MOTIF VATIONS

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ACTIVE MOTIF®

Tools to Analyze Cellular Function

ChIP-IT™ Express

magnetic beads for a faster, more streamlined ChIP method

IN THIS ISSUE

New: Chromatin Assembly Kit – Test Regulation of Your DNA in its Native Form

The new Chromatin Assembly Kit enables you to generate chromatin *in vitro* from your linear or supercoiled DNA. It yields chromatin that closely mimics natural *in vivo* chromatin, so you can discover which histone modifications and associated proteins are crucial to regulation of your target sequence.

It's easy to generate chromatin

Now you can investigate regulation of your gene of interest in its native form by assembling it into chromatin with this easy *in vitro* Chromatin Assembly Kit. This kit includes all the recombinant proteins, core histones, buffers and ATP-utilizing factors to generate chromatin from your DNA sequence and also to verify successful assembly. High-quality chromatin with more than 6 regularly spaced nucleosomes is made by adding the supplied components to 1 µg of your linear or supercoiled DNA, then incubating for 4 hours. A simple partial enzymatic digestion of the resulting chromatin reveals the ordered spacing of nucleosomes (Figure 1).

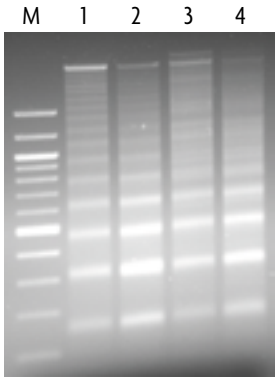


Figure 1: Enzymatic digestion of assembled chromatin. Chromatin assembled from 1 µg samples of circular DNA (Lanes 1 & 2) and linear DNA (Lanes 3 & 4) were digested for 2 and 4 minutes, respectively, deproteinated, phenol/chloroform extracted and run on an agarose gel. Each sample type resulted in regularly spaced nucleosomes.

Chromatin Assembly Kit advantages

- Generate chromatin from linear or supercoiled DNA
- ATP-dependent method results in an extended array of regularly spaced nucleosomes
- Easy protocol, simply incubate the supplied components with your DNA
- Excellent substrate for gene regulation experiments

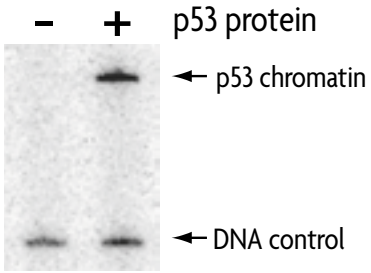


Figure 2: *In vitro* transcription of assembled chromatin. Chromatin was incubated with p300 and acetyl-CoA in the presence (+) or absence (-) of p53 at 30°C for 30 min. HeLa nuclear extract and NTPs (^{32}P-labeled CTP) were then added, along with a control DNA template, to initiate transcription. Transcription was p53-dependent.

Why bother to make chromatin?

When DNA sequences are assembled into chromatin by ATP-dependent factors, the resulting structure closely resembles the natural chromatin configuration. DNA that is in a bare or unassembled state often can not reveal the mechanism of transcriptional activation or repression with the associated factors and relevant histone modifications. However, properly assembled chromatin with regularly ordered nucleosomes is an excellent substrate for subsequent assays such as *in vitro* transcription assays (Figure 2), chromatin immunoprecipitation (ChIP) (Figure 3), and histone acetyltransferase (HAT) assays.

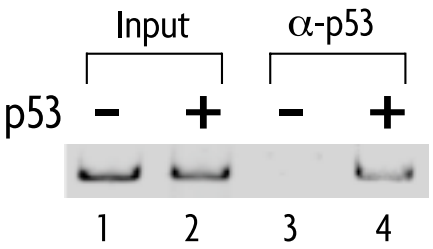


Figure 3: ChIP of *in vitro* assembled chromatin. Chromatin was incubated with p300 and acetyl-CoA in the presence (+) or absence (-) of p53 at 30°C for 30 min. ChIP was then performed with p53 antibody. Pull-down and Input DNAs were then PCR amplified with primer pairs surrounding the p53-binding site. Pull down of chromatin by p53 antibody was p53-dependent.

What's in the kit?

The kit includes recombinant h-NAP-1 chaperone protein, ACF assembly complex, HeLa core histones, and the buffers and enzymes necessary to generate assembled chromatin from your input DNA. Control Supercoiled DNA is also provided, and all of the kit components have been quality control tested and proven. To verify that your chromatin assembly reaction has been successful, reagents are provided to perform an easy partial enzymatic digestion to visualize the regular spacing of nucleosomes by agarose gel electrophoresis (Figure 1).

Try it today!

With Active Motif's Chromatin Assembly Kit, you can easily assemble chromatin on your sequence of interest to investigate gene regulation in a context that closely resembles *in vivo* chromatin. For more information, give us a call or visit us at www.activemotif.com.

Product	Format	Catalog No.
Chromatin Assembly Kit	10 rxns	53500

ChIP-IT™ Express: Magnetic Beads Make Chromatin Immunoprecipitation Easy, Saving You Hours of Time

Active Motif's ChIP-IT™ Express kits use magnetic protein G-coated beads to make chromatin immunoprecipitation (ChIP) faster and easier than traditional methods, which use agarose beads. Magnetic beads have made it possible to streamline the protocol and optimize buffers so time-consuming steps have been eliminated or reduced. With ChIP-IT Express, you can get results in half the normal time with much less sample manipulation. And, ChIP-IT Express makes it easy to perform ChIP on many samples at the same time (Figure 1).

