MOTIF VATIONS

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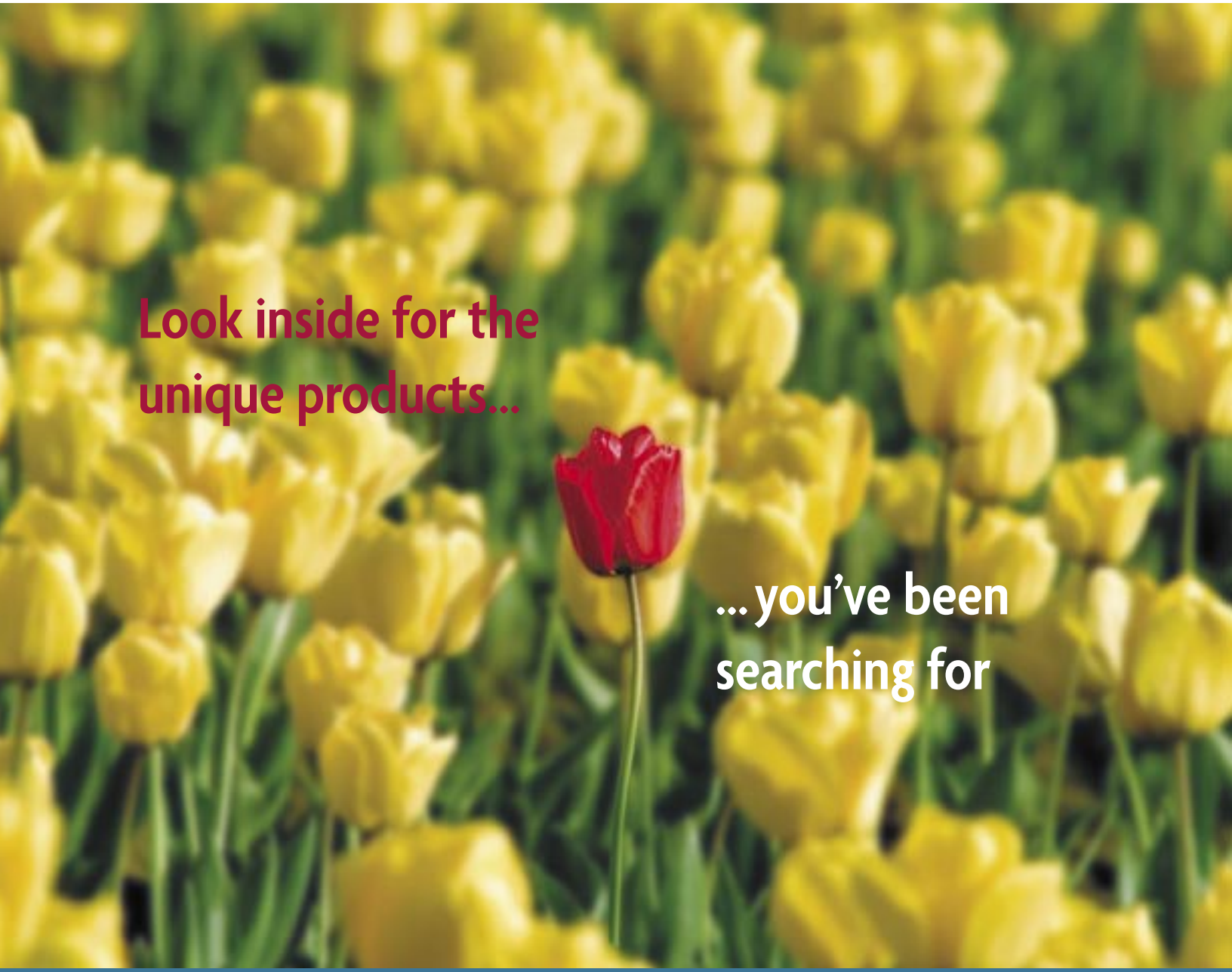
ACTIVE MOTIF®

Tools to Analyze Cellular Function



IN THIS ISSUE

NEW: CodeQuest™ Brings High-throughput Bioinformatics to Your Desktop

CodeQuest™ is an accelerated desktop workstation that makes it easy to build and process sophisticated informatics pipelines. The system combines computer cluster performance with drag-and-drop pipeline development, bringing high-throughput bioinformatics capabilities to your lab in a compact, easy-to-use system.

