MOTIF VATIONS

ACTIVE MOTIF®

Innovative Tools for Cell & Molecular Biology

THE NEWSLETTER OF ACTIVE MOTIF — SEPTEMBER 2004 • VOLUME 5 • NUMBER 3

ChIP-IT™ – Chromatin Immunoprecipitation Made Easy

Classically, chromatin immunoprecipitation (ChIP) has been performed using reagents prepared "in house", or with commercial kits that do not contain everything that is needed to complete the experiment. This can be time consuming and frustrating, particularly as there are no guarantees that the separate components will work well together. To overcome this problem, Active Motif has developed its ChIP-IT™ Kit. ChIP-IT is a complete solution that contains all of the reagents required for DNA shearing, "pull down" and purification. Plus, the kit includes positive and negative control antibodies and PCR primers that help you interpret your results. With ChIP-IT you get a complete package of high-quality, optimized reagents, ensuring that you get the best results possible, while saving you both time and money.

The power of ChIP

Transcriptional regulation is one of the main methods used by cells to control gene expression. Traditionally, researchers have analyzed transcription factors and

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Phospho-specific ELISAs for Bad, c-Src, FAK, FKHR, GSK3β, JAK & PI3K

FACE™ (Fast Activated Cell-based ELISA) Kits offer a better alternative to Western blots and Sandwich ELISAs for the study of protein phosphorylation because the cell-based format of FACE allows you to grow, fix and assay your cells all in the same plate. This keeps protein modifications to a minimum, enabling you to assess the exact protein state within the cell at your chosen time point. Now, Active Motif's popular FACE Kits are available for monitoring both the phosphorylated and native forms of Bad, c-Src, FAK, FKHR, GSK3β, JAK1 and PI3 Kinase p85. And, for added convenience, FACE Kits are offered in both colorimetric and ultra-sensitive chemiluminescent formats, giving you the flexibility to choose the kit that best fits your needs and your in-house equipment. Studying phosphoproteins has never been so simple.

The FACE method

In FACE, cells are cultured in the provided 96-well plates and stimulated to induce the pathway of interest (Figure 2, pg. 6). The cells are then rapidly fixed with formaldehyde to preserve protein modifications, including phosphorylation. Each well is then incubated with a primary antibody specific for the phospho-protein of interest. This is followed by secondary HRP-conjugated antibody and developing reagent, which provides a sensitive colorimetric or chemiluminescent readout. FACE Kits come complete with everything needed to perform 96 assays using a phospho-antibody that is directed against the phosphorylated form of the protein of interest, as well as 96 assays using a total antibody. Using two different antibodies enables simultaneous analysis of both the native and activated protein levels (Figure 1).

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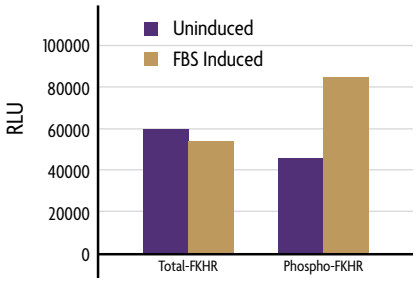

Figure 1: Measurement of phosphorylated and total FKHR. CHO-K1 cells were cultured in 96-well plates and serum-starved for 16 hours. Cells were then treated with 20% FBS for 30 minutes and fixed. Total and phospho FKHR were each assayed in triplicate using the phospho and total FKHR antibodies included in the FACE FKHR Chemi Kit. Data was plotted after correction for cell number (performed through use of the kit's Crystal Violet reagent).

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Sensitive, Non-radioactive Method for Studying Transcription Factor Activation

Active Motif's TransAM™ Kits are ELISA-based assays* that simplify the study of transcription factor activation in mammalian tissue and cell culture extracts. The TransAM method is up to 100-fold more sensitive than the gelshift technique and provides results in less than 5 hours. TransAM Kits eliminate the use of radioactivity, while their flexible format makes it possible to screen from 1 to 96 samples in a single experiment. And, unlike reporter assays, TransAM Kits can be used to study all sample types, including cell lines and tissues, giving you unsurpassed flexibility.