ChIP-IT Express advantages

- No pre-clearing, blocking or DNA purification
- Simple wash steps improve consistency
- High throughput compatible
- Dramatically reduced hands-on-time

Magnetic power

ChIP-IT Express Kits include a strong bar magnet which can be adapted to work with any tip box or rack of your choice (Figures 1 and 2). Other commercially available racks like those from Ambion and Promega can also be used.

ChIP-IT Express improved method

Not only does ChIP-IT Express save you time, but the simple method generates more consistent results than traditional ChIP. The ChIP-IT Express magnetic beads have much less background than agarose beads, and this means pre-clearing and blocking steps are no longer necessary. The magnetic pull-down occurs in just seconds, and re-formulated buffers allow steps to be combined and DNA purification to be eliminated. ChIP-IT Express is available in both sonication and enzymatic shearing formats.

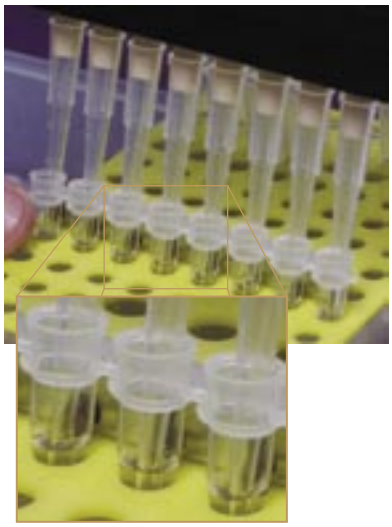


Figure 1: Multiple-sample ChIP using ChIP-IT Express. Washing magnetic beads is fast and easy because the pellet forms against the side of the tube in seconds. This makes it possible to ChIP multiple samples in 8-well PCR tubes using a multi-channel pipettor.

Ultimate controls ensure success

Interpreting ChIP results can be difficult, so Active Motif provides a complete set of controls to help you understand your results and troubleshoot your assays. To provide you with controls that are appropriate for *your* research, we removed the human-only controls from ChIP-IT Express Kits and now offer human and mouse ChIP-IT Control Kits separately. These provide positive and negative control antibodies and species-specific primer sets, PCR buffer and a convenient 10X DNA loading dye so your PCR reactions are gel-ready. All reagents are quality control tested and validated to ensure your ChIP assay is working properly. In addition, we offer convenient Ready-to-ChIP HeLa Chromatin, so you can be certain that the only variable in validating a new antibody for ChIP applications is the antibody itself.

Figure 2: Use of a standard tip box for magnetic ChIP. ChIP-IT Express Kits include a strong bar magnet that can be used to convert tip boxes into a magnetic stand for washing the protein G-coated magnetic beads.

Start your ChIP today

Active Motif's ChIP-IT products provide you with everything you need to make your ChIP experiments easier and your results more reproducible. No ChIP method is faster than ChIP-IT Express, and the validated reagents and controls help ensure success. For more complete information and to see what else is new, please give us a call or visit us online at www.activemotif.com/chip.

Product	Format	Catalog No.
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014
ChIP-IT™ Control Kit - Human	5 rxns	53010
ChIP-IT™ Control Kit - Mouse	5 rxns	53011
Ready-to-ChIP HeLa Chromatin	10 rxns	53015

Investigate SUMOylation with Efficient SUMOlink™

Active Motif's SUMOlink™ Kits provide a simple, effective method for generating SUMOylated proteins *in vitro*. These fast, efficient and robust assays contain all the necessary reagents for SUMOylation of target proteins, and include positive and negative controls that help ensure your success.

The SUMOlink method

SUMOlink™ Kits enable you to easily perform and detect post-translational modifications by SUMO (small ubiquitin-like modifier). With SUMOlink, you simply add the assay components to a microcentrifuge tube with your protein of interest. After a 3-hour incubation, the reaction is stopped and results can be analyzed by Western blot (Figure 1). With the kit's p53 antibody and either SUMO-1 or SUMO-2/3 antibodies, you can easily see the extent to which your target protein has been SUMOylated.

Everything you need to study SUMO

The kits contain E1 activating and E2 conjugating enzymes along with wild-type and mutant SUMO-1 (SUMO-1 Kit) or SUMO-2 and -3 proteins (SUMO-2/3 Kit). Antibodies for SUMO-1 or SUMO-2/3 modifications, as well as control p53 protein and antibody, are included. For complete information, please give us a call or visit us at www.activemotif.com.

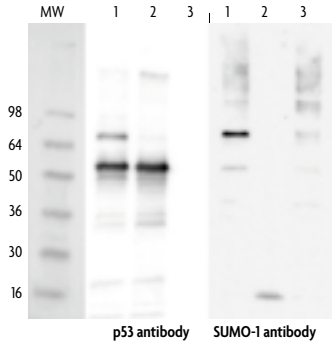


Figure 1: Specific SUMO-1 labeling of p53 by SUMOlink.
Western blot analysis of *in vitro* SUMOylation of p53 protein by wild-type and mutated isoforms of SUMO-1: The two Western blots were incubated with p53 antibody (1:5000 dilution) and SUMO-1 antibody (1 :4000 dilution)
Lane 1: Wild-type SUMO protein conjugation reaction.
Lane 2: Mutated SUMO protein conjugation reaction.
Lane 3: No p53 control protein used in conjugation.

