MOTIF VATIONS

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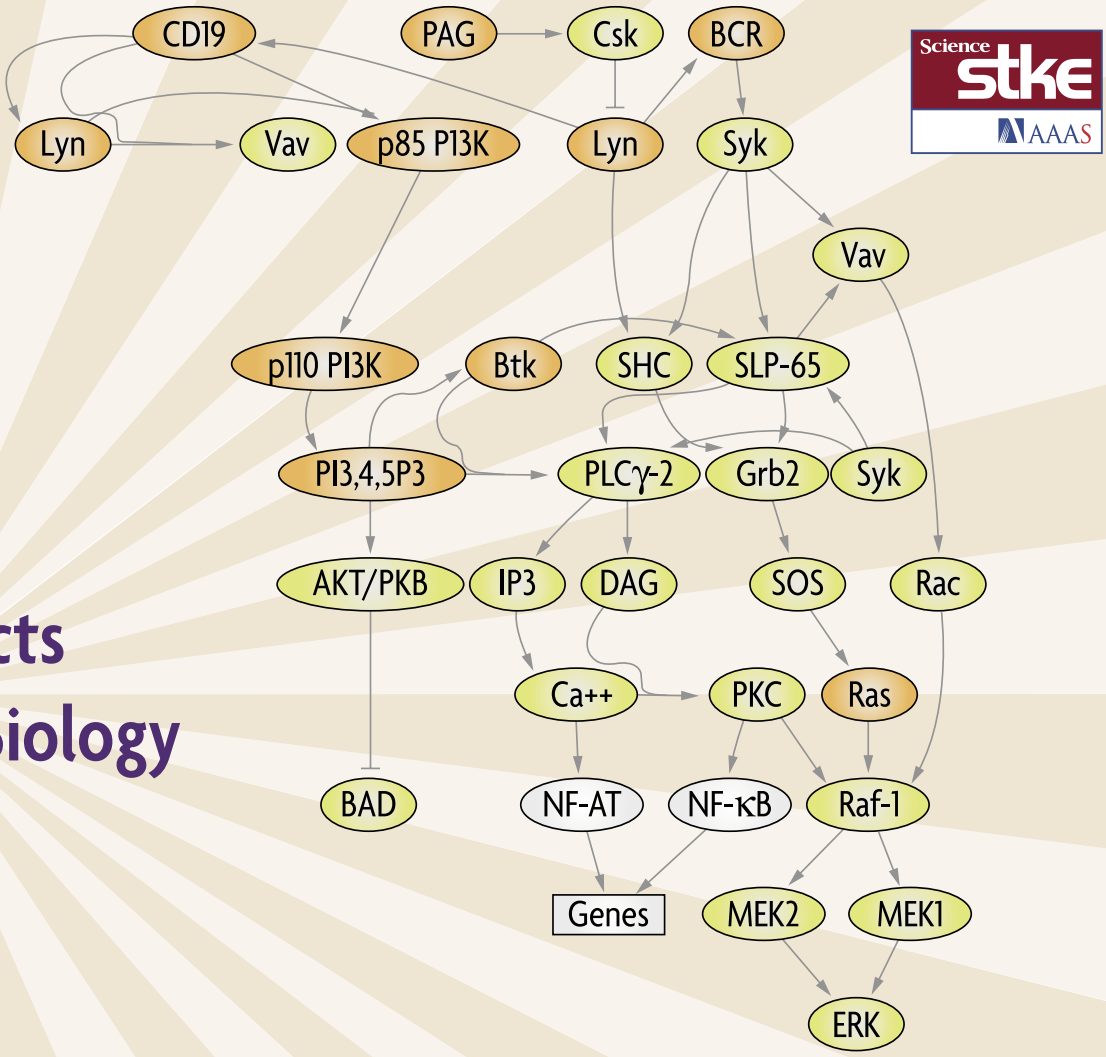
ACTIVE MOTIF®

Tools to Analyze Cellular Function

New Products for Kinase Biology



Science STKE AAAS

IN THIS ISSUE

Fast, Efficient Analysis of Protein Phosphorylation

As interest in kinase activation and their effect on cellular regulation increases, so does the need for fast, efficient and robust assays to measure protein phosphorylation. That is why Active Motif developed its Fast Activated Cell-based ELISA (FACE™) Kits. FACE Kits provide a simple, sensitive, cell-based method for monitoring protein phosphorylation.

The FACE method

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

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.