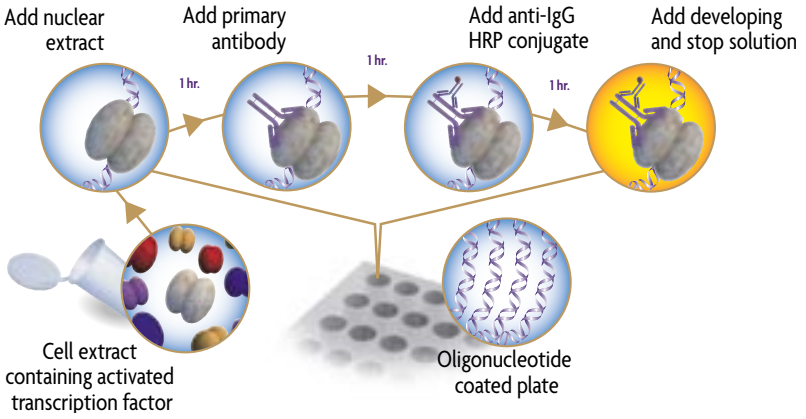

Figure 1: Flowchart of the TransAM procedure.

Simple, specific method
Each TransAM Kit includes a 96-well plate in which multiple copies of a specific double-stranded oligonucleotide have been immobilized. When nuclear or whole-cell extract is added to the wells of the plate, the transcription factor of interest binds to its consensus-binding site on the oligonucleotide. A primary antibody that is specific for the activated form of the transcription factor is then added, followed by incubation with a secondary HRP-conjugated antibody and developing reagent. The colorimetric change is read using a spectrophotometer, providing a sensitive, quantitative measurement of transcription factor activation (Figure 1). The novel ELISA format is readily adaptable to automated high-throughput screening, which further reduces the time and labor normally associated with quantifying levels of activated transcription factors.

Sensitive, quantitative assay
Because small changes in transcription factor levels have a significant impact on cellular function, it is vital to use a sensitive assay. TransAM Kits are 10-fold more sensitive than gelshift assays, while TransAM Chemi Kits provide 100-fold better sensitivity. And, because TransAM assays provide quantitative results, you can subtract the level of endogenous factor present in your untreated samples from your test samples, further improving your results.

TransAM assay formats
TransAM Kits are offered in a variety of formats – individual kits assay single transcription factors, while Family Kits make it possible to profile multiple members of a transcription factor family. And, TransAM Chemi Kits utilize a luminometer to provide the maximum sensitivity possible. Currently available TransAM assays are listed below.

TransAM advantages
- Colorimetric method provides sensitive, quantifiable results
- Eliminates the use of radioactivity and the need to run and develop gels
- Results in less than 5 hours
- Profile multiple members of a transcription factor family simultaneously
- Ability to assay cell and tissue samples

Complete solution for activation assays
TransAM Kits will improve your transcription factor activation research because they are faster, more sensitive and easier to use than existing methods. Please visit our website at www.activemotif.com/transam to learn about the latest additions or to download TransAM manuals and a list of publications that cite the use of TransAM. For a better method for studying transcription factor activation, try TransAM today.

* Technology covered under AAT-filed patents licensed to Active Motif.

TransAM Product Line

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

** TransAM Chemi Kits require the use of a luminometer.

Chariot™ – A Fast, Convenient Alternative to DNA Transfection

Despite their wide-spread use, DNA transfection and gene expression are suboptimal techniques for determining what effects are caused by introducing recombinant proteins into cells. Lipid-based transfection methods have long been known to be cytotoxic to most cell lines. This, coupled with overexpression of the recombinant protein, can cause a number of changes in the cell that are completely independent of the actions of the introduced protein. Thus, it can be difficult to determine if any observed changes are caused by the recombinant protein or by the transfection/expression method itself. In addition, the need to wait days to weeks after transfection to assay for the recombinant protein is not only inconvenient, it is a waste of both time and resources.