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

MethylDetector™ – Fast, Reproducible DNA Methylation Analysis

Active Motif's MethylDetector™ Bisulfite Modification Kit makes DNA methylation analysis fast and efficient by combining optimized reagents for performing DNA conversion with time-saving DNA purification columns and positive control PCR primers to help you validate your results.

Proven controls ensure success

DNA methylation analysis typically involves using bisulfite to convert unmethylated cytosines to uracils, while leaving methylated cytosines unchanged. The DNA is then amplified by PCR and analyzed by sequencing or restriction digest. However, bisulfite conversion can be technically challenging, and confirming the process was successful before sample analysis is preferred. MethylDetector provides positive control PCR primers that are specific for bisulfite-converted DNA, so you can confirm the procedure worked before starting further costly analysis (Figure 1).

Advantages

- **Flexible** – use high G/C content sequences and uncut DNA
- **Reproducible** – 99% conversion efficiency
- **Efficient** – reagents and protocol with proven controls
- **Easy** – DNA purification columns mean no more precipitations

Try MethylDetector today

MethylDetector will speed and simplify your methylation analysis. Learn more at www.activemotif.com.

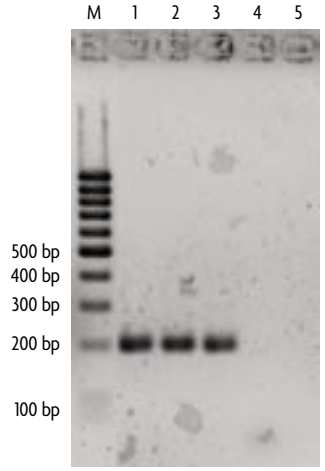


Figure 1: Agarose gel analysis of PCR products generated with MethylDetector.
Three different DNA conversions were performed (Lanes: 1-3) and compared to an unconverted DNA control (Lane: 5) and to a no DNA control (Lane: 4). The presence of PCR product in only the converted samples demonstrates the conversion efficiency and reproducibility of the MethylDetector Kit.

Product	Format	Catalog No.
MethylDetector™	50 rxns	55001

Multiple Methods to Study Nuclear Receptor 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 drug targets is important for nuclear receptor-targeted drug discovery. Active Motif's NR Peptide ELISAs 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 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).

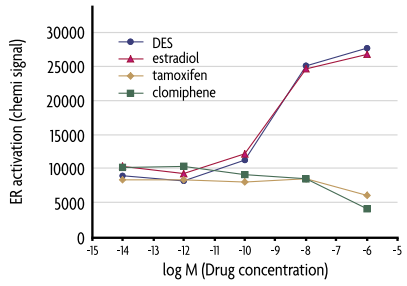


Figure 1: ERα agonism/antagonism dose-response curves. Nuclear extracts from the breast cancer cell line MCF-7 are incubated in wells of the NR Peptide ELISA ER plate in the presence of 100-fold serial dilutions (10^{-6} to 10^{-14} M) of the agonist compounds diethylstilbestrol (DES) and estradiol,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

Assess DNA-binding activity of NRs

Inappropriate nuclear receptor signaling is associated with numerous diseases including cancer, asthma and arthritis. This 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 is an ideal biomarker. 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 (see page 8).

Quantify total NR with Sandwich ELISAs

In order to fully examine activation of a given nuclear receptor, it is important to be able to quantify its total levels within a sample. 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 improves 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).

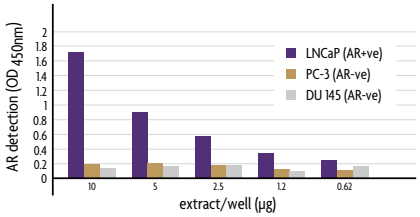


Figure 2: Monitoring expression levels of AR. Different amounts of nuclear extracts from three human prostate cancer cell lines, LNCaP, PC-3 and DU 145, were analyzed for levels of AR protein using the NR Sandwich AR Kit.

A host of NR antibodies & proteins, too

In addition to its assays, Active Motif offers over 200 highly characterized antibodies to transcription factors and nuclear receptors, as well as recombinant nuclear receptor proteins that are ideal for use as positive controls and in *in vitro* screening. Contact us today for more complete information on any of our tools for studying nuclear receptors.

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

New: RapidReporter® – Double Destabilization and *Gaussia* Luciferase Improve Response & Sensitivity

Active Motif's new RapidReporter® is a patented* method that provides a faster and more pronounced response to stimulation than other reporter gene assays. Because RapidReporter vectors include destabilization elements for both the luciferase protein and its mRNA, the assay yields more accurate kinetic and drug concentration-dependent responses. This vastly improves the monitoring of transcription factor activation and the detection of active compounds during high-throughput screening. In addition, RapidReporter vectors feature *Gaussia* luciferase, which is brighter and much smaller than firefly and *Renilla* luciferases.