Complete bioinformatics workstation

CodeQuest™ utilizes the DeCypher Engine™ accelerator card to drive the included BLAST, Smith-Waterman, HMM, GeneDetective and RepeatMasker algorithms with the performance of 50-750 CPUs. So, you'll get answers to your bioinformatics questions in minutes or hours, rather than in days or weeks.

Easy-to-use across the lab

The PipeWorks™ user interface enables you to combine accelerated algorithms with open source applications into rapid analysis pipelines (Figure 1). This frees you from the laborious steps required to integrate data from multiple web searches. And, because PipeWorks can be run from any desktop computer, the entire lab can use CodeQuest to validate results or annotate sets of genes.

CodeQuest speeds many common bioinformatics tasks (Table 1), including:

- Screening specific microarray probes
- Annotating new genomes
- Fast Swiss-Prot protein searches
- Identifying microRNAs
- Mapping SNPs to a genome

Get more done, and fast!

CodeQuest's high performance and ease of use makes it possible to complete projects you hadn't thought possible. Visit www.timelogic.com/codequest to learn how CodeQuest can speed your research informatics activities.

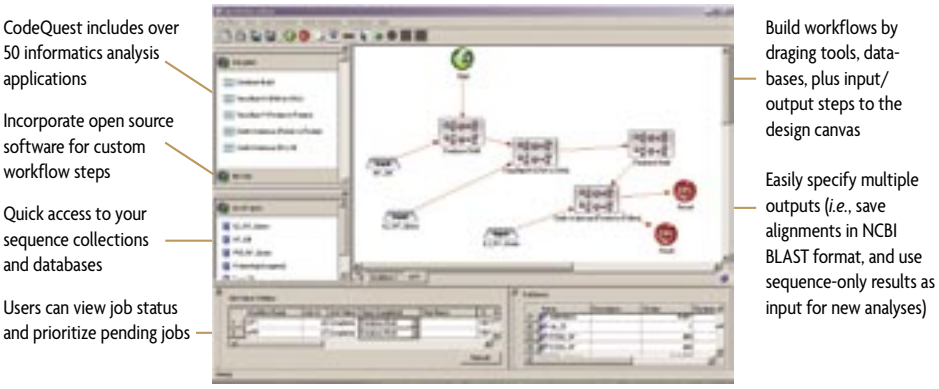


Figure 1: The PipeWorks interface simplified analysis pipeline design with drag-and-drop ease.

Application	Analysis	Time
BLASTX	Annotate 50 BACs against the Human genome	6 hours
T-BLASTN	Compare 4,500 bacterial protein sequences with 192 translated bacterial genomes	2 hours
BLASTN	Screen 26,000 50-mers against the rat genome	1 hour
HMM	Compare 25,000 sequences to PFAM for protein domain classification	5 hours
Smith-Waterman	Compare 25,000 mouse proteins to 49,000 Human protein sequences	7 hours
GeneDetective	Build intron/exon maps for 200 genes to explore alternative splicing	3 hours

Table 1: What can I accomplish on CodeQuest in a single day?

Uncover gene-to-disease links with AKS

AlmaKnowledgeServer (AKS) is a powerful literature mining system for elucidating connections between gene function, disease and related chemical compounds. AKS helps you visualize complex relationships through interactive diagrams, and streamlines your exploration of CodeQuest results.

Your personal informatics supercomputer

CodeQuest includes a powerful dual-CPU HP workstation with 2 GB RAM, 800 GB of storage, and a 19" flat panel monitor. And because the system includes PipeWorks, a DeCypher Engine and all of our accelerated algorithms, CodeQuest is a much better value than a computer cluster, and is significantly easier to use.

Product	Description	Catalog No.
CodeQuest™	Biocomputing workstation	75002
AlmaKnowledgeServer for CodeQuest™	Literature mining module	75105

Sensitive Protein Quantification Using Fluorescence

Active Motif's ProStain™ Protein Quantification Kit is a simple, sensitive alternative to traditional methods for determining protein levels. Offering high signal strength, unique spectral properties and robust conjugation, the kit offers limits of detection that are superior to other fluorescent-based systems, as well as traditionally used methods, such as the Bradford assay.