Keep in control

In stark contrast, Active Motif's Chariot™ is an innovative alternative that is fast, convenient and overcomes the limitations of classical DNA transfection. Chariot is a non-cytotoxic peptide carrier that efficiently transports biologically active proteins, peptides and antibodies directly into cells in less than two hours. Not only do you get your results sooner, but you can control the timing and amount of your protein that is delivered. This makes Chariot the delivery vehicle of choice for functional studies.

Fast, stable protein delivery

During a brief 30-minute incubation, Chariot and the macromolecule of interest form a stable, non-covalent complex. This complex protects the macromolecule from degradation during delivery, which helps to preserve 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 fully functional and free to proceed to its target organelle (Figure 1). By directly delivering the protein, peptide or antibody, Chariot completely bypasses the transcription-translation process associated with gene expression, saving hours to days of time.

Cell line or model	Cell type	Macromolecule delivered	Delivery efficiency (%)
3T3-L1	Mouse fibroblast	Antibody	80%
A549	Human lung carcinoma	Antibody	95%
Arabidopsis	Primary plant protoplasts	Protein	Not provided
COS-7	Monkey kidney	Protein, oligopeptide	80%
CV-1	Monkey kidney	Antibody	55%
HCA2	Human fibroblasts	Protein	90%
HeLa	Human cervix carcinoma	Protein, peptide, antibody	95%
HMSC	Primary human mesenchymal stem cells	Peptide	80%
HS-68	Human foreskin fibroblast	Protein, peptide, antibody	95%
IMR90	Human fibroblast	Protein, peptide	80%
Jurkat	Human T-cell leukemia	Protein, peptide, antibody	75%
Mono Mac 6	Primary human monocyte	Protein	50%
Mouse hepatocytes	Primary liver	Protein	95%
Mouse model (*in vivo*)	Alveolar wall tissue	Protein	88%
Neural retina cells	Primary chicken	Protein	80%
NIH/3T3	Mouse embryo	Protein, peptide	80%
NRK	Normal rat kidney	Antibody	30-60%
PC-12	Rat pheochromocytoma	Protein, peptide	80%
Sensory neurons (DRG)	Primary chick	Antibody, peptide	80%
Sensory neurons (DRG)	Primary rat	Protein	80%
Thyrocytes	Primary human	Protein	90%
WI-38	Human lung fibroblast	Protein, peptide, antibody	95%
WISH	Human placenta carcinoma	Protein	30%

Do more with Chariot

The ability to deliver functionally active protein, peptides and antibodies into cells makes it easy to perform studies that are not possible using classical methods. There is an extensive list of publications on our website describing such novel research, as well as a complete list of delivery efficiencies provided by Chariot users (Table 1).

To find out more about how Chariot can improve your research, give us a call today or send in the enclosed reply card.

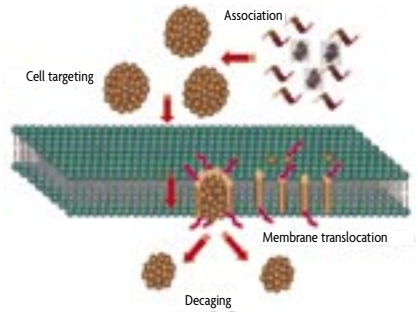

Figure 1: Schematic model for translocation of the Chariot complex. Figure generously provided by Dr. Gilles Divita, Biophysics Dept., CNRS, Montpellier, France.[1]

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

1. Deshayes *et al.* (2004) *Biochemistry* 43:1449-1457.

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

continued from page 1 — ChIP-IT™ – Chromatin Immunoprecipitation Made Easy

gene promoter activity using methods such as reporter gene assays, EMSA, Western blotting and, more recently, using DNA microarrays. However, although these methods have led to significant advances in the scientific understanding of transcription, there is still a need for improved assays. Specifically, there is a need for better understanding of *in vivo* interactions between DNA and transcription factors. The ChIP method overcomes this limitation through a formaldehyde cross-linking step, which "freezes" and preserves cellular protein/DNA interactions.

