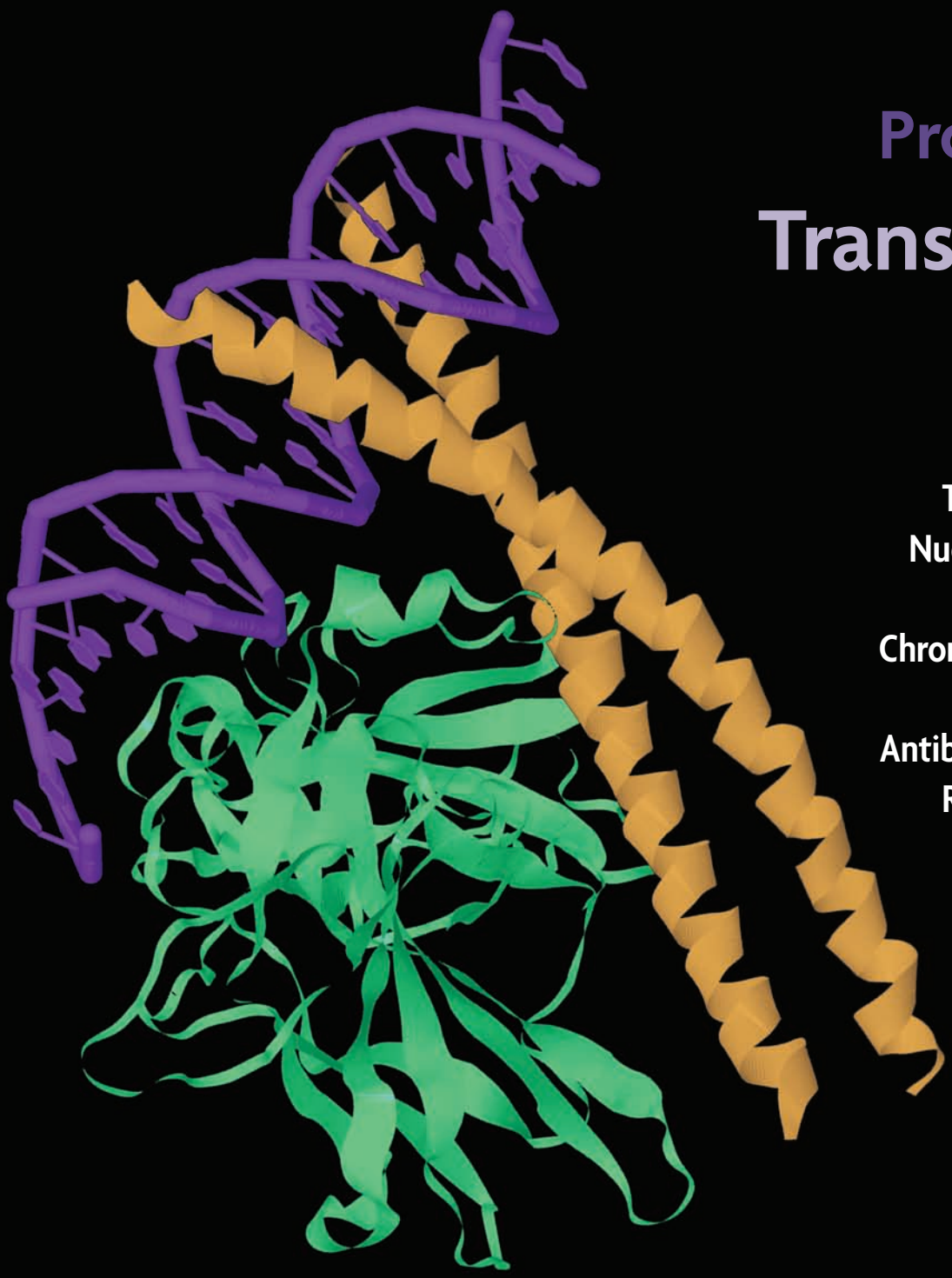

Products for Transcription Biology

Transcription Factor & Nuclear Receptor ELISAs

Chromatin Assays & IP Kits

Antibodies, Cell Extracts & Recombinant Proteins

Luciferase Assays

Supershift & Gelshift

ACTIVE MOTIF®

Tools to Analyze Nuclear Function

Active Motif Offices

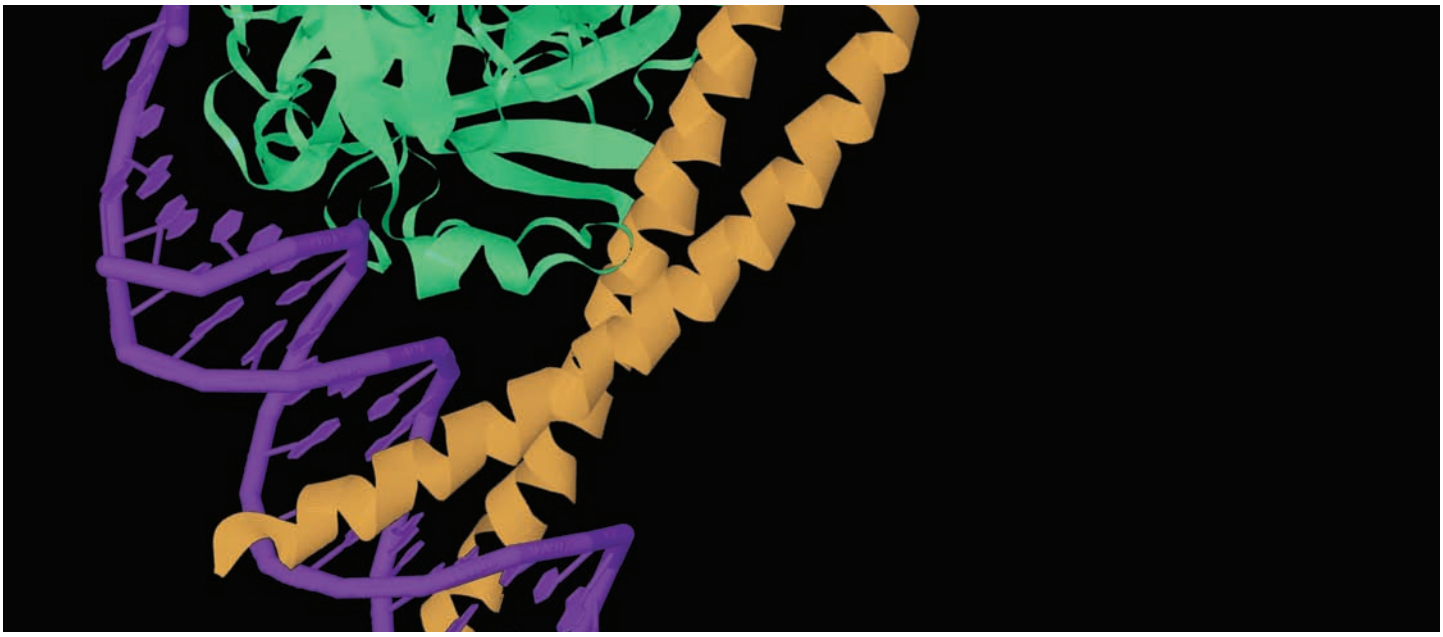

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Table of Contents

On the Cover

Crystal structure of NFAT1 (green) and Fos-Jun (yellow) on the IL-2 ARRE1 (A-T–rich purine-box/antigen receptor response element) (purple) binding site.

TransAM®

sensitive, non-radioactive transcription factor ELISAs

TransAM® Kits are highly sensitive ELISA-based assays* that facilitate the study of transcription factor activation in mammalian tissue and cell extracts. They combine a fast, user-friendly format with a sensitive, specific assay. The standard TransAM Kits are 10-fold more sensitive than conventional gelshift techniques (Figure 2), while TransAM Chemi Kits utilize chemiluminescent detection to improve assay sensitivity to only 40 ng of extract. The TransAM method eliminates the use of radioactivity and provides quantitative results in less than five hours, while its high-throughput format offers the flexibility to screen up to 96 samples simultaneously.

Until now, transcription factors have been studied using primarily three methods: gelshift assays, immunoblotting and reporter gene assays. These methods are time consuming and provide, at best, only semi-quantitative results. Moreover, they don't support high-throughput methods and lack sensitivity, specificity and reproducibility. The TransAM technology eliminates these shortcomings, making transcription factor research faster and more precise.

* TransAM is licensed from EAT under issued and pending worldwide patents. Purchase includes the right to use for basic research only. Other-use licenses available, please contact Technical Services.