Because of their simplicity and versatility, reporter gene assays are an important tool for studying signal transduction pathways and gene expression. In such assays, a promoter or enhancer element is cloned into a vector upstream of a reporter gene, commonly luciferase. After transfection, the cells are stimulated to induce transcription from the cloned promoter element. The assay uses the increase in luciferase protein levels as a measure of transcriptional activity.

Standard luciferase assays, however, are limited by the fact that basal activity of the cloned promoter results in accumulations of luciferase mRNA and protein. The slow clearance rate of these pre-existing reporter molecules substantially delays and dilutes the measurable response to stimulation. This is because the short, rapid increase in luciferase that occurs in response to stimulation has proportionally little impact on the high steady-state levels already present. As a result, with standard reporter gene assays, transient or relatively minor effects are hidden and kinetic assays are inaccurate.

Double destabilization is the key

To solve the problem of long half-life proteins, some assays incorporate a protein-destabilizing element in the vector, causing the luciferase to degrade more rapidly. Destabilizing the reporter protein, however, only partly addresses the problem as reporter clearance rates are also dependent on the half-life of the reporter mRNA. As long as the pre-existing reporter mRNA remains intact, it continues to produce new reporter protein. To address this issue, the patented RapidReporter® method utilizes vectors that include both protein AND mRNA destabilizing elements. This makes the assay much more responsive to both increases and decreases in transcriptional activity. Thus, RapidReporter provides a more accurate measurement of the actual transcriptional activity of the cloned promoter.

RapidReporter advantages

- Increased magnitude of response provides better separation of active vs. inactive compounds, which will reduce false positives
- Effect of weak to moderate stimulations, which are not found by other assays, are easily detected
- Faster response time enables the detection of transient effects and of drugs that decompose rapidly

Your choice of stringency

RapidReporter vectors are offered in two different stringencies. pRR-High is highly destabilized, while pRR-Low has fewer elements, which is appropriate when studying weaker stimulation.

Pre-made vectors & complete kits

In addition to empty RapidReporter vectors, Active Motif offers vectors that contain widely studied promoters. All vectors are available separately or in complete assay kits, which also include a positive control vector, Lysis and Assay buffers, and a substrate optimized for *Gaussia* luciferase. For more complete information on this exciting new technology, please give us a call or visit our website at www.activemotif.com.

Product	Format	Catalog No.
RapidReporter® Gaussia Luciferase Assay	100 rxns	33001
	1000 rxns	33002
RapidReporter® pRR-High vector	10 µg	33003
RapidReporter® pRR-High Assay	100 rxns	33004
RapidReporter® pRR-Low vector	10 µg	33005
RapidReporter® pRR-Low Assay	100 rxns	33006
RapidReporter® pRR-High-CRE vector	10 µg	33007
RapidReporter® pRR-High-CRE Assay	100 rxns	33008
RapidReporter® pRR-High-NFκB vector	10 µg	33009
RapidReporter® pRR-High-NFκB Assay	100 rxns	33010

* RapidReporter is covered under U.S. Patent No. 7,157,272 and various other patents worldwide, and is sold under license granted by GeneStream Pty Ltd. Purchasers are subject to a Limited-use License; please contact Active Motif's Technical Services or download a product manual from our web site for details. RapidReporter is a registered trademark of GeneStream Pty Ltd.

Deliver Functional Proteins Directly into Living Cells

Chariot™ is Active Motif's patented* protein delivery reagent that 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).

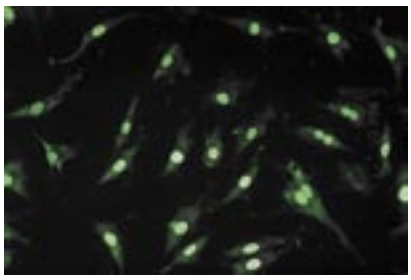


Figure 1: Targeted protein delivery.
50 ng of a 10 kDa nuclear protein that was labeled with Lucifer yellow at the C-terminus was complexed with Chariot and delivered into HS-68 cells. Unfixed cells were observed 90 minutes post-delivery.

Non-covalent delivery of native protein

Many protein delivery systems require that you begin by fusing a carrier protein to your macromolecule. In addition to being time-consuming, this can alter 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 protein is shown using a 119 kDa subunit of β-galactosidase. β-galactosidase is composed of four subunits that must assemble to form functional protein. HeLa cells turn blue when X-gal is added after delivery of the Chariot-galactosidase complex, demonstrating successful delivery of functional β-galactosidase (Figure 2).

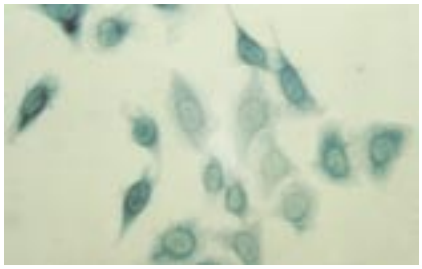


Figure 2: Chariot delivery of β-galactosidase.
One µg of a 119 kDa subunit of β-galactosidase was complexed with Chariot for 30 minutes and delivered into HeLa cells. Cells were fixed and stained with X-gal 2 hours post-delivery.

Advantages

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

Why use protein delivery?

Direct delivery of active protein makes it easy to perform studies not even possible using DNA transfection and expression. Successful Chariot 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. For a list of papers that cite the use of Chariot, simply return the enclosed reply card or download the list at www.activemotif.com/chariot.