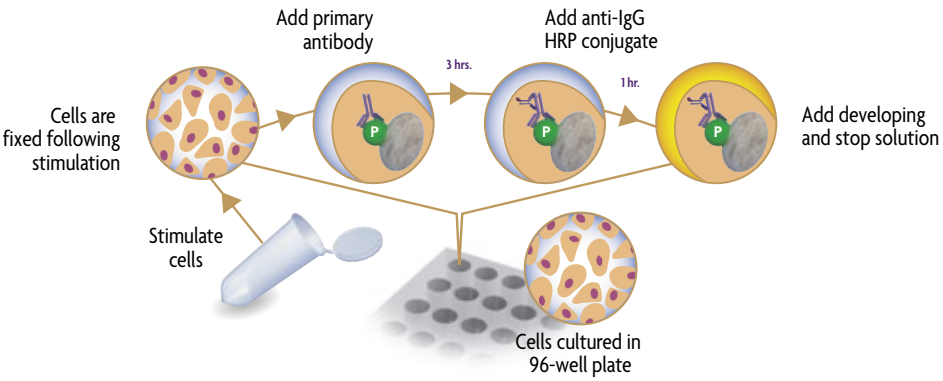


Figure 1: Flow chart of the FACE process.
Cells are grown, stimulated and fixed in the same 96-well plate. Addition of primary and secondary antibodies detects phosphorylated protein.

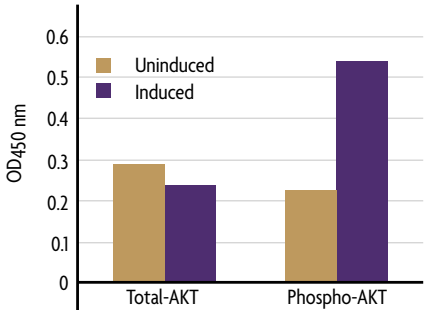


Figure 2: Measurement of phosphorylated and total AKT.
NIH/3T3 cells were cultured in 96-well plates and serum-starved for 16 hours. Cells were then treated with 50 ng/ml PDGF for 5 minutes and fixed. Total and phospho-AKT were each assayed in triplicate using the phospho and total AKT antibodies from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

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 both phosphorylated and native protein levels in the same kit
- **Quantitative results** – ELISAs provide more meaningful data than Westerns

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

High-throughput analysis

Many cellular kinase cascades contain critical control points, where multiple downstream components can be phosphorylated by a single kinase. The phosphorylation of one or other substrates can cause significant shifts in the cellular response, which means that monitoring phosphorylation events across multiple proteins can become

commonplace. However, trying to monitor this type of "signaling switch" using Western blotting or classical radioactive kinase assays is a labor intensive and expensive undertaking, especially if there are multiple treatments to be measured. To overcome this problem using FACE can be as simple as an extra pipetting step. This is because in the FACE method, cells are cultured and analyzed within the same well and therefore do not require the use of multiple culture plates, extract preparations, gels, membranes, *etc*. And, because you can easily increase sample throughput, your results will be statistically more relevant.

Eliminate unwanted sample modifications

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

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 (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™ JAK1 (Y1022/Y1023)	FACE™ JNK (T183/Y185)
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)	

NEW: Highly Active Recombinant Kinases

Active Motif provides an extensive line of recombinant transcription factor and cell signaling-related proteins. These recombinant proteins are ideal for all your research needs and can be used in a variety of applications including as probes in the new TransArray™ transcription factor protein array (see page 10) or as protein standards in ELISAs. The c-Fos, c-Jun, c-Myc, CREB, NFκB p50, NFκB p65, p53 and Sp1 proteins have been validated for use in making standard curves in our TransAM™ Transcription Factor ELISAs (see page 5). Complete information on the recombinant proteins, including detailed technical data sheets that specify the protein length, the method used for purification, *etc*. can be found at www.activemotif.com.

Recombinant Kinase & Transcription Factor Product Line					
ABL	AKT	ATF	B-RAF	BRCA	BRK
AP-1	c-Myc	CHK	CK2	CREB	CSK
CTF1	EGFR	eIF2	ER	ErbB	ERK
FAK	FGF	FGR	FXR	GR	GSK3
HCK	IGF1-R	IκBα	IKK	INS-R	IRAK
ITK	JAK	JNK	LCK	LXR	Lyn
MAPKAPK	MEK	MKK	NFκB	p38	p53
p300	PAK	PDGFR	PDK	PKA	PKC
PPAR	PXR	Rad	RAR	pRb	RXR
S6K	SNK	Sp1	Src	STAT	SYK
TR	VEGF-R	VRK	WEE	Yes	ZAP70

NEW: Colorimetric Quantitation of Activated Kinases

Active Motif's KineActive™ ELISAs improve the study of highly conserved members of kinase families in cell and tissue extracts by providing unmatched sensitivity and specificity in a non-radioactive, 96-well format.

Specific kinase detection

A major limitation when using traditional kinase assays is that many kinase families, such as Src, contain members that phosphorylate a common target. Therefore, to accurately measure the activity of a specific kinase, it is necessary to isolate it from others that can act upon the same peptide substrate. The high specificity of the KineActive Kits enable the study of conserved kinase families whose activity is otherwise difficult to measure accurately.