The TransAM® advantage

TransAM Kits offer a novel method for rapid and sensitive quantitative measurement of transcription factor activation. TransAM Kits are available in the original format that contains a 96-well plate to which an immobilized oligonucleotide containing a consensus-binding site has been immobilized or in a Flexi format that enables you to bind any oligonucleotide to the plate. In both formats, cell extract is added to each well and the transcription factor of interest binds specifically to the oligonucleotide that is immobilized on the plate. Each well is then incubated with primary antibody specific for the active form of the bound transcription factor. Subsequent incubation with an HRP-conjugated secondary antibody and either the standard or chemiluminescent Developing Solution provides an easily quantified readout (Figure 1).

TransAM Kits are available for study of a variety of individual transcription factors and transcription factor families. TransAM eliminates the need for time consuming cloning, cell transfections, and gel exposures. The use of messy radioactive probes is also unnecessary. There is also no need for constructing stable reporter cell lines, or for dealing with the variability in expression of transfected reporter genes.

WHY USE TRANSAM®?

- Up to 100-fold greater sensitivity than gelshift assays
- Results in less than 5 hours
- Non-radioactive, colorimetric readout easily quantified by spectrophotometry
- No cloning or cell transfections required
- Ability to assay tissue samples
- 96-stripwell format is compatible with high-throughput automation

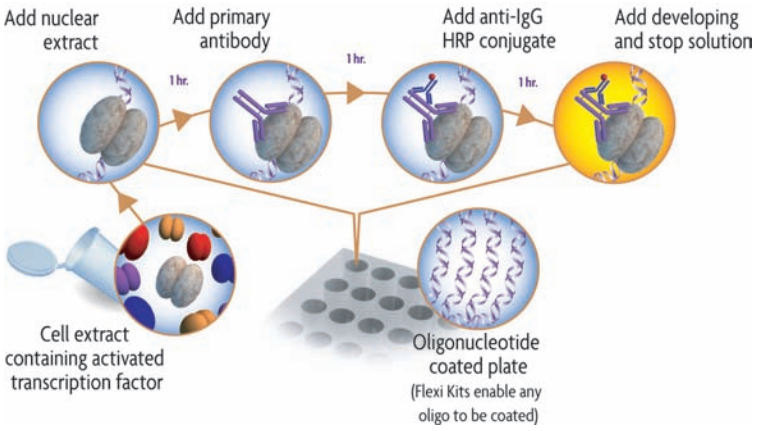

FIGURE 1:
Flow chart of the TransAM process.
Activated transcription factor in cell extract binds to oligonucleotides immobilized in the well. Incubation with primary and secondary antibodies quantifies the amount of activated transcription factor in a sample.

Increased sensitivity & accuracy

Small changes in transcription factor levels can have a significant impact on cellular function. Therefore, it is important to use a sensitive assay when studying transcription factor activation. TransAM Kits are 10-fold more sensitive than gelshift assays and also yield more quantitative results. To illustrate, NFκB p50 activity was assayed using the TransAM NFκB p50 Kit and EMSA (Figure 2). These data clearly demonstrates that the DNA binding ELISA format of the TransAM Kits yields more sensitive and quantitative results than traditional assays such as EMSA.

In addition, TransAM Kits provide more accurate measurements than traditional assays because of their broad linear range of detection. This eliminates the need to run multiple assays to obtain accurate results. Both the increased sensitivity and accuracy of the TransAM Kits will drastically improve your transcription factor analysis. The combination of a fast, user-friendly format with high sensitivity and specificity is validated by over 900 journal citations (Figure 4).

Proven specificity

To accurately study transcription factor activation, it is vital to be able to determine which isoform of the transcription factor of interest is involved in pathway regulation. All TransAM Kits are tested for specificity by performing the assays in the presence of an excess of oligonucleotide containing a wild-type or mutated consensus-binding site (Figure 3). This competitive assay proves that the transcription factor detected is binding specifically to the probe that has been immobilized to the TransAM plate. All TransAM antibodies are also assayed for cross-reactivity with other closely related family members to ensure that only the isoform of interest is detected.

Applications of TransAM® Kits

TransAM Kits have unsurpassed flexibility, which is why they have been used successfully with both primary and transformed cell lines as well as tissue samples. Common applications include:

- Drug screening and/or potency studies
- Monitoring of transcriptional regulation activity
- Protein structure/function studies

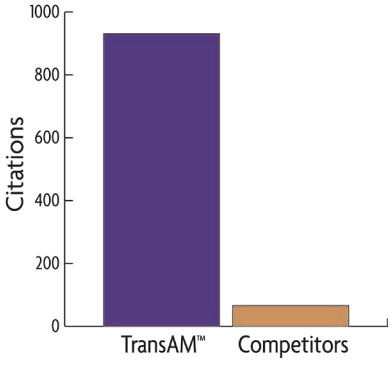

FIGURE 4:
TransAM citations.
Using HighWire Press, http://highwire.stanford.edu, a comparison was made by searching for citations of TransAM from Active Motif versus the tradenames of all competitor kits combined. TransAM is clearly the leader.