"The ability to deliver active proteins directly into living cells and then assay their effect makes feasible studies not even possible with other methods."

Chariot delivers results

Chariot speeds and simplifies a variety of functional studies because it efficiently delivers biologically active proteins, peptides and antibodies directly into mammalian cells, even into hard-to-transfect and non-dividing cells. To learn what your protein is really doing, study it using Chariot.

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

* 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.

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

A Complete Solution for Transcription Factor Analysis

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

Save time and money

Transcription factor activity is often studied using Electrophoretic Mobility Shift Assays (EMSA), immunoblotting and reporter gene assays. But, these methods are time consuming and, at best, provide only semi-quantitative results. Moreover, determining the activity of transcription factor subunits by these methods is costly in terms of time and money, and may not even be possible. TransAM™ Kits, however, use a unique plate-based format to capture activated transcription factors, which are analyzed using antibody specific to your isoform of interest (Figure 1). As a result, levels of entire transcription factor families can be measured in a matter of hours, saving you time and money.

The TransAM advantage

- Up to 100-fold more sensitive than gelshift assays
- Eliminates the use of radioactivity and the need to run gels
- Results in less than five hours
- Colorimetric readout enables easy, quantitative analysis
- 96-stripwell format enables both high and low throughput

Widest selection of targets and formats

Active Motif is proud to offer the broadest selection of transcription factor-binding ELISAs, including kits for NFκB (p65, p50, p52, c-Rel and RelB), AP-1 (c-Jun, JunB, JunD, c-Fos, FosB, Fra-1 and Fra-2), STAT (1α, 3, 5A and 5B), PPARγ, HIF-1, NFATc1, C/EBP and many others (Table 1). In addition, TransAM Kits are available in either colorimetric or ultra-sensitive chemiluminescent* readout. And for the ultimate flexibility, TransAM Flexi NFκB Kits let you use any capture oligo you choose.

Functional assay for all sample types

Many commonly used transcription factor analysis tools, such as gelshift and reporter assays, either lack sensitivity, or cannot be used with primary material, such as tissues or peripheral blood mononuclear cells (PBMCs). This can make comparing primary and cell model data difficult and lead to inaccuracies. TransAM, however, is up to 100-fold more sensitive than gelshift assays and can be used with cell or nuclear extract to functionally monitor transcription factor activation in any sample type. Consequently, TransAM is the most widely cited DNA-binding ELISA available today. So, why not give us a call or visit www.activemotif.com/transam to find out more, including getting the list of the 100's of papers that cite TransAM.

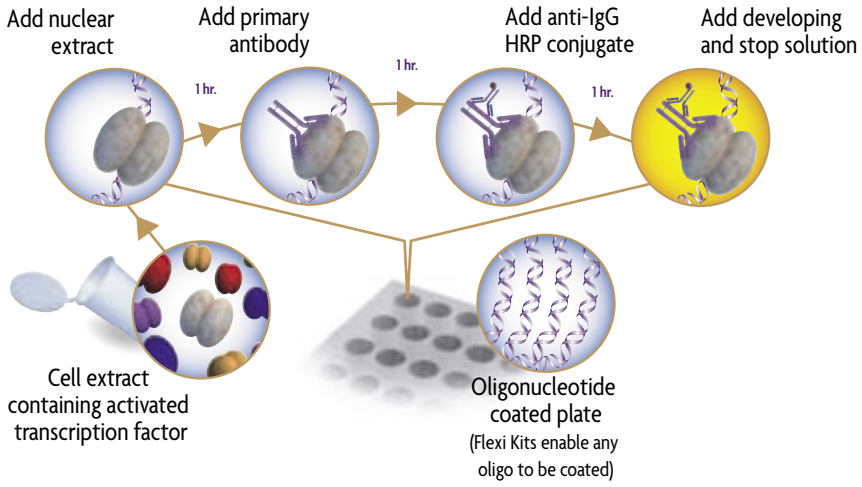


Figure 1: Flow chart of the TransAM process.

Table 1: TransAM™ Product Line

TransAM™ AP-1 Family	TransAM™ AML-1/Runx1	TransAM™ C/EBP α/β	TransAM™ HNF-1	TransAM™ Flexi NFκB p65
TransAM™ GATA Family	TransAM™ AML-3/Runx2	TransAM™ CREB & pCREB	TransAM™ IRF-3	TransAM™ NFκB p65*
TransAM™ HNF Family	TransAM™ AP-1 c-Fos	TransAM™ Elk-1	TransAM™ MEF2	TransAM™ Oct-4
TransAM™ MAPK Family	TransAM™ AP-1 c-Jun	TransAM™ ER	TransAM™ MyoD	TransAM™ p53
TransAM™ Flexi NFκB Family	TransAM™ AP-1 FosB	TransAM™ FKHR (FOXO1/4)	TransAM™ NF-YA	TransAM™ PPARγ
TransAM™ NFκB Family	TransAM™ AP-1 JunD	TransAM™ GATA-4	TransAM™ NFATc1	TransAM™ Sp1 & Sp1/Sp3
TransAM™ STAT Family	TransAM™ ATF-2	TransAM™ GR	TransAM™ Flexi NFκB p50	TransAM™ STAT3
	TransAM™ c-Myc	TransAM™ HIF-1	TransAM™ NFκB p50*	

* The Original TransAM NFκB p50 & p65 Kits are offered in both colorimetric and chemiluminescent formats. TransAM Chemi Kits require the use of a luminometer.