"KineActive provides rapid, specific detection of activated kinases in a 96-well format."

The KineActive method

In the KineActive ELISA method, a cellular extract or recombinant kinase is added to the wells of the provided 96-well, antibody-capture plate. This is followed by addition of a kinase-specific antibody, which binds to the plate and captures the kinase of interest. After the unbound proteins are washed away, a biotinylated, unphosphorylated peptide substrate is added in the presence of ATP. The immobilized kinase phosphorylates this substrate peptide, which is then captured by the addition of a second antibody that is specific for the phosphorylated form of the peptide. Addition of streptavidin-HRP and subsequent developing solution provides a quantitative colorimetric readout that is directly proportional to the activity of the kinase (Figure 1).

Advantages

- Quantitative
- Unmatched specificity
- Non-radioactive detection
- High throughput compatible
- Improved sensitivity
- Assay cell and tissue extracts or purified protein

Improved sensitivity

KineActive offers improved sensitivity over other methods, which enables you to choose from a variety of sample sources including purified protein or cell and tissue extracts. For example, while traditional immunoprecipitation-based radioactive kinase assays may require several hundred micrograms of whole-cell extract in order to detect Src activity, KineActive Src can measure Src activity using less than 20 µg of extract (Figure 2). Try KineActive today to simplify your kinase biology research.

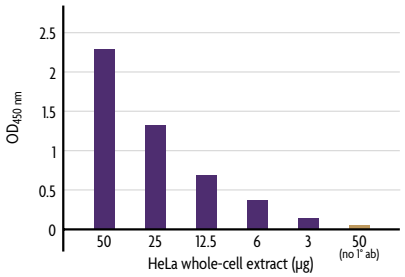


Figure 2: Sensitive detection of activated Src in HeLa cells using KineActive.
HeLa whole-cell extract was diluted down to 3 µg per well and assayed using the KineActive Src ELISA Kit.

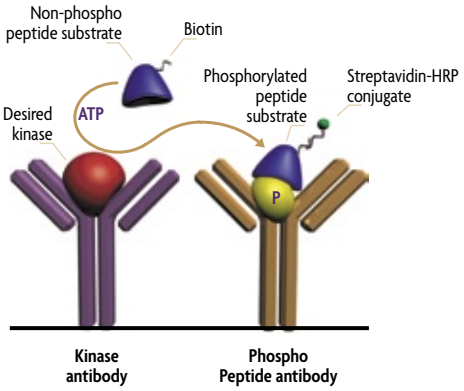


Figure 1: Flow chart of KineActive ELISA process.
In KineActive, sample containing the desired kinase is added to a 96-well antibody capture plate. This is followed by addition of kinase antibody, which binds to the plate and specifically captures the kinase of interest. After washing, a biotinylated, non-phosphorylated peptide substrate is added in the presence of ATP. The immobilized kinase phosphorylates this peptide, which is captured by addition of an antibody against the phosphorylated peptide. Addition of Streptavidin-HRP and developing solution provides a quantitative colorimetric readout.

Product	Format	Catalog No.
KineActive™ Lyn	1 x 96 rxns	56096
KineActive™ Src	1 x 96 rxns	56196
KineActive™ Yes	1 x 96 rxns	56296

Sensitive, Specific Transcription Factor ELISAs

Active Motif's TransAM™ Kits simplify the study of activated transcription factors by combining high sensitivity with convenience in a non-radioactive, ELISA-based assay.

Traditionally, transcription factor activity has been studied using either Electrophoretic Mobility Shift Assays (EMSA), immunoblotting or reporter gene assays. However, these methods are quite time-consuming and, at best, provide only semi-quantitative results. Moreover, use of these methods to determine the activity of multiple transcription factor family members would require the investment of significant amounts of both time and money; in many cases, it may not even be possible.

Simple, quantitative assay

In contrast, Active Motif's TransAM Kits make it straightforward to measure the activity of transcription factors. You can even study multiple members of a transcription factor family in a single experiment that is complete in less than 5 hours. In addition to being fast, the TransAM method is non-radioactive and provides quantitative results (Figure 1). Plus, TransAM Kits can be used on all sample types, including cell lines and tissues, giving you unsurpassed flexibility.

"TransAM Kits are sensitive, non-radioactive DNA-binding ELISAs that facilitate the study of transcription factor activation in cell and tissue samples."