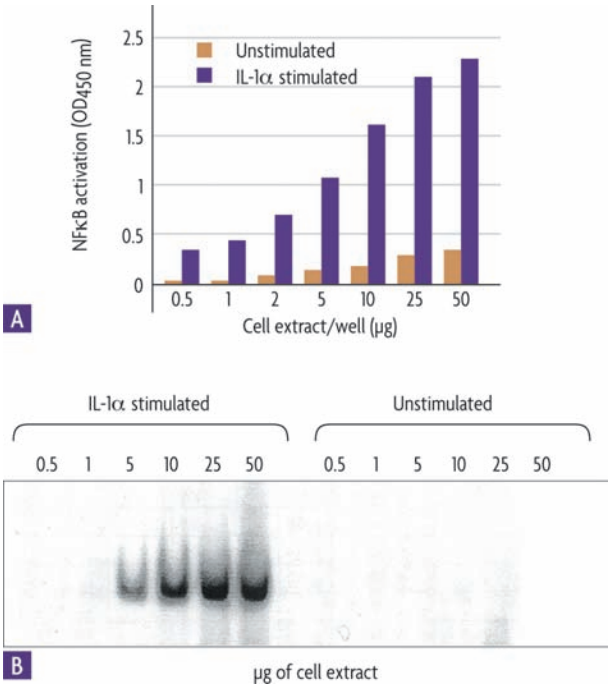

FIGURE 2:
TransAM Kits are more sensitive than gelshift.
Human fibroblast WI-38 cells are stimulated with IL-1α for 30 minutes. Increasing amounts of whole-cell extract are assayed using the TransAM NFκB p50 Kit (A) or gel retardation (B). The TransAM method is 10-fold more sensitive and provides more quantitative data.

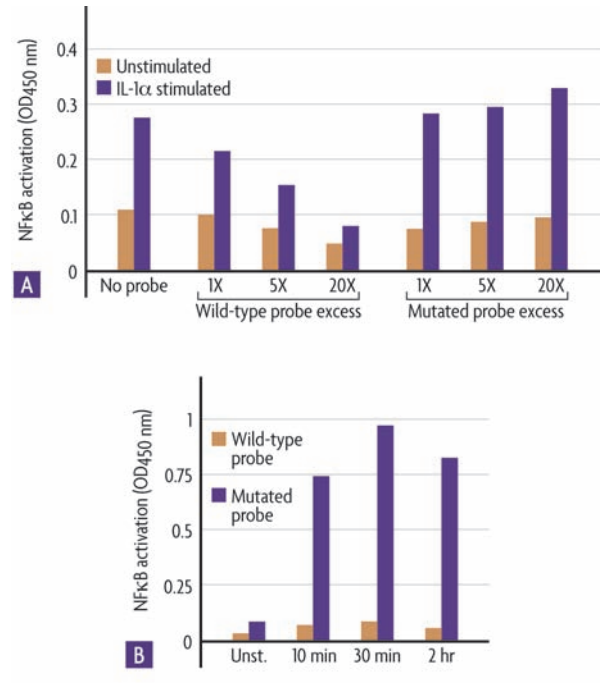

FIGURE 3:
Specificity of TransAM Kits.
TransAM NFκB p50 Kit assays are performed in the presence of wild-type and mutated competitor oligonucleotides using 10 µg/well whole-cell extract from human fibroblast WI-38 cells stimulated with IL-1α for 30 minutes (A) and 5 µg/well whole-cell extract from HeLa cells stimulated with TNF-α for 10 minutes, 30 minutes and 2 hours (B).