Clone Your Target Once, Then Easily Label It with Multiple Tags

Active Motif's LigandLink™* Universal Labeling technology enables you to label your target gene in living cells with multiple fluorescent tags and just clone your sequence into one vector. The easily changed tags make LigandLink fusion proteins ideal for co-localization studies.

Superior and flexible dye options

With LigandLink™, the gene of interest is cloned into the vector once, and this single clone can be labeled with different LigandLink fluorescent tags. The fluorescent tags are cell permeable and highly specific for the LigandLink fusion protein, so there is very low background (Figure 1). And, the fusion protein is small (~18 kDa), so protein activity is less likely to be affected than with GFP (27 kDa). LigandLink Labels are available with both red and green fluorescent dyes (Figures 1 and 2). These labels are stable and ideal for co-localization research.

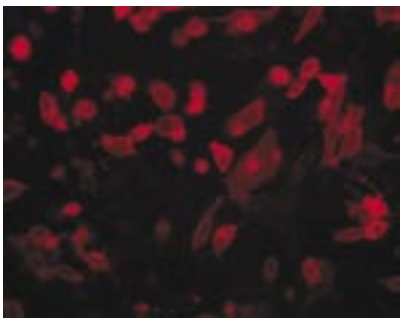
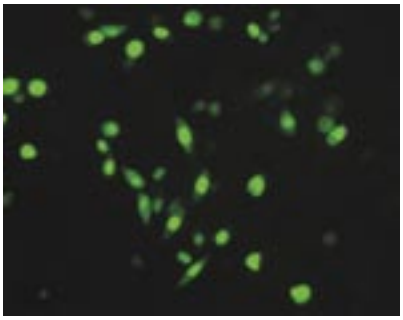


Figure 1: Membrane-localized eDHFR labeling by LigandLink Hexachlorofluorescein.
The myristoylation/palmitoylation sequence from Lyn was cloned into pLL-1 and transfected into CHO cells. Twenty-four hours post-transfection, 10 nM LigandLink Hexachlorofluorescein was added to the cells; 2 hours later,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

LigandLink advantages

- Clone once, but obtain different functionalities by using different LigandLink labels
- Small label is unlikely to interfere with protein function
- LigandLink labels are cell permeable
- LigandLink does not interact with other mammalian proteins



Figured 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 for 10 minutes.

The LigandLink method

Simply clone your gene of interest in frame with the *E. coli* dihydrofolate reductase (eDHFR) gene in the LigandLink pLL-1 vector. Then, transfect the vector into mammalian cells to express the fusion protein. Twenty-four hours later, label the protein of interest by adding a LigandLink label to the cell culture medium. Depending on the cell type, the labeled fusion protein can be visualized in as little as 10 minutes. The LigandLink fluorescent label is a TMP (trimethoprim) ligand that specifically and rapidly binds to eDHFR and does not interact with mammalian proteins. This is because TMP is an antibiotic designed to inhibit the bacterial eDHFR gene with a high binding specificity ($K_i \approx 1$ nm).

Try LigandLink today

LigandLink is an improved way to label proteins in living cells. It is easy to change labels by adding a different LigandLink tag. Find out more by visiting www.activemotif.com or calling us today.

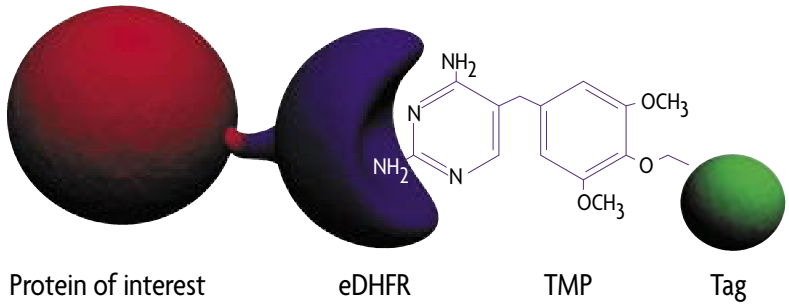


Figure 3: Specific protein labeling using LigandLink.
The gene of interest is cloned into pLL-1 in frame with the vector's *E. coli* dihydrofolate reductase (eDHFR) gene. After transfection into cells, the protein of interest is expressed as a fusion to eDHFR. Addition of one of the cell-permeable LigandLink Labels to the medium results in rapid, specific binding of the label by the fusion protein.

Product	Format	Catalog No.
LigandLink™ pLL-1 Kit	1 kit	34001
LigandLink™ pLL-1-AKT1 Kit	1 kit	34002
LigandLink™ pLL-1-AKT2 Kit	1 kit	34003
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

* Patent pending.

FACE™: Quantify Phospho-Proteins Without Making Lysates

Active Motif's Fast Activated Cell-based ELISA (FACE™) Kits provide you with a simple, sensitive, cell-based method for detecting protein phosphorylation directly in the cell, without making cell extracts or the trouble of running gels.