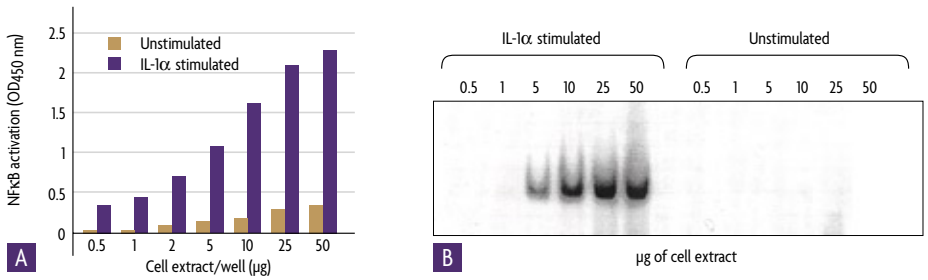


Figure 1: TransAM NFκB is more sensitive than gelshift, and provides more quantitative results.
Human fibroblast WI-38 cells are stimulated with IL-1α for 30 minutes. Increasing amounts of whole-cell extract are assayed using either the TransAM NFκB p50 Kit (A) or gel retardation (B).

Flexi or Original format

The Original TransAM Kits offer a fast, non-radioactive alternative to gelshift by providing a 96-well plate that is precoated with oligonucleotide that contains a consensus-binding site for the factor of interest. Activated transcription factor binds the oligo and is quantified on a spectrophotometer using the supplied antibodies and developing reagent. This format is convenient for measuring binding at the consensus site, but does not enable you to study alternative sites. For this reason, we developed TransAM Flexi Kits; they contain all of the optimized reagents provided in the Original kits, including antibodies, but give you the flexibility to immobilize any oligo in the 96-well plate.

TransAM advantages

- Non-radioactive, colorimetric method provides quantifiable results
- Results in less than 5 hours
- 10-fold greater sensitivity than gelshift (100-fold with Chemi Kits)
- Simultaneous profiling of multiple family members
- Assay both cell and tissue samples

Try sensitive, quantitative ELISAs

TransAM Kits make it simple to quantify activated transcription factors. Please give us a call or visit our website to learn more about the TransAM product line.

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™ IRF Family	TransAM™ AP-1 c-Jun	TransAM™ ER	TransAM™ MyoD	TransAM™ p53
TransAM™ MAPK Family	TransAM™ AP-1 FosB	TransAM™ FKHR (FOXO1)	TransAM™ NF-YA	TransAM™ PPARγ
TransAM™ Flexi NFκB Family	TransAM™ AP-1 JunD	TransAM™ GATA-4	TransAM™ NFATc1	TransAM™ Sp1 & Sp1/Sp3
TransAM™ NFκB Family	TransAM™ ATF-2	TransAM™ GR	TransAM™ Flexi NFκB p50	TransAM™ STAT3
TransAM™ STAT Family	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.

Use ChIP-IT™ for More Successful Chromatin Immunoprecipitation

Chromatin immunoprecipitation (ChIP) is a powerful, yet technically challenging tool used to study protein/DNA interactions. Active Motif's ChIP-IT™ Kits make your ChIP more successful by combining nearly everything you need in a single kit, including positive control antibodies and primers, DNA purification columns and a comprehensive protocol.

The ChIP method

In ChIP, intact cells are fixed using formaldehyde, which cross-links and preserves protein/DNA interactions. The DNA is then sheared into small, uniform fragments. The protein/DNA complexes are then immunoprecipitated using antibodies directed against the DNA-binding protein(s) of interest. Following immunoprecipitation, the cross-links are reversed and DNA fragments are purified and screened to determine which gene, or group of genes, was bound by the protein of interest (Figure 1).

ChIP-IT advantages

- Easy to use – all critical buffers, Protein G beads and DNA purification columns are provided
- No need to optimize reagents and protocol
- Your choice of enzymatic or sonication shearing
- Direct measurement of transcription factor/DNA interactions or histone modifications

Choose enzymatic or sonication shearing

The key to a successful ChIP experiment begins with correctly shearing the DNA into 200-1000 bp fragments. This can be achieved by using one of two methods: enzymatic digestion or sonication. Sonication shearing is an effective method for shearing DNA but can be difficult to optimize due to complications from overheating, emulsification and dependence on sonicator type. In contrast, enzymatic digestion can be used to quickly and easily digest DNA into fragments suitable for ChIP. And, as digestion is dependent only on time and temperature, very little optimization is required. For your convenience, ChIP-IT Kits are available with your choice of either sonication or enzymatic shearing.

Start your ChIP today

Active Motif's ChIP-IT Kits provide you with everything you need to make your ChIP experiments easier and more reproducible. For more information on ChIP-IT visit www.activemotif.com/chip.

"ChIP-IT makes performing ChIP with transcription factors more successful by combining all of the critical components you need in a single kit."