Broad spectral shift for better results

A disadvantage of many protein quantification methods, such as Bradford assays, is that the absorbance spectra of the free and conjugated forms of the dye partially overlap. This causes non-linear protein measurement because 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 the fluorescent dye provided in ProStain are separated by 108 nm. This means that when conjugated sample is excited at ~500 nm (for example, at 488 nm), only conjugated dye is excited. In addition, the emission intensity of free dye is 50-fold lower than conjugated dye; taken together, these features effectively eliminate background (Figure 1).

"The spectral properties and robust conjugation of the ProStain Protein Quantification Kit make measurement fast and accurate."

Fast, simple conjugation

Using ProStain is fast and easy; the kit provides fluorescent dye, dilution buffer and a protein standard. Simply resuspend the dye and add it to the wells of a microplate, then add a serial dilution of the standard protein to produce a Standard Curve, along with your sample. After a 30-minute, room-temperature incubation, simply read the fluorescence to quantify your samples.

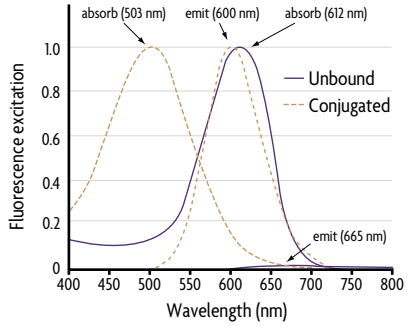


Figure 1: Absorption/emission spectra of free/bound dye. Normalized absorption and emission spectra of free (solid lines) and conjugated dye (dotted lines) in phosphate buffer of pH 7.2.

Contaminating substances

Unlike many protein determination methods, the ProStain Protein Quantification Kit has been shown to be resistant to the effects of many contaminating agents, such as detergents and salts (Figure 2). However, to achieve the best results the Standard Curve should be created using the same conditions as your samples. For example, if your samples are prepared in an unusual buffer, then your Standard Curve should be prepared using the same buffer.

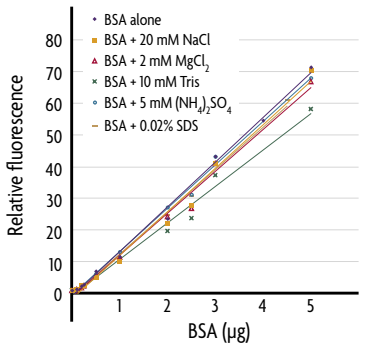


Figure 2: Standard Curves of BSA. Increasing amounts of BSA protein were quantified using the Fluorescent Protein Quantification Kit in the presence of a variety of contaminants.

Consistent complex quantification

To be effective, a protein quantification method must be consistent when used on different samples. To show the advantages of ProStain, it and the Bradford assay were used to produce BSA Standard Curves and then to quantify known amounts of three different nuclear extracts. Results obtained with ProStain showed a greater degree of accuracy than the Bradford assay, which displayed inconsistent results between different samples types and concentrations (Table 1). This type of inconsistency will impair downstream analyses performed with your samples as it will cause significant loading errors in your Westerns, ELISAs and other assays. To improve the results of your downstream assays, quantify using ProStain.

HeLa (µg/ml)	ProStain	Bradford
0.5	0.57 (13.8%)	1.39 (177.0%)
1.0	1.18 (17.9%)	0.82 (18.3%)
5.0	5.31 (6.2%)	5.21 (4.2%)
Jurkat (µg/ml)	**ProStain**	**Bradford**
0.5	0.46 (7.7%)	0.88 (75.8%)
1.0	0.98 (1.9%)	1.63 (63.2%)
5.0	5.15 (2.9%)	4.84 (3.2%)
MCF-7 (µg/ml)	**ProStain**	**Bradford**
0.5	0.51 (1.1%)	0.48 (3.2%)
1.0	1.02 (1.6%)	0.79 (20.7%)
5.0	5.63 (12.7%)	3.52 (29.6%)

Table 1: ProStain and Bradford quantification of extracts. Known amounts of 3 nuclear extracts were assayed in triplicate. The average concentration calculated, and its error from the known amount quantified, are shown.

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

Novel Dyes for Improved Capillary Electrophoresis

Active Motif's novel CE Dyes are a significant improvement over existing stains used to label proteins prior to capillary electrophoresis (CE). Their ease of use, spectral shift upon conjugation, high quantum yield and ability to maintain the natural ionic character of labeled protein make the CE Dyes ideal for use in CE.