The ChIP-IT method

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

The keys to successful ChIP

Successful ChIP depends on a variety of different factors, including correct fixation of the DNA, shearing of the DNA to an optimal length and possessing both an antibody that recognizes the exposed motifs of fixed, DNA-bound protein as well as a PCR primer pair that specifically amplifies the region of interest. A variety of specialized buffers, inhibitor cocktails and blocking reagents are required during these steps to minimize non-specific enrichment and reduce background. Should any of your reagents fail to work properly or not be optimized for use with one another, your ChIP experiment may fail, wasting precious time and resources. However, the ChIP-IT Kit was designed to eliminate such worries. All reagents in the kit are optimized for use together and are rigorously QC'd to ensure that you get the best results possible.

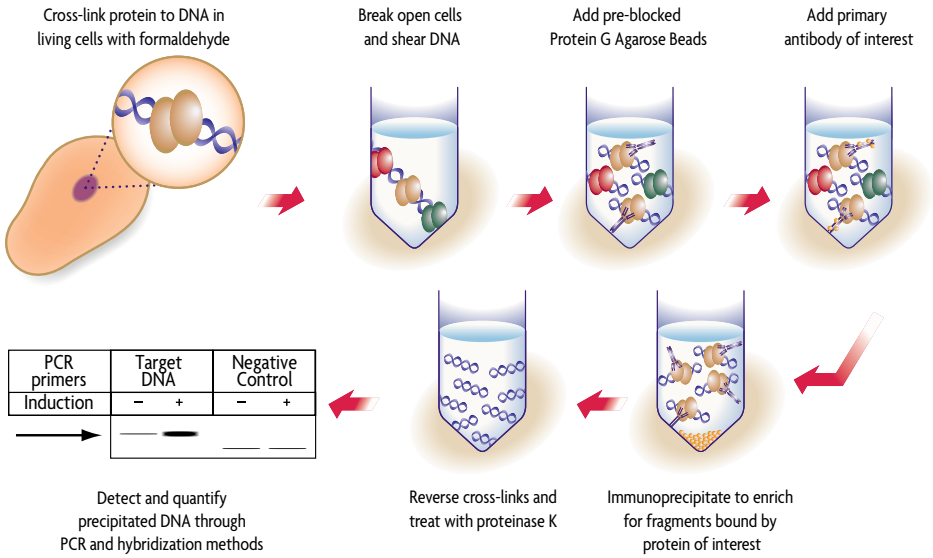

Figure 1: Schematic of chromatin immunoprecipitation. In ChIP, protein/DNA interactions are fixed, and the DNA is then sheared and precipitated using an antibody. After reversing the cross-links, the DNA is purified and then screened to determine which genes were bound by the protein of interest.

ChIP-IT advantages

- Complete solution for ChIP – all critical reagents and controls are supplied
- Direct measurement of transcription factor/DNA interactions or histone modifications
- Compatible with genome-wide profiling or selective PCR-based screening
- No need to optimize your own reagents and protocol

More specific promoter analysis using transcription factor antibodies

ChIP has typically been performed using antibodies directed against abundant chromatin components, such as acetylated histones. While this method can indicate the transcriptional state of a given promoter, it cannot be used to identify which transcription factor(s) were bound to the promoter(s) of interest. To do that, ChIP must be performed using antibodies directed against specific transcription factors. However, this is more technically challenging because the amount of bound transcription factor is far less than that of acetylated histones. To achieve success, every aspect of the ChIP procedure must be efficient. Because the reagents included in Active Motif's ChIP-IT have been optimized to work well together, you can be confident of success, even when performing such difficult assays.