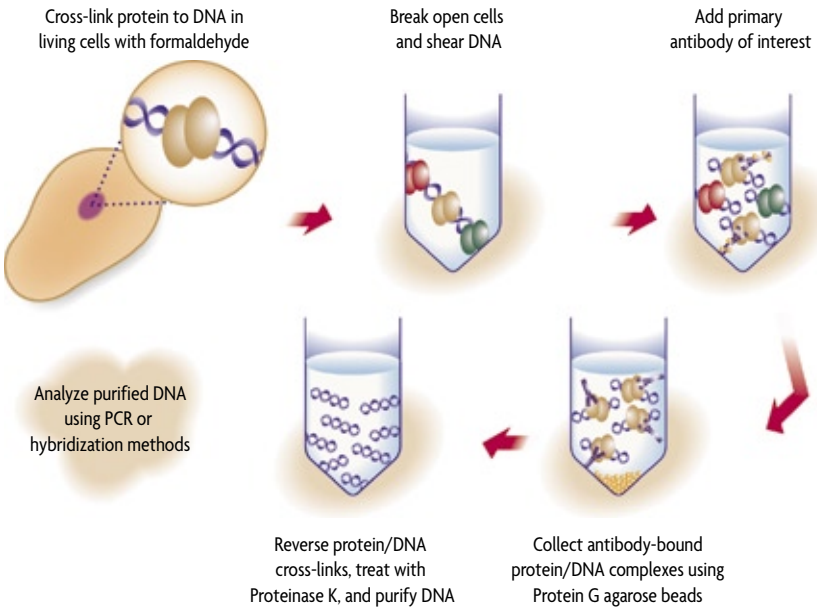


Figure 1: Flow chart of chromatin immunoprecipitation.
In ChIP, protein/DNA interactions are fixed, and the DNA is sheared and precipitated using an antibody. After cross-link reversal, the DNA is purified and screened to determine which genes were bound by the protein of interest.

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

NEW: Rapid DNA Methylation Detection

Active Motif's MethylDetector™ Bisulfite Modification Kit simplifies analysis of DNA methylation. The kit provides optimized reagents for performing DNA conversion with bisulfite, plus time-saving DNA purification columns and positive control PCR primers to validate your results.

Methylation is important

DNA methylation is a naturally occurring event that affects cell function by altering gene expression. As aberrant methylation is prevalent in many human cancers, much research depends on accurately quantifying DNA methylation.

Proven controls ensure success

Many DNA methylation analysis methods begin by using bisulfite to convert unmethylated cytosines to uracils. During conversion, methylated cytosines remain unchanged. The DNA is then amplified by PCR and analyzed by sequencing or restriction digest. However, bisulfite conversion can be technically challenging, and it is desirable to confirm that the process was successful before spending time and money on sample analysis. To help ensure your success, the MethylDetector Kit provides optimized conversion reagents, an easy-to-use protocol and positive control PCR primers that are specific for bisulfite-converted DNA. Because these primers produce PCR product only if conversion has occurred, you can confirm the procedure worked before starting sequencing or other analysis methods (Figure 1).

"MethylDetector improves DNA methylation analysis by providing optimized reagents and proven controls."

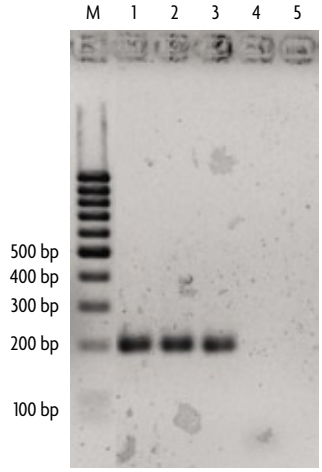


Figure 1: Agarose gel analysis of PCR products generated with MethylDetector.
Three different DNA conversions were performed (Lanes: 1-3) and compared to an unconverted DNA control (Lane 5) and to a no DNA control (Lane 4). The presence of PCR product in only the converted samples demonstrates the conversion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

Advantages

- Works efficiently with high G/C content sequences and uncut DNA
- Reproducible assay consistently provides 99% conversion efficiency
- Optimized reagents and protocol with proven controls
- Combined thermal denaturation and conversion reaction streamlines procedure
- DNA purification columns eliminate precipitation and a separate desulfonation step
- High yield of converted DNA is ideal for downstream analysis

The MethylDetector method

In the MethylDetector method, DNA of interest is rapidly heat denatured in a thermocycler in the presence of the bisulfite conversion reagent. The temperature is then lowered and the conversion reaction is performed. Unlike other methods, MethylDetector does not require an initial acid denaturation step as the conversion reagent includes a DNA denaturant, which saves you time and effort. After DNA conversion, the sample is added to the included DNA purification columns, and a simple, on-column desulfonation is performed. Ready-to-use DNA is then eluted from the columns (Figure 2).

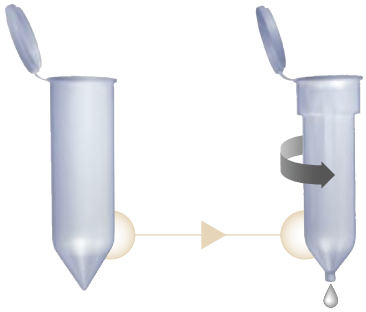


Figure 2: Flow chart of the MethylDetector process.

