluorescent plate-based assays. Providing sensitivity and flexibility, Active Motif Chromeon dyes and reagents exhibit superior luminescence properties, including a broad range of fluorescence excitations and emissions, large Stokes shifts, limited photobleaching and a broad pH tolerance. We identified the need for improved fluorescence-based assays and developed some unique tools like Py-Dyes, which are chameleon-type pyrylium dyes that change their color upon binding to proteins.

The fluorescent dye products shown below are but a small subset of those offered by Active Motif. For more comprehensive information, please visit **www.activemotif.com/fluor**.

Conjugated Secondary Antibodies

For the utmost in convenience, our dyes are all available already conjugated to high-quality goat anti-mouse and goat anti-rabbit secondary antibodies. Because of the wide range of dyes offered (see Table 1), you can use multiple secondaries at the same time for multi-color staining (Figure 1). HRP conjugates are also available. For details, please visit **www.activemotif.com/secondary**.

Chromeo™ Dyes

Active Motif's Chromeo™ Dyes are also offered ready to use to covalently label biomolecules. For added flexibility, the dyes are offered in 6 different formats: as amine-reactive NHS-esters, which are useful to label amino groups on peptides, proteins or amino-modified DNA; as azides and alkynes for use in click chemistry; as carboxylic acids, and as biotin and streptavidin conjugates. For details, please visit **www.activemotif.com/dyes**.

Chromeo™ Py-Dyes

Py-Dyes are a set of 5 fluorescent chameleon-type dyes that are ideal for labeling proteins; they undergo a large shortwave spectral shift, as well as a large increase in quantum yield, upon reacting with primary amines. This eliminates background from any unbound dye, so there is no need for purification following the simple one-step, room-temperature labeling reaction. For more information, please visit **www.activemotif.com/pydye**.

Get 12-fold Greater Resolution Using STED

The *Abbe Law of Diffraction Limiting Resolution* restricts the ability of classical confocal microscopy to visually resolve objects separated by less than ~200 nm. Leica Microsystems' **ST**imulated **E**mission **D**epletion (**STED**) microscopy, however, improves resolution by up to 12-fold, enabling separation of sub-cellular structures that previously could not be resolved. Leica recommends Active Motif's Chromeo™ 488 and 494 dyes and secondary antibody conjugates, and its fluorescent ATTO (STED) secondary antibody conjugates for use in STED. For more details, please visit **www.activemotif.com/sted**.

Dye	Absorption	Emission	ε L/(mol-cm)	Stokes Shift
Chromeo™ 488	488 nm	517 nm	73,000	29 nm
Chromeo™ 494	494 nm	628 nm	55,000	134 nm
Chromeo™ 505	505 nm	628 nm	70,000	21 nm
Chromeo™ 546	545 nm	561 nm	98,800	16 nm
Chromeo™ 642	642 nm	660 nm	180,000	18 nm
ATTO 594	601 nm	627 nm	120,000	26 nm
ATTO 647N (STED)	644 nm	669 nm	150,000	25 nm
ATTO 655 (STED)	663 nm	684 nm	125,000	21 nm

TABLE 1:
Fluorescent properties of dyes when conjugated to secondary antibodies.

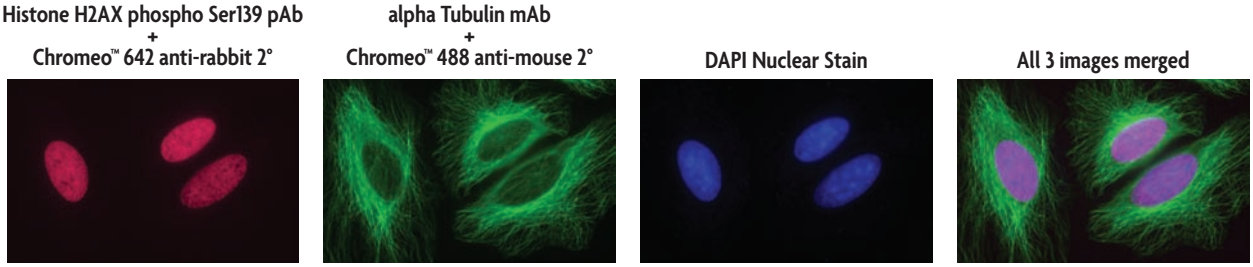


FIGURE 1:
Concurrent staining using Chromeo 488 and Chromeo 642 fluorescent secondaries.
HeLa cells were treated with 100 µM etoposide for 6 hours, prior to fixation with methanol. The histone variant H2AX was then stained with Histone H2AX phospho Ser139 rabbit pAb (Cat. No. 39117) and Chromeo 642 goat anti-rabbit IgG (Cat. No. 15044), while tubulin was visualized using alpha Tubulin mouse mAb (Cat. No. 39527) and Chromeo 488 goat anti-mouse IgG (Cat. No. 15031). The nuclei were stained using DAPI and the three separate images were merged.

ACTIVE MOTIF®

Tools to Analyze Nuclear Function

U.S.

1914 Palomar Oaks Way, Suite 150
Carlsbad, CA 92008 USA
Toll Free: 1 877 222 9543
Direct: 1 760 431 1263
Fax: 1 760 431 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
UK 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