Controls to validate your results

ChIP is a complicated procedure that takes several days to complete. Troubleshooting the assay and interpreting its results can be difficult unless you have reagents and controls that have proven to work in ChIP. To help minimize your time and effort, the ChIP-IT Kit includes a comprehensive manual and positive and negative control antibodies and PCR primer sets. These are ideal for verifying that your DNA was fixed and sheared correctly, as well validating that your own antibodies and primer sets function in ChIP. No other kit provides such a complete solution for ChIP. For more successful ChIP, try the ChIP-IT Kit.

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

Improved Gene Silencing Through Higher Specificity Binding

Active Motif's custom gripNA™ oligonucleotides bind to nucleic acids with higher affinity and more stringent mismatch discrimination than other gene silencing reagents. This improves the results of your gene silencing experiments by minimizing non-specific interactions that can cause unintended, mutant phenotypes. gripNAs have proven to be effective at silencing genes in mammalian cells[1], zebrafish, *Xenopus* and in *in vitro* translation systems. Because gripNAs are resistant to nuclease degradation, they are stable in the *in vivo* environment, enabling them to silence genes expressed over a range of developmental stages. And, you can deliver gripNAs into cells using Chariot™ II, lipids, electroporation, microinjection and the scrape method, making gripNAs the most versatile tool available for gene silencing.

Enhanced PNA chemistry

gripNAs are a negatively charged form of Peptide Nucleic Acids (PNAs). PNAs are DNA analogs in which the nucleoside bases are attached to a synthetic backbone rather than to deoxyribose, as in DNA. Like DNA and RNA, PNAs bind complementary nucleic acids by conventional Watson-Crick base pairing. However, they hybridize to their target with a greater affinity and an increased sequence specificity. Their binding affinity is significantly reduced (or completely eliminated) if there is even a single base-pair mismatch with their target nucleic acid (Table 1). This improves your gene silencing results by minimizing the non-specific silencing of genes with sequences that are nearly identical, but not homologous, to your target sequence.

Why choose gripNAs?

- Unsurpassed sequence specificity
- Resistant to nucleases
- Flexible synthesis modifications
- Multiple delivery options
- Simple online ordering

	DNA/DNA		gripNA/DNA		
	T_m (°C)	ΔT_m (°C)	T_m (°C)	ΔT_m (°C)	Sequence of DNA oligonucleotide
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	3.1	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	7.1	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: Increased binding specificity of gripNAs.
Identical 16-mer gripNA and DNA probes were synthesized with the sequence 5´-TGG-TCT-CAA-GTC-AGT-G-3´. These were annealed to a complementary DNA oligo (5´-CAC-TGA-CTT-GAG-ACC-A-3´) in Hybridization Buffer (20 mM Tris, pH 7.5, 500 mM NaCl, 10 mM $MgCl_2$). Each sample was 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 the DNA/DNA and gripNA/DNA duplexes. The experiment was then repeated by hybridizing the gripNA and DNA probes to a series of DNA oligos containing one- or two-base mismatches (shown above, with the mismatches 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 and no ΔT_m.

Hit your target only

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

Order anytime

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

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

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

continued from page 1 — Phospho-specific ELISAs for Bad, c-Src, FAK, FKHR, GSK3β, JAK & PI3K

Specificity you can count on

A concern for many researchers studying phosphorylated proteins is the lack of specificity of phospho-specific antibodies. To be certain that you detect only the protein you are interested in, all antibodies in FACE Kits are stringently tested for cross-reactivity using Western Blot analysis. All phospho-specific antibodies are verified to detect only the activated form of the target protein. In addition, the phospho-specific and total antibodies are used in tandem to ensure the phospho-specific antibody doesn't interact with other phosphorylated proteins. This ensures that FACE Kits are highly specific and detect only the protein you are interested in, at the specific phosphorylated site.