Order one today

The MethylDetector Kit will improve your bisulfite conversions. Find more information about this new innovative kit from Active Motif by giving us a call or visiting www.activemotif.com.

Product	Format	Catalog No.
MethylDetector™	50 rxns	55001

NEW: Fluorescent Detection Products

Active Motif is pleased to announce the launch of the new Active Motif Chromeon fluorescent detection product line. This new product range offers innovative fluorescent tools using the proprietary fluorescent dyes and substrates developed at Chromeon GmbH.

The Chromeon advantage

The dyes developed at Active Motif Chromeon exhibit superior luminescence properties, including a broad range of fluorescence excitation and emission spectrums, large Stokes shifts, limited photobleaching and pH stability. These features enable the development of a versatile platform for novel fluorescent assays such as the new ProStain™ Protein Quantification Kit (see page 9), CE Dyes for capillary electrophoresis and the Albumin Blue Fluorescent Assay Kit. These products and the Chromeo™ Dyes represent only a small fraction of dyes available from Active Motif Chromeon. To find more information about other fluorescent dyes, visit www.chromeon.com.

Albumin Quantitation Kit

Serum albumin is a reliable prognostic indicator for morbidity and mortality, liver disease, kidney disease and malnutrition. The new Albumin Blue Fluorescent Assay Kit from Active Motif Chromeon is a quantitative assay designed to measure albumin levels in biological samples such as serum and urine. The kit combines unmatched sensitivity, high specificity and a quick and easy protocol. Learn more at www.activemotif.com.

Chromeo Dyes

Fluorescent labels for bioanalysis

The Chromeo Dyes – Chromeo 494, Chromeo 546 and Chromeo 642 – provide unmatched photostability, which enables multiple exposures and increased exposure time. They are compatible with most excitation sources including diode lasers, LEDs, tungsten lamps and xenon arc lamps (Table 1). In addition, Chromeo Dyes display a large tolerance to pH. Their luminescent properties together with their low toxic effects make Chromeo Dyes ideal for use as labels of biomolecules in cell culture staining experiments. Chromeo Dyes are available as amine reactive NHS-Esters, used for conjugation with amino-groups in peptides, proteins and amino-modified DNA, or as carboxylic acids.

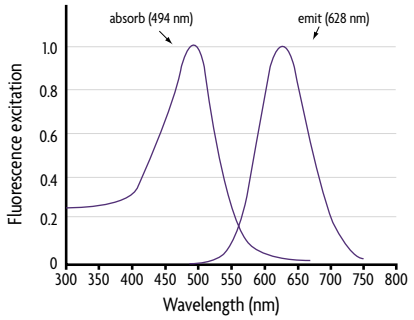


Figure 1: Chromeo 494 enables fluorescent multiplexing. Chromeo 494 is excited at 488 nm. With its large Stokes shift of 124 nm, Chromeo 494 is the ideal partner for multiplexing with other, short-shifted 488-excitable dyes.

Dye	Absorption	Emission	e L/(mol-cm)	Quantum Yield (%)	Stokes Shift
Chromeo™ 494	494	628	20,000	25	124 nm
Chromeo™ 546	545	561	110,000	8	16 nm
Chromeo™ 642	642	660	170,000	17	18 nm

Table 1: Chromeo Dye properties.

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™ 546 Carboxylic Acid	1 mg 5 mg	15210 16210
Chromeo™ 546 NHS-Ester	1 mg 5 mg	15211 16211
Chromeo™ 642 Carboxylic Acid	1 mg 5 mg	15310 16310
Chromeo™ 642 NHS-Ester	1 mg 5 mg	15311 16311
Albumin Blue Fluorescent Assay Kit	1 kit	15002

You can find additional Chromeon Dyes at www.chromeon.com

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.

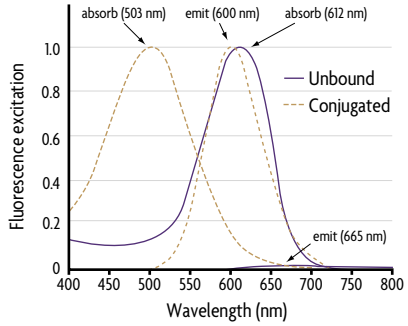


Figure 1: Absorption/emission spectra of free/bound dye. Normalized absorption and emission spectra of free (solid lines) and conjugated dye (dotted lines) in phosphate buffer of pH 7.2.

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.

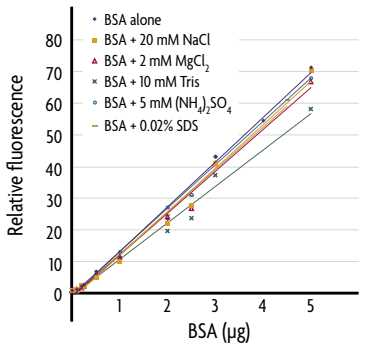


Figure 2: Standard Curves of BSA. Increasing amounts of BSA protein were quantified using the Fluorescent Protein Quantification Kit in the presence of a variety of contaminants.