TransAM® Ordering Information

FACTOR	PRODUCT	FORMAT	Cat. No.
AML/Runx	TransAM® AML-1/Runx1	1 x 96-well plate	47396
		5 x 96-well plates	47896
	TransAM® AML-3/Runx2	1 x 96-well plate	44496
		5 x 96-well plates	44996
AP-1	TransAM® AP-1 Family (c-Fos, FosB, Fra-1, Fra-2, c-Jun, JunB & JunD)	2 x 96-well plates	44296
	TransAM® AP-1 c-Fos	1 x 96-well plate	44096
		5 x 96-well plates	44596
	Recombinant c-Fos protein	5 µg	31115
	TransAM® AP-1 c-Jun	1 x 96-well plate	46096
		5 x 96-well plates	46596
	Recombinant c-Jun protein	5 µg	31116
	TransAM® AP-1 FosB	1 x 96-well plate	45096
		5 x 96-well plates	45596
	TransAM® AP-1 JunD	1 x 96-well plate	43496
		5 x 96-well plates	43996
ATF-2	TransAM® ATF-2	1 x 96-well plate	42396
		5 x 96-well plates	42896
c-Myc	TransAM® c-Myc	1 x 96-well plate	43396
		5 x 96-well plates	43896
	Recombinant c-Myc protein	5 µg	31117
C/EBP	TransAM® C/EBP α/β	1 x 96-well plate	44196
		5 x 96-well plates	44696
CREB	TransAM® CREB	1 x 96-well plate	42096
		5 x 96-well plates	42596
	TransAM® pCREB	1 x 96-well plate	43096
		5 x 96-well plates	43596
Elk-1	TransAM® Elk-1	1 x 96-well plate	44396
		5 x 96-well plates	44896
ER	TransAM® ER	1 x 96-well plate	41396
		5 x 96-well plates	41996
FKHR	TransAM® FKHR (FOXO1)	1 x 96-well plate	46396
		5 x 96-well plates	46896
GATA	TransAM® GATA Family (GATA-1, GATA-2 & GATA-3)	2 x 96-well plates	48296
	TransAM® GATA-4	1 x 96-well plate	46496
		5 x 96-well plates	46996
GR	TransAM® GR	1 x 96-well plate	45496
		5 x 96-well plates	45996
HIF	TransAM® HIF-1	1 x 96-well plate	47096
		5 x 96-well plates	47596
HNF	TransAM® HNF Family (HNF-1, HNF-3α, HNF-3β & HNF-4α)	2 x 96-well plates	46296
	TransAM® HNF-1	1 x 96-well plate	46196
		5 x 96-well plates	46696
IRF-3	TransAM® IRF-3 (Human)	1 x 96-well plate	48396
		5 x 96-well plates	48896
	TransAM® IRF-3 (Mouse)	1 x 96-well plate	48496
		5 x 96-well plates	48996
IRF-7	TransAM® IRF-7	1 x 96-well plate	50196
		5 x 96-well plates	50696
MAPK	TransAM® MAPK Family (ATF-2, c-Jun, c-Myc, MEF2 & STAT1α)	2 x 96-well plates	47296

TransAM® Ordering Information, continued

FACTOR	PRODUCT	FORMAT	Cat. No.
MEF2	TransAM® MEF2	1 x 96-well plate 5 x 96-well plates	43196 43696
MyoD	TransAM® MyoD	1 x 96-well plate 5 x 96-well plates	47196 47696
NF-YA	TransAM® NF-YA	1 x 96-well plate 5 x 96-well plates	40396 40896
NFAT	TransAM® NFATc1	1 x 96-well plate 5 x 96-well plates	40296 40796
NFκB	TransAM® NFκB Family (p50, p52, p65, c-Rel & RelB)	2 x 96-well plates	43296
	TransAM® Flexi NFκB Family (p50, p52, p65, c-Rel & RelB)	2 x 96-well plates	43298
	TransAM® NFκB p50	1 x 96-well plate 5 x 96-well plates	41096 41596
	TransAM® NFκB p50 Chemi	1 x 96-well plate 5 x 96-well plates	41097 41597
	TransAM® Flexi NFκB p50	1 x 96-well plate	41098
	Recombinant NFκB p50 protein	5 µg	31101
	TransAM® NFκB p52	1 x 96-well plate 5 x 96-well plates	48196 48696
	TransAM® NFκB p52 Chemi	1 x 96-well plate 5 x 96-well plates	48197 48697
	TransAM® NFκB p65	1 x 96-well plate 5 x 96-well plates	40096 40596
	TransAM® NFκB p65 Chemi	1 x 96-well plate 5 x 96-well plates	40097 40597
	TransAM® Flexi NFκB p65	1 x 96-well plate	40098
	Recombinant NFκB p65 protein	5 µg	31102
Nrf2	TransAM® Nrf2	1 x 96-well plate 5 x 96-well plates	50296 50796
Oct-4	TransAM® Oct-4	1 x 96-well plate 5 x 96-well plates	42496 42996
p53	TransAM® p53	1 x 96-well plate 5 x 96-well plates	41196 41696
	Recombinant p53 protein	5 µg	31103
PPAR	TransAM® PPARγ	1 x 96-well plate 5 x 96-well plates	40196 40696
Sp1 and Sp3	TransAM® Sp1	1 x 96-well plate 5 x 96-well plates	41296 41796
	TransAM® Sp1/Sp3	1 x 96-well plate 5 x 96-well plates	40496 40996
	Recombinant Sp1 protein	5 µg	31136
STAT	TransAM® STAT Family (STAT1α, STAT3, STAT5A & STAT5B)	2 x 96-well plates	42296
	TransAM® STAT3	1 x 96-well plate 5 x 96-well plates	45196 45696
T-bet	TransAM® T-bet	1 x 96-well plate 5 x 96-well plates	51396 51896
	Nuclear Extract Kit (see page 16 for details)	100 rxns 400 rxns	40010 40410
	ProStain™ Protein Quantification Kit (visit www.activemotif.com for details)	1000 rxns	15001