Protein analysis using CE

Derivatization of proteins with fluorescent labels prior to CE is commonly used to overcome the lack of sensitivity when analyzing proteins via UV absorbance or natural fluorescence. In general, the proteins are labeled by attaching dye molecules to the primary amines of lysine residues. Lysine is used primarily because it is a relatively abundant amino acid, and also because lysine residues are often located at the surface of the protein. However, as dye labeling is non-uniform, and because proteins usually contain many lysine residues, inconsistent labeling of multiple lysine residues within a single protein is common. If the dye used affects the overall protein charge, then a single protein will give rise to a series of products, each with a different charge. This can cause problems during separation and other steps.

One charge – for clear separation

Like other dyes, Active Motif's CE Dyes utilize highly abundant and accessible lysine residues for their attachment chemistry. However, CE Dyes overcome the limitation of other dye systems by maintaining the positive charge of the amine group following dye conjugation (Figure 1). This means that proteins labeled with CE Dyes will not display band broadening or require adjusted ionic character calculations to be performed. The result is that CE Dyes provide a highly sensitive labeling system that maintains the natural charge properties of every protein within your sample.

Non-toxic – for user-friendly labeling

A further disadvantage of commonly used dyes, such as *o*-pthaldialdehyde, naphthalene-2,3-dicarboxaldehyde and 3-(2-furoyl)quinoline-2-carboxaldehyde, is that derivatization must be performed in the presence of cyanide. Use of such a highly toxic compound is not only undesirable, it creates significant disposal problems for the unused material. In contrast, the CE Dye labeling process is simple to perform, non-toxic and requires only a 30-minute incubation at either room temperature or 50°C, depending on the CE Dye used.

The CE Dye method

Labeling samples with CE Dyes is fast and convenient. The protein sample of interest is dissolved in bicarbonate solution (pH unadjusted) and stock CE Dye is added, followed by incubation at either room temperature or 50°C depending on the CE Dye used. Completion of the reaction can be observed by eye due to a change in sample color from blue to red. It's as simple as that!

Large Stokes Shift minimizes background

In addition to their non-toxic and charge properties, CE Dyes also undergo a significant change in spectral properties upon conjugation to amine groups. In their free state, CE Dyes have very weak fluorescence (quantum yield < 1%), with an absorbance maximum of 612 nm and an emission maximum of 665 nm. However, upon reaction with a primary amine the absorbance maximum shortwave-shifts by more than 100 nm to 503 nm and emits strongly with a quantum yield of more than 50% at 600 nm. This change in spectra and quantum yield means that the presence of unconjugated label does not affect downstream analysis and significantly reduces background effects.

Get better CE results starting now

To improve your CE results, give us a call to order CE Dyes today.

"Labeling proteins for capillary electrophoresis is easy with CE Dyes, while the large Stokes Shift and charge maintenance help improve your results."

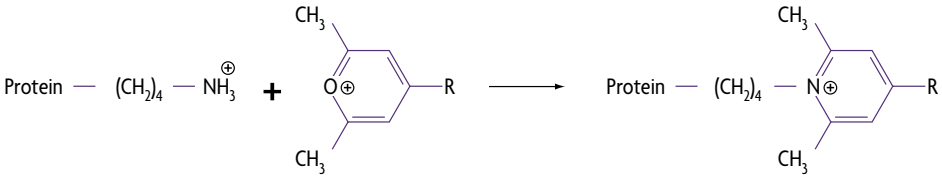


Figure 1: Labeling with CE Dyes maintains each protein's natural charge.
Chemical equation depicting the chemistry of CE Dye labeling, which demonstrates the preservation of the positive charge of the lysine residue of a protein. R stands for the respective chromogenic/fluorogenic group.

Product	Format	Catalog No.
CE Dye 503	1 kit	15101
CE Dye 540	1 kit	15102

Optimized Co-IP of Nuclear Protein Complexes

The Nuclear Complex Co-IP Kit simplifies co-immunoprecipitation studies of nuclear protein complexes by providing you with optimized reagents for both nuclear extract preparation and immunoprecipitation.

Better Co-IP method and reagents

The extraction process of the Nuclear Complex Co-IP Kit was designed to isolate intact protein complexes from the nuclear compartments of the cell, especially complexes that had been bound to DNA. And, the kit's versatile Co-IP reagents offer you the flexibility to vary the stringency of the Co-IP buffer compositions. This gives you the ability to study tightly bound or weak protein complexes with equal ease.