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 sample 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

NEW: Transcription Factor Protein Arrays

Active Motif's new line of TransArray™ TF Protein Arrays make it possible to simultaneously study 38 activated transcription factors in a fast and easy-to-use format.

Multiplexed detection

Post-transcriptional regulation and translational modifications can produce dramatic changes in protein levels and activity that are not detectable in DNA arrays. This makes identifying protein: protein and protein:DNA interactions in a high-throughput format an enormous challenge as current assays enable only a single protein to be characterized at a time. In contrast, the new TransArray Kits enable high-throughput characterization of multiple transcription factor proteins.

Why use TransArray?

- Simultaneously measure 38 activated transcription factors (see Table 1)
- Highly specific
- Quantitative detection
- Simple alternative to pull-down assays, yeast two-hybrids and Co-IPs
- Study protein:protein or protein: DNA interactions

The TransArray method

Each array consists of highly active purified proteins that are spotted in triplicate on a nitrocellulose membrane. A protein, DNA or ligand of interest is then used as a probe to search for interactions with the immobilized proteins. These interactions are assessed either by tagging the probe with a small molecule, by radioactivity or through using an antibody to the probe (Figure 1). Currently, the signals are visualized by radioactive detection using photographic film or by a phosphor imager.

"TransArray enables multiplexed analysis of transcription factor interactions with both DNA and proteins "

	A	B	C	D	E
1	STAT3	Rap74	hRPB5	c-Fos	BRG-1 Mutated
2	STAT1	Rap30	Negative	c-Jun	PC4
3	BRCA1	TFIID	Sp1	AP-1	PC4 F77P
4	TFIIA-p55	TBP	CTF-1	DR1	p52
5	TFIIA-p12	TFIIH	E2F-1	HMG-1	p75
6	TFIIB	TFIIH-p62	pRB	TOPO WT	p75-CTD
7	TFIIEα-p56	RNA pol II	p53	TOPO Y723F	p300
8	TFIIEβ-p34	RNA pol II CTD	c-Myc	BRG-1 WT	Negative

Table 1: Activated transcription targets included in the TransArray™ TF Protein Arrays

Product	Format	Catalog No.
TransArray™ TF membrane	2 arrays	31400

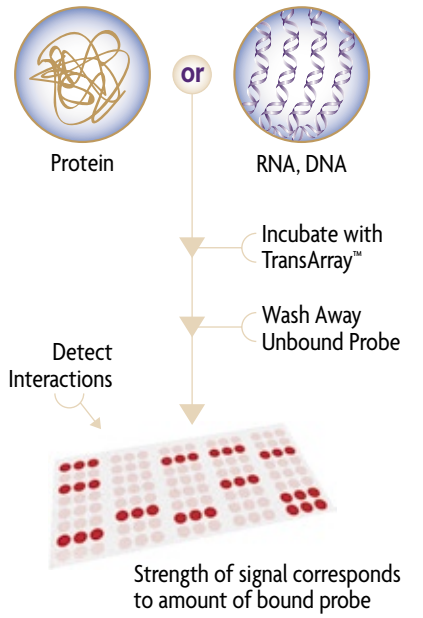


Figure 1: Flow chart of the TransArray process.

Try TransArray today

The innovative TransArray format enables multiplexed detection and interaction assessment of transcription factor proteins with DNA, RNA and/or ligands of interest. For your convenience, Active Motif also offers a full line of highly active recombinant proteins (see page 3) that are ideal for use as protein probes in TransArray. To find more information about these new protein arrays, visit www.activemotif.com.

NEW: Learn More Faster with AlmaKnowledgeServer

AlmaKnowledgeServer (AKS) is a powerful text mining system that makes it possible for you to uncover relationships that exist in the scientific literature between genes, proteins, chemical compounds and diseases. AKS can even discover links that exist, but that have not yet been published.

A new way to keep pace

Researchers read journal articles to learn about new developments in their field. But, with 1000's of journals and more than 15 million abstracts on Medline (plus over 2,000 more added daily), it is physically impossible to keep current, let alone to explore all of the areas that may provide new and useful insights.

The answer is AlmaKnowledgeServer (AKS), a powerful text mining system that, unlike you, reads every paper every day. AKS examines all abstracts added to PubMed, then applies statistics and rules-based analysis to uncover relationships that exist in the scientific literature between genes, proteins, chemical compounds and diseases. This enables you to make better informed decisions about your research in far less time.

Bioentity and synonym recognition

AKS understands and retrieves biological concepts (bioentities) instead of simple words. The bioentities recognized by AKS include genes and proteins, diseases and chemical compounds. AKS has been taught the many synonyms, acronyms and other scientific naming ambiguities that plague traditional searching. For example, searching PubMed for IL-2, IL2, IL 2 or interleukin 2 return different results, though all are the same gene. So, an exhaustive search for a single gene may actually require 5-10 individual searches. With AKS, one search returns all documents that are relevant to IL-2.