Universal Magnetic Co-IP Kit

lower background; suitable for both nuclear & whole-cell complexes

The Universal Magnetic Co-IP Kit improves co-immunoprecipitation (Co-IP) through the use of protein G-coated magnetic beads, which speed and simplify the IP and wash steps while greatly reducing background. The kit includes optimized reagents for making both nuclear & whole-cell extracts from cells or tissue. This gives you the flexibility to Co-IP any protein complex, whether it was originally bound to DNA (Figure 1) or in the cytoplasm.

Co-IP cytoplasmic AND nuclear complexes

Co-IP is often used to study cytoplasmic protein complexes. But, traditional methods are not optimal for studying DNA-binding proteins because nuclear complexes are very fragile, causing them to be disrupted during extraction. For this reason, in addition to containing components for preparing whole-cell extracts, the Universal Magnetic Co-IP Kit provides nuclear extraction reagents that have been optimized to preserve nuclear protein complexes. The kit's Enzymatic Shearing Cocktail uses DNA digestion to gently release the nuclear protein complexes from the DNA, so they are intact and ready for Co-IP.

Universal Magnetic Co-IP Kit advantages

- Magnetic beads simplify procedure and reduce background
- Optimized extraction method maintains nuclear protein complexes
- Preserve protein modifications

Simpler procedure, lower background

The Universal Magnetic Co-IP Kit utilizes protein G-coated magnetic beads, which simplify Co-IP by enabling the IP and wash steps to be performed in seconds, rather than having to use centrifugation. Because these beads have very low non-specific binding, background is reduced even while using the kit's low-salt Co-IP/Wash Buffer, which helps maintain weaker complexes.

Complete kit for better results

The Universal Magnetic Co-IP Kit has both nuclear and whole-cell extraction reagents, so you can perform IP on all types of protein complexes. The kit also includes protein G-coated magnetic beads, a unique Co-IP/Wash Buffer as well as phosphatase, protease and deacetylase inhibitors that preserve the integrity of the proteins and protein modifications (Figures 2 & 3). Finally, the kit includes a strong bar magnet, so you can take advantage of the improved wash and IP steps enabled by the magnetic beads. This makes the Universal Magnetic Co-IP Kit a simple, flexible and complete solution for getting more from your Co-IP.

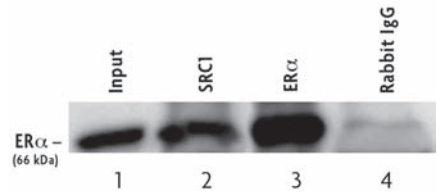

FIGURE 1:
Nuclear Co-IP of SRC-1 and ERα.
The Universal Magnetic Co-IP Kit was used to make nuclear extract from MCF-7 cells induced 1 hour with 10 nM Estradiol. IP was performed on 300 µg samples using 2 µg SRC-1 pAb, ERα pAb and rabbit IgG (as a negative control). Western blot was then performed using the ERα pAb on 10 µg Input Extract (Lane 1), SRC-1 IP (Lane 2), ERα IP (Lane 3) and the rabbit IgG IP (Lane 4).