Maintain the complex

Co-Immunoprecipitation (Co-IP) is often used to find and study protein/protein interactions. In Co-IP, a first antibody is used to immunoprecipitate a target antigen, which also co-precipitates any associated, interacting proteins. The interacting proteins are then detected by Western blot using antibodies targeted against the interacting proteins of interest. However, traditional methods for performing Co-IP are not optimal for studying complexes of DNA-binding proteins because these complexes are frequently disrupted during the extraction process. The immunoprecipitation process can also be problematic because many protein complexes are altered or disrupted by the salt and detergent composition of the immunoprecipitation buffers. In response, the protocol and reagents included in the Nuclear Complex Co-IP Kit were designed to help maintain nuclear protein complexes during their extraction and immunoprecipitation.

"The kit improves Co-IP of DNA-binding proteins by providing extraction and immunoprecipitation components that help maintain nuclear protein complexes."

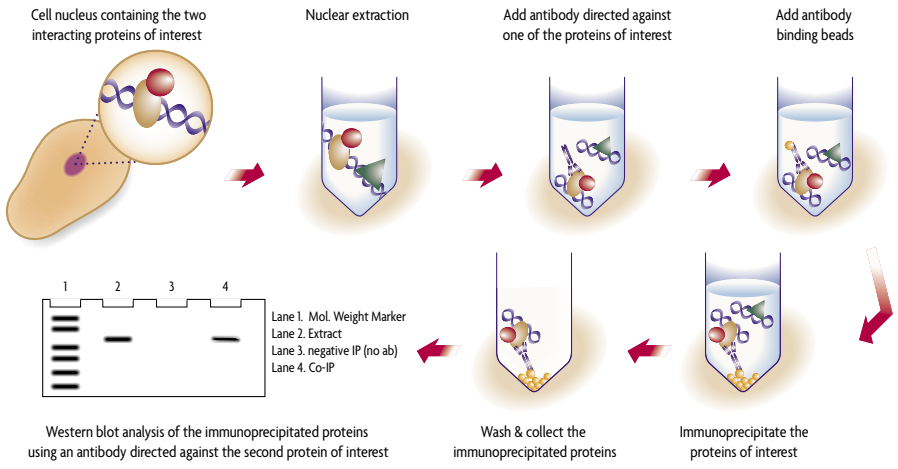


Figure 1: Flowchart of the Co-Immunoprecipitation process.

Product	Format	Catalog No.
Nuclear Complex Co-IP Kit	50 rxns	54001

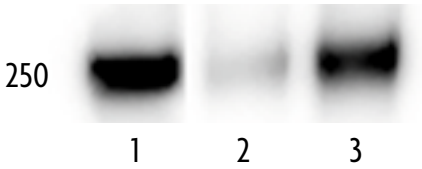


Figure 2: Western blot analysis of the IP'd p33 subunit of the RNA pol II complex.
HeLa cells were grown to confluence on 100 mm plates and nuclear extracts were prepared using the kit's extraction reagents. For IP experiments, the stringency of the IP High Buffer was increased by supplementing with NaCl and Detergent. 100 µg of nuclear extract was used per IP reaction and incubated with either 2 µg p33 antibody or no antibody. Following the IP, Western blot analysis was performed using RNA pol II mouse mAb at 0.1 µg/ml followed by anti-mouse HRP at 1:1000. Detection of the p33/RNA pol II complex by the RNA pol II antibody (lane 3) demonstrates that the Co-IP was successful in maintaining the protein complex. The input HeLa extract (lane 1) was run as a control for the Western blot using 0.1 µg/ml RNA pol II.

Lane 1 Western blot control
Lane 2 Negative Control (no antibody used in IP)
Lane 3 Co-IP: IP using p33/WB using RNA pol II

Easily modify stringency

The Co-IP Kit contains high and low stringency IP buffers, as well as salt and detergent. Addition of salt and detergent is ideal for robust protein/protein interactions because it reduces background. However, because unstable complexes may not withstand high stringencies, the kit's convenient format makes it simple to modify stringency as required for each particular protein complex.