Search first, then analyze

After a search, AKS ranks the relevant concepts and bioentities. Documents can be explored by viewing sentences in which two or more bioentities occur (Figure 1). By reading the top ranking sentences, it's possible to build a significant understanding of the interrelations between bioentities. You can also read abstracts with co-occurring bioentities highlighted, and apply filters to fine tune your results. AKS also provides links to Medline, so you can quickly access the most essential papers.

Visualize biological relationships

Because new insights often come from exploring data from different perspectives, AKS also provides a graphical view of the relationships between bioentities (Figure 2). The links between terms represent documents or sentences where multiple entities co-occur. Though this visual representation may contain the content of thousands of documents, it can be interpreted quickly and easily, helping you to obtain new insights.

Achieve more with better information

AKS can help answer questions that are important to your research, like if a given protein is involved in a disease, or who are the thought leaders in an emerging field? To learn more, give us a call or visit Active Motif's biocomputing division, TimeLogic, at www.timelogic.com.

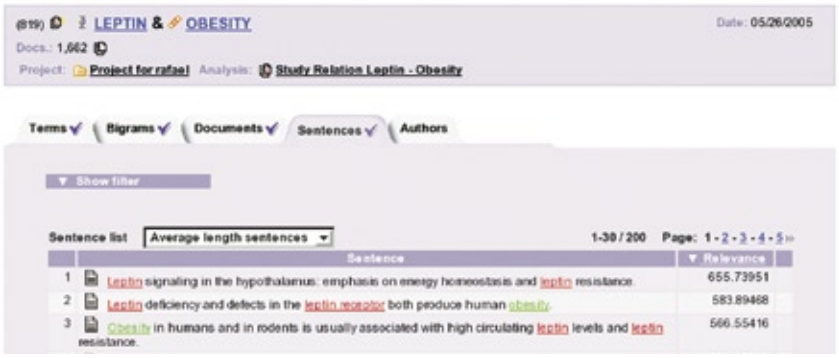


Figure 1: Sentences displaying co-occurrences of "leptin" and "obesity" bioentities.

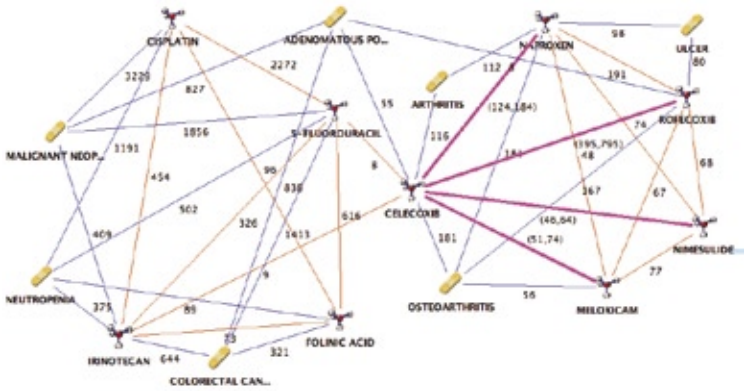


Figure 2: AKS includes a powerful visual environment to map relationships between bioentities.

Improved Co-IP of Nuclear Protein Complexes

Using the Nuclear Complex Co-IP Kit from Active Motif will improve your co-immunoprecipitation studies of nuclear protein complexes because it provides optimized reagents for both nuclear extract preparation and immunoprecipitation.

Optimized method and reagents

Co-immunoprecipitation (Co-IP) is often used to analyze protein/protein interactions. However, traditional Co-IP methods are not optimal for studying DNA-binding protein complexes as these complexes are unstable. The Nuclear Complex Co-IP Kit helps maintain these complexes by providing both high and low stringency buffers, as well as additional salt and detergent. Addition of salt and detergent is ideal for robust protein interactions as it reduces background. However, many complexes may not withstand high stringencies, thus stringency can be modified as required.

Advantages

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

Try the new Co-IP Kit today

The Nuclear Complex Co-IP Kit enables you to study tightly bound or weak protein complexes with equal ease, which improves your results. To get more information on this innovative product please give us a call or visit our website at www.activemotif.com.

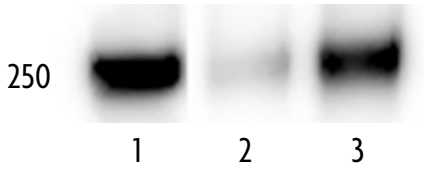


Figure 1: Western blot analysis of the IP'd p33 subunit of the RNA pol II complex.
HeLa cells were grown to confluence on 100 mm plates and nuclear extracts were prepared using the kit's extraction reagents. For IP experiments, the stringency of the IP High Buffer was increased by supplementing with 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

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