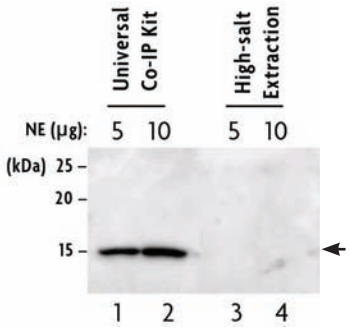

FIGURE 2:
Detection of acetylated Histone H3.
HeLa nuclear extracts were made using the Universal Magnetic Co-IP Kit and a traditional high-salt extraction protocol, each supplemented with 1 µM trichostatin A, a deacetylase inhibitor. Five and ten µg samples of each extract were used in Western blot with Histone H3 acetyl rabbit pAb (Cat. No. 39139). The pan acetyl-H3 (arrow) was detected only in samples made using the kit's gentle nuclear extraction procedure.

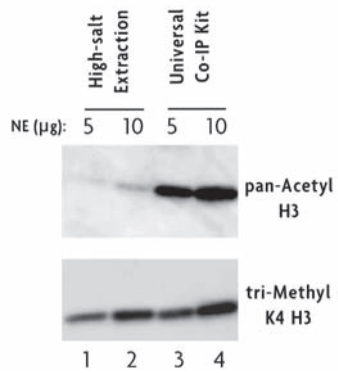

FIGURE 3:
Preserve acetylation and methylation.
Nuclear extracts were made from HeLa cells treated with 500 nM trichostatin A for 20 hours using either the Universal Magnetic Co-IP Kit (with its deacetylase inhibitor) or traditional high-salt extraction. Five and ten µg samples of these extracts were used in Western blot with Histone H3 acetyl pAb (Cat. No. 39139) and Histone H3 trimethyl Lys4 pAb (Cat. No. 39159). The acetylated protein was detected only in the sample made using the kit. Methylation was slightly better maintained in the sample made using the kit.

Product	Format	Cat. No.
Universal Magnetic Co-IP Kit	25 rxns	54002

ChIP-IT® Express & ChIP-IT Express HT

improved kits greatly reduce background

Active Motif has improved its ChIP-IT® Express Kits by greatly reducing the background, which improves your results and enables you to use less starting material than ever before. In addition, the provided magnetic beads have made it possible to streamline the protocol so you can get results in half the normal time with much less sample manipulation. These advances have made possible the new ChIP-IT Express HT Kit, which enables you to perform true high-throughput ChIP in 96-well plates.

ChIP-IT Express advantages

- No more need for pre-clearing, blocking or DNA purification steps
- Reduced background
- High throughput compatible
- Dramatically reduced hands-on time

The most efficient ChIP enrichment kit

ChIP is an enrichment technique, not a purification method. Thus, the less efficient your enrichment, the higher the sample background and the more material you will need to obtain an interpretable result. Conventional ChIP requires at least 2 million cells as starting material, which can be problematic with some cell lines. At the least, growing this many cells is labor intensive. Active Motif's improved ChIP-IT Express Kits, however, have been optimized to provide superior target gene enrichment, resulting in unmatched sensitivity. Using ChIP-IT Express, it is routine to perform ChIP on material from as few as 750,000 cells and the kits have even been shown to work with as few as 12,500 cells (Figure 1).

High-throughput ChIP

The efficient ChIP-IT Express method has lead to the recent development of ChIP-IT Express HT, which enables you to perform ChIP in 96-well plates using multi-channel pipettors.

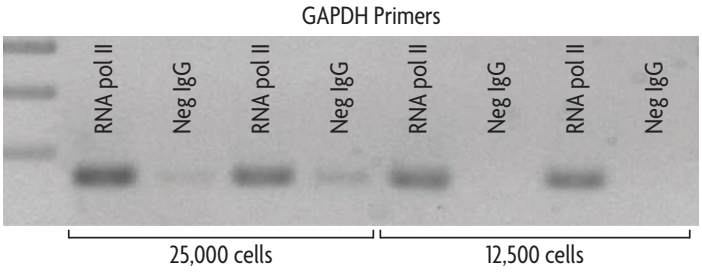

FIGURE 1:

ChIP-IT Express works with 12,500 cells.

ChIP-IT Express was performed in duplicate on decreasing amounts of sonicated HeLa cell chromatin. Two µg of RNA pol II and Neg IgG antibody was used for IP. GAPDH PCR primers were used to analyze the immunoprecipitated DNA. Using the improved ChIP-IT Express reagents and protocol, positive ChIP data was obtained from as few as 12,500 cells.

The magnetic bead advantage

The ChIP-IT Express magnetic beads have less background than standard agarose beads, so pre-clearing and blocking steps are no longer necessary. The magnetic pull-down occurs in just seconds, and re-formulated buffers allow steps to be combined and DNA purification to be eliminated. ChIP-IT Express is available in both sonication and enzymatic shearing formats.