Advantages

- Simple and efficient
- Optimized extraction procedure preserves nuclear protein complexes
- Easily alter IP stringency to detect interactions of varying strengths

Order one today

The Nuclear Complex Co-IP Kit offers a simple, flexible alternative to traditional immunoprecipitation. To find out more about the Nuclear Complex Co-IP Kit, visit us at www.activemotif.com.

Multiple Tools to Study Kinase Activity

As interest in kinase activation and its effect on cellular regulation increases, so does the need for optimized tools to measure phosphorylation and the activity of kinases. In response, Active Motif has released several new active recombinant kinase proteins. In addition, the innovative FACE™ Cell-based ELISA Kits enable the detection of phosphorylated kinases directly within the cell without the need for extracts, gels or blotting.

Simplified phospho-detection

FACE™ (Fast Activated Cell-Based ELISA) Kits offer you a simple, sensitive and efficient method for monitoring kinase phosphorylation (Figure 1). They enable modification-specific analysis to be performed directly within the cell, which eliminates the need for time-consuming extract preparation and Western blotting. In addition, FACE Kits yield results that are more quantitative than Westerns, further improving your results. All FACE Kits provide both the phosphorylated and total antibody for the kinase of interest, so you can compare phospho to native protein levels in the same kit. FACE Kits are available in both Colorimetric and Chemiluminescent formats.

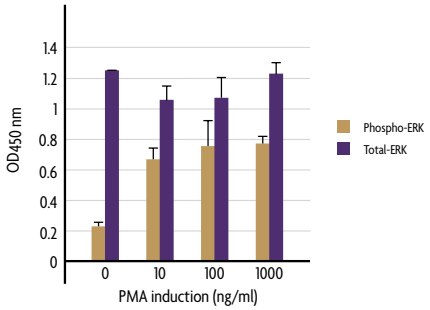


Figure 1: Colorimetric measurement of phosphorylated ERK and total ERK.
Murine Macrophage 4/4 cells were cultured in 96-well plates and serum-starved for 16 hours. Cells were then stimulated with the indicated amounts of Phorbol 12-myristate 13-acetate (PMA) for 10 minutes and fixed. Total ERK and phospho ERK were each assayed in triplicate using the phospho-ERK and total-ERK antibodies included in the FACE ERK1/2 Kit. Data was plotted after correction for cell number (performed through use of Crystal Violet). Note that the induction treatment did not affect the overall level of total ERK.

NEW - Active Recombinant Kinases			
Recombinant AKT1	Recombinant AKT2	Recombinant AKT3	Recombinant CHK2
Recombinant CK2α1	Recombinant CK2α2	Recombinant CK2β	Recombinant eIF2α
Recombinant ERK1	Recombinant ERK2	Recombinant JNK2α1	Recombinant JNK2α2
Recombinant MEK1	Recombinant MKK6 mutant	Recombinant p38α	Recombinant PKA
Recombinant PKCα			

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

How FACE works

In FACE, cells are cultured in 96-well plates and stimulated to induce the pathway of choice. Following stimulation, cells are rapidly fixed to preserve activation-specific protein modifications. Each well is then incubated with a primary antibody specific for the protein of interest. Subsequent incubation with secondary HRP-conjugated antibody and developing solution provides a colorimetric or chemiluminescent readout.

Active proteins for kinase assays

In addition to FACE, Active Motif now offers an extensive range of active recombinant kinase proteins. All of these new proteins are pre-validated for use in both Western blotting and activity assays, so you can feel confident in your results (Figure 2). Give us a call or visit us at www.activemotif.com for more details, including downloadable technical data sheets.

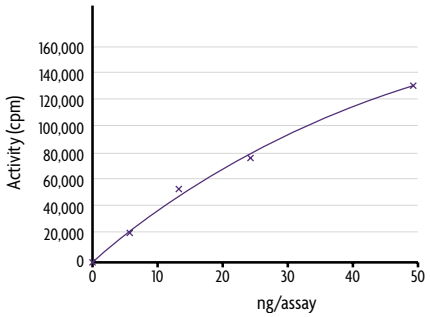


Figure 2: ERK1 Kinase Assay.
Varying amounts of the ERK1 enzyme were used in a kinase assay to phosphorylate MBP (Myelin Basic Protein).

All your kinase needs

Active Motif's broad line of products for studying both kinase phosphorylation and activity provide you with the tools you need. To learn more, please return the enclosed reply card or visit us at www.activemotif.com.