FACE advantages

- **Cell-based ELISA** – no extracts, gels, blotting or radioactivity
- **Fast** – requires less than 2 hours of hands on time
- **Flexible** – high-throughput chemi and colorimetric formats
- **2 Antibodies** – compare phosphorylated and native protein levels in the same kit
- **Quantitative results** – ELISAs provide more meaningful data than Westerns

Order today

FACE Kits provide you with a simple, efficient, quantitative method to monitor proteins activated by phosphorylation. No other phospho-specific assay available offers you such a quick and convenient solution. And, because the assay is so sensitive, FACE Kits can accurately monitor even the smallest changes in protein phosphorylation. Active Motif has recently released new kits for studying the activation of Bad, c-Src, FAK, FKHR, GSK3β, JAK1 and PI3 Kinase p85, with more on the way. To see the latest additions to this growing product line, be sure to check our website at www.activemotif.com/face. For the most accurate and convenient phospho-specific assay available, try a FACE Kit today.

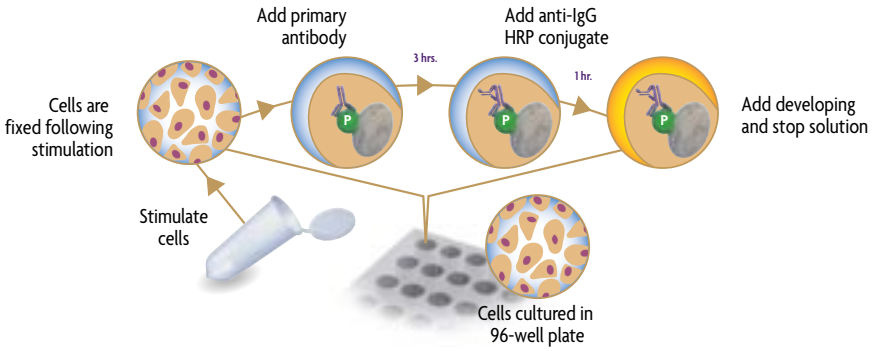

Figure 2: Flowchart of the FACE process.
Cells are grown, stimulated and fixed in the same 96-well plate. Addition of primary and secondary antibodies detects phosphorylated protein.

Product	Format	Colorimetric Kit Catalog No.	Chemiluminescent Kit Catalog No.
FACE™ AKT	1 x 96 rxns 5 x 96 rxns	48120 48620	48220 48720
FACE™ ATF-2	1 x 96 rxns 5 x 96 rxns	48115 48615	48215 48715
FACE™ Bad	1 x 96 rxns 5 x 96 rxns	48165 48665	48265 48765
FACE™ c-Jun (S63)	1 x 96 rxns 5 x 96 rxns	48125 48625	48225 48725
FACE™ c-Jun (S73)	1 x 96 rxns 5 x 96 rxns	48135 48635	48235 48735
FACE™ c-Src	1 x 96 rxns 5 x 96 rxns	48155 48655	48255 48755
FACE™ EGFR (Y992)	1 x 96 rxns 5 x 96 rxns	48150 48650	48250 48750
FACE™ EGFR (Y1173)	1 x 96 rxns 5 x 96 rxns	48190 48690	48290 48790
FACE™ ErbB-2 (Y877)	1 x 96 rxns 5 x 96 rxns	48130 48630	48230 48730
FACE™ ErbB-2 (Y1248)	1 x 96 rxns 5 x 96 rxns	48105 48605	48205 48705
FACE™ ERK1/2	1 x 96 rxns 5 x 96 rxns	48140 48640	48240 48740
FACE™ FAK	1 x 96 rxns 5 x 96 rxns	48145 48645	48245 48745
FACE™ FKHR (FOXO1)	1 x 96 rxns 5 x 96 rxns	48160 48660	48260 48760
FACE™ GSK3β	1 x 96 rxns 5 x 96 rxns	48170 48670	48270 48770
FACE™ MEK1/2	1 x 96 rxns 5 x 96 rxns	48180 48680	48280 48780
FACE™ JAK1	1 x 96 rxns 5 x 96 rxns	48185 48685	48285 48785
FACE™ JNK	1 x 96 rxns 5 x 96 rxns	48110 48610	48210 48710
FACE™ p38	1 x 96 rxns 5 x 96 rxns	48100 48600	48200 48700
FACE™ PI3 Kinase p85	1 x 96 rxns 5 x 96 rxns	48175 48675	48275 48775