FACE advantages

- No cell extracts or immunoblotting
- Less than 2 of hours hands on time
- Total and phospho-specific antibodies included

The "in-cell" Western method

In FACE™, cells are grown in a 96-well plate and stimulated to induce the pathway of interest. After stimulation, the cells are fixed to preserve protein activation and modification states. Each well is then incubated with a primary antibody specific to the protein of interest. An antibody that recognizes all forms of the protein is included as well as an antibody that recognizes the phosphorylated site of the target protein so you can compare the stimulation effect in your cells. After the primary antibody incubation, an HRP-conjugated secondary is added along with either colorimetric or chemiluminescent developing solutions (Figure 1). The results provided are both quantitative and reproducible (Figure 2).

Prevent unwanted sample modifications

The FACE method eliminates the need to prepare cell lysates, which greatly reduces the possibility of introducing undesired alterations of the protein of interest. FACE uses a fixation step that "freezes" the cellular state and prevents further protein modifications. This enables detection of the exact protein state in the cells at your chosen time points, providing more accurate results.

"FACE Kits enable detection of phosphorylated proteins within the cell, eliminating the need for cell extractions, gels & Western blotting."

A variety of kits to choose from

FACE kits are available in both chemiluminescent and colorimetric formats for over 20 different kinase and receptor targets (Table 1). And, with the FACE Maker Kits, you can use your own primary and secondary antibodies to detect any target or modification state of interest. For complete information, visit www.activemotif.com/face.

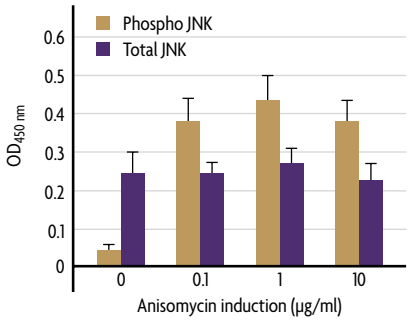


Figure 2: Monitoring the effects of anisomycin treatment on JNK phosphorylation.
The FACE JNK assay was used to assay the levels of phosphorylated JNK in response to different concentrations of anisomycin treatment on macrophage 4/4 cells.

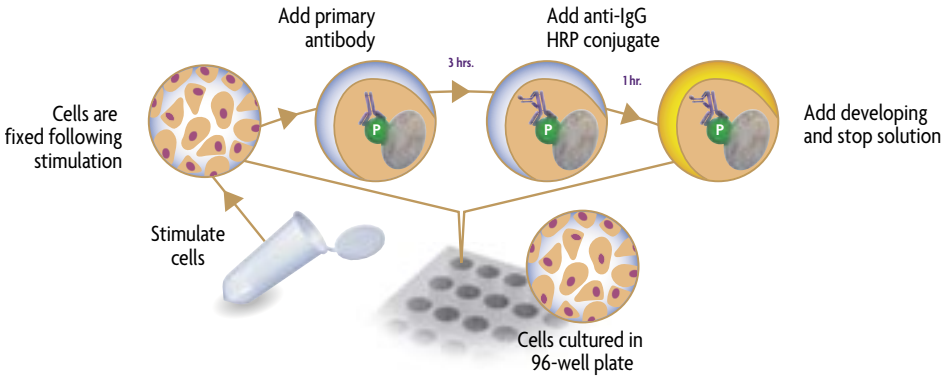


Figure 1: Flow chart of the FACE process.
Cells are grown, stimulated and fixed in the same 96-well plate. Addition of primary and secondary antibodies detects phosphorylated protein.

Table 1: The FACE™ Product Line

FACE™ AKT (S473)	FACE™ ATF-2 (T71)	FACE™ Bad (S112)	FACE™ c-Jun (S63)	FACE™ c-Jun (S73)	FACE™ c-Src (Y418)
FACE™ EGFR (Y845)	FACE™ EGFR (Y992)	FACE™ EGFR (Y1173)	FACE™ ErbB-2 (Y877)	FACE™ ErbB-2 (Y1248)	FACE™ ERK1/2 (T202/Y204 & T185/Y187)
FACE™ FAK (Y397)	FACE™ FKHR (T24)	FACE™ GSK3β (S9)	FACE™ HSP27 (S82)	FACE™ JAK1 (Y1022/Y1023)	FACE™ JNK (T183/Y185)
FACE™ Maker	FACE™ MEK1/2 (S217/S221)	FACE™ NFκB Profiler (S468 & S536)	FACE™ p38 (T180/Y182)	FACE™ PI3 Kinase p85	FACE™ STAT2 (Y689)
FACE™ STAT4 (Y693)	FACE™ STAT6 (Y641)				

Chromeo™ Dyes: Excellent Fluorescent Labels for Bioanalysis

Active Motif's Chromeo™ Dyes are superb labels for cell culture with their robust photostability, low cell toxicity and broad range of pH stability. And, they can be used with many common excitation sources.

Superior fluorescent properties

The Chromeo™ Dyes, Chromeo 494, Chromeo 546 and Chromeo 642, are ideal for any fluorescent application because of their superior luminescent properties and broad Stokes shifts (Table 1). Chromeo Dyes are suitable for use with many common excitation sources such as diode lasers, LEDs, tungsten and Xenon arc lamps.

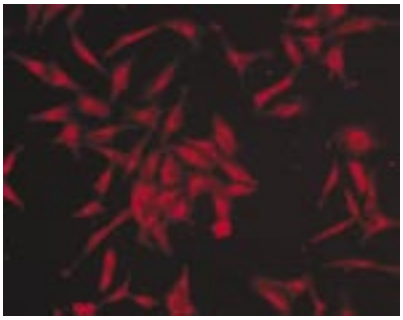


Figure 1: Cytosolic staining of HeLa cells using Chromeo 494.