Study NFκB Binding at Any DNA Sequence

Active Motif's TransAM™ Flexi NFκB Kits provide a fast, quantitative method for profiling the binding of the NFκB Family members p65, p50, p52, c-Rel and RelB at any DNA-binding site you choose.

Flexi Kits give you options

Flexi Kits are the latest version of our original TransAM Kits, which are the method of choice for studying transcription factor binding activity. Original TransAM Kits offer a non-radioactive alternative to gelshift assays by providing a 96-well plate that is pre-coated with the consensus-binding site for the factor of interest. While this is convenient for measuring binding activity at a consensus site, it does not enable you to study alternative sites. In contrast, Flexi Kits provide all of our proven TransAM reagents (antibodies, controls and reaction buffers), but give you the flexibility to test the relative binding of NFκB at various promoters, or at mutated sites.

How do TransAM Flexi Kits work?

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

"TransAM Flexi Kits make it possible to quickly quantify the binding of NFκB family members at any binding site you choose."

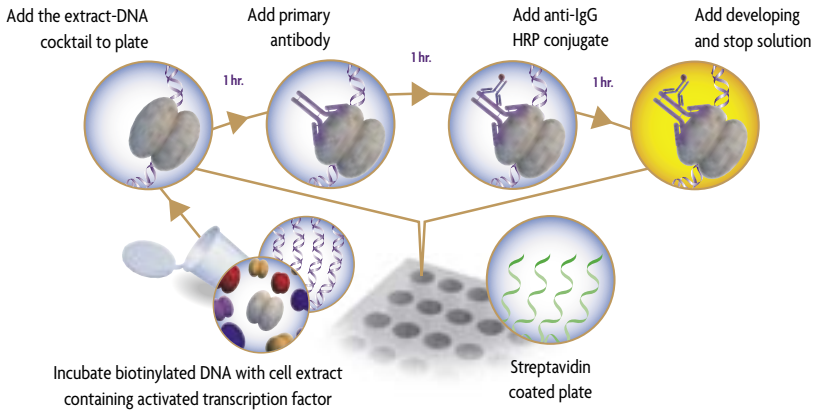


Figure 1: Flowchart of the TransAM Flexi procedure.

Product	Format	Catalog No.
TransAM™ Flexi NFκB Family	2 x 96-well plate	43298
TransAM™ Flexi NFκB p50	1 x 96-well plate	41098
TransAM™ Flexi NFκB p65	1 x 96-well plate	40098

TransAM Flexi NFκB advantages

- Monitor NFκB binding at any site
- Sensitive & quantitative
- Results in less than 5 hours
- Assay cell or tissue samples

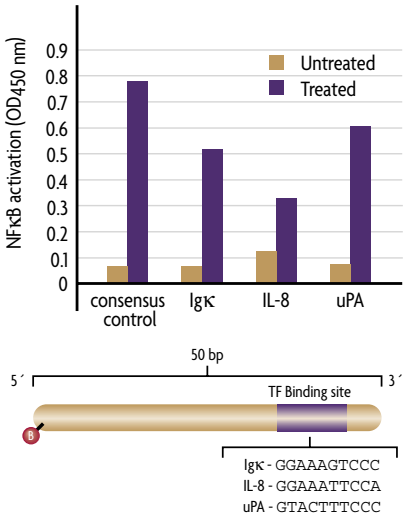


Figure 2: Measuring NFκB p65 binding at different sites. Five µg of nuclear extract from untreated and TNF-α-treated HeLa cells were used to assay the binding affinity of NFκB p65 for four different biotinylated 50-mer oligos. Each of the 3 test oligos (Igκ, IL-8 and uPA) contained the wild-type binding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

Compare binding at multiple sites

Flexi Kits make it easy to quantitate the binding of NFκB family members at any binding site. To demonstrate, three different oligos, each containing different native NFκB binding sites and known to have different affinities for activated NFκB p65, were synthesized. Following induction by TNF-α, the relative binding of activated p65 at each site was measured. The results confirm that NFκB p65 had its weakest affinity for the IL-8 promoter (Figure 2).

Get Flexi today

TransAM Flexi Kits make it fast and easy to study binding of NFκB members (p50, p65, p52, c-Rel and RelB) at any sequence you choose. For complete details, visit us at www.activemotif.com/transam.

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