Faster, More Accurate Measurement of Nitric Oxide Production

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

Nitric oxide is a key signaling molecule that, either directly or indirectly, stimulates host defenses in the immune system, maintains blood pressure in the cardiovascular system and modulates neural transmission in the brain. Consequently, NO has become the subject of extensive research. However, because NO has an extremely short half-life (< 10 seconds), it is difficult to detect and study. Therefore, because NO is rapidly metabolized to nitrite and nitrate, quantitation of these stable anions is frequently used to indirectly measure the amount of NO originally present.

The old way

The best index of total NO production is the sum of both the nitrite and nitrate in the sample, commonly quantified in a Two-Step assay. The first step is conversion of nitrate to nitrite by the use of NADH- or NADPH-dependent nitrate reductase. The nitrite is then converted into a purple-colored azo compound by the addition of Griess Reagent. Quantitation of this azo compound by spectrophotometry provides an indirect measure of the original NO concentration (Figure 1). However, the sensitivity of the Two-Step assay is limited because NADPH, which is an essential cofactor in step one, interferes with the Griess Reagent in step two. Assay sensitivity can be increased by the addition of a third step, in which lactate dehydrogenase (LDH) is used to eliminate excess NADPH prior to the Griess reaction. However, while inclusion of this additional LDH step increases assay sensitivity, it also increases the time required to perform each assay.

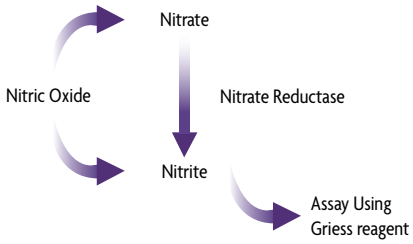

Figure 1: Measurement of nitric oxide by Griess Reagent.
Nitric oxide is converted to nitrite, then assayed using Griess Reagent.

Save time and money

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

Improved accuracy for better results

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

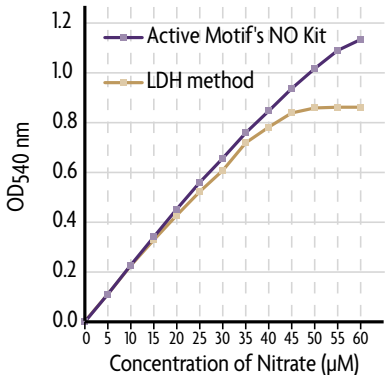

Figure 2: Dynamic range of nitrate standard curves.
Nitrate standard curves produced using the Nitric Oxide Quantitation Kit and a conventional Three-Step LDH-method kit.

Get better results today

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

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

Efficient, Affordable Competent *E. coli*

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

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

RapidTrans advantages

- Freedom to use anywhere from 1 to 96 reactions eliminates wasted reactions
- Choice of > 1 x 10^8 or > 3 x 10^8 cfu/µg
- Economical pricing (Figure 1)
- Adaptable for high-throughput use

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

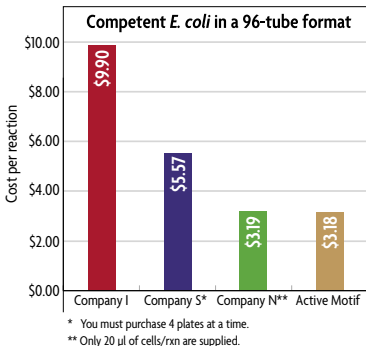

Figure 1: RapidTrans Competent *E. coli* are priced economically. The graph above shows the cost per reaction of 96-tube competent *E. coli* from various manufacturers.

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

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