Better for biology

Chromeo Dyes exhibit key features that make them better than other labels for fluorescent microscopy. Chromeo conjugates are bright and readily soluble (Figure 1). Their brightness intensity, limited photobleaching (Figure 2), and broad Stokes shifts make them easy to pair with other fluorescent probes. In particular, Chromeo 494 has a Stokes shift of 124 nm that makes it an ideal partner for multiplexing.

Chromeo capabilities

As a leader in providing fluorescent tools for bioanalysis, Active Motif Chromeon also offers several other dyes to meet your research needs, including RuLabels and Py-Dyes. RuLabels are phosphorescent ruthenium probes that emit strong polarized light with a long decay time, which enhances sensitivity and reduces background. The Py-Dyes possess unique physical and spectral properties that make conjugation simple, while greatly reducing background. To find out more about Active Motif Chromeon products visit www.chromeon.com.

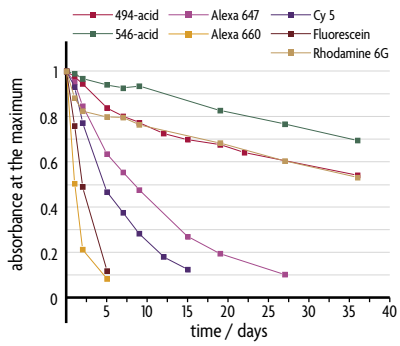


Figure 2: Photostability test.
Dyes were dissolved in PBS (22 mM, pH 7.2) and the solutions were allowed to stand at room temperature in daylight. Absorbance at the respective maximum was measured over a period of 36 days.

Dye	Absorption	Emission	ε L/(mol-cm)	Quantum Yield (%)*	Stokes Shift
Chromeo™ 494	494	628	55,000	25	124 nm
Chromeo™ 546	545	561	98,800	10	16 nm
Chromeo™ 642	642	660	180,000	15	18 nm

Table 1: Chromeo Dye properties. (* when conjugated to BSA)

Product	Format	Catalog No.
Chromeo™ 494 Carboxylic Acid	1 mg 5 mg	15110 16110
Chromeo™ 494 NHS-Ester	1 mg 5 mg	15111 16111
Chromeo™ 494 Biotin	1 mg 5 mg	15112 16112
Chromeo™ 494 Streptavidin	1 mg 5 mg	15113 16113
Chromeo™ 546 Carboxylic Acid	1 mg 5 mg	15210 16210
Chromeo™ 546 NHS-Ester	1 mg 5 mg	15211 16211
Chromeo™ 546 Biotin	1 mg 5 mg	15212 16212
Chromeo™ 546 Streptavidin	1 mg 5 mg	15213 16213
Chromeo™ 642 Carboxylic Acid	1 mg 5 mg	15310 16310
Chromeo™ 642 NHS-Ester	1 mg 5 mg	15311 16311
Chromeo™ 642 Biotin	1 mg 5 mg	15312 16312
Chromeo™ 642 Streptavidin	1 mg 5 mg	15313 16313

New: Rapid Staining of Live or Fixed Cells

Active Motif's new LavaCell™ stain is a unique, non-toxic, cell permeable dye, ideal for use in the study of cell proliferation, cell counting, chemotaxis, cell morphology or any application that requires cellular imaging.

LavaCell™ is a small, naturally fluorescent compound with inherent properties that make it ideal for cell imaging:

- Non-cytotoxic
- Does not alter cell growth
- Cell permeable
- 405, 488 and 532 nm excitable
- Brightly fluorescent at biological pH (Figure 1)
- Non-fluorescent prior to cellular internalization

Ideal for multiplexing

LavaCell has a significant Stokes shift (488 nm ex:610 nm em), which means that it is ideally suited for use with other green-emitting fluorophores that are commonly used in cell biology (Figure 2). It is also excited by a variety of common laser sources (405, 488 and 532 nm).

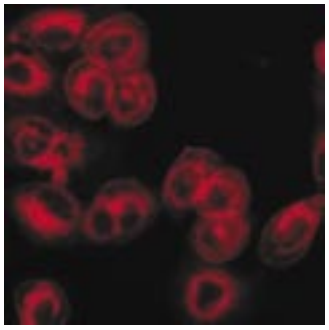


Figure 1: Staining of live CHO cells with LavaCell.

Just add and image

Using LavaCell is simple; just add it to your cell culture, wait 30 minutes and analyze. Nothing could be easier! The dye readily diffuses into live or fixed cells to produce bright orange fluorescence on the plasma and internal membranes. And, there is no need to wash away unbound dye. Please call or visit us online for more complete information.

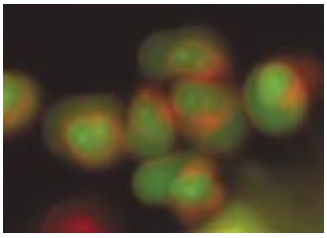


Figure 2: LavaCell and Calcein AM in live CHO cells.

Product	Format	Catalog No.
LavaCell™	200 µg	15004

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Tools to Analyze Cellular Function