Positive controls ensure success

Because interpreting ChIP data can be difficult, Active Motif has developed a complete set of controls to help you understand your results and troubleshoot your assays. To provide you with controls that are appropriate for your research, we offer our human, mouse and rat ChIP-IT Control Kits separately from ChIP-IT Express Kits. These provide positive and negative control antibodies and species-specific primers, PCR buffer and a convenient 10X DNA loading dye so your PCR reactions are gel-ready. All reagents are quality control tested and validated to ensure your ChIP assay is working properly. In addition, we also offer convenient Ready-to-ChIP Chromatin from a number of ENCODE cell lines, so you can be certain that the only variable in validating a new antibody for ChIP is the antibody itself.

Try the best ChIP kits today

For additional information on the ChIP-IT Express and ChIP-IT Express HT Kits, please visit us at **www.activemotif.com/chipit**.

Product	Format	Cat. No.
ChIP-IT® Express	25 rxns	53008
ChIP-IT® Express Enzymatic	25 rxns	53009
ChIP-IT® Express HT	96 rxns	53018
ChIP-IT® Protein G Magnetic Beads	25 rxns	53014
ChIP-IT® Control Kit – Human	5 rxns	53010
ChIP-IT® Control Kit – Mouse	5 rxns	53011
ChIP-IT® Control Kit – Rat	5 rxns	53012
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
Ready-to-ChIP Hep G2 Chromatin	10 rxns	53019
Ready-to-ChIP K-562 Chromatin	10 rxns	53020
Ready-to-ChIP NIH/3T3 Chromatin	10 rxns	53021

Re-ChIP-IT®

identify protein co-localization *in vivo* using sequential chromatin IP

Performing sequential chromatin IP (also called Re-ChIP) was technically challenging and difficult, until now. Active Motif's Re-ChIP-IT® Kit makes it easy to perform sequential ChIP, so you can localize two different proteins or histone modifications to the same genomic locus.

Extend the utility of ChIP

When performing chromatin immunoprecipitation (ChIP) experiments, it is often useful to prove that two different proteins or histone modifications are present at the same site in the genome. Or, you may want to determine if a protein coincides with a specific histone modification at the same regulatory element. Re-ChIP (aka Sequential ChIP, Chromatin Re-IP and ChIP Re-ChIP) is a relatively new technique in which sequential chromatin immunoprecipitations are performed using two different antibodies. This enables you to you assay for the simultaneous presence of two proteins or distinct histone modifications at the same genomic region of interest.

Active Motif's new Re-ChIP-IT Kit makes it simple for you to perform these types of experiments. All buffers for chromatin IP are included, making it easy to get started. And, the detailed protocol ensures you're successful the first and every time. Plus, Active Motif offers a variety of ChIP controls kits (page 9) to help you validate the results of your Re-ChIP experiments.

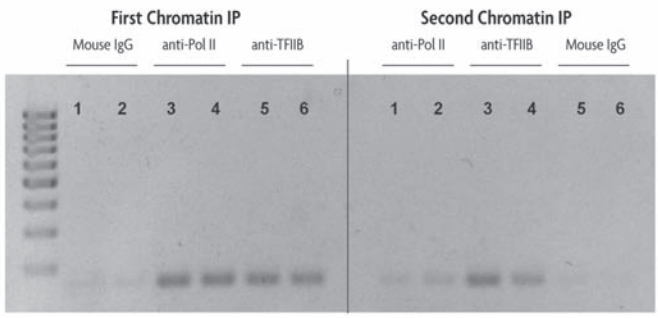

FIGURE 1:
Sequential chromatin immunoprecipitation using Re-ChIP-IT.
The lane numbers are the same in each panel to indicate that the DNA is from the same chromatin sample. The left panel shows the results of PCR performed on an aliquot of DNA removed from the experiment after the first ChIP step; the right panel represents PCR results on DNA from chromatin samples after both ChIP steps. For example, chromatin samples subjected to first ChIP using Mouse IgG as a negative control (lanes 1 and 2 in the left panel) were then subjected to a second ChIP with an RNA Pol II antibody (lanes 1 and 2 in the right panel). Chromatin samples in which Mouse IgG was used as either the first antibody (lanes 1 and 2) or second antibody (lanes 5 and 6) show little amplification of GAPDH DNA in either the left (first ChIP) or right panel (first and second ChIP). Chromatin samples in which the first antibody used was anti-RNA Pol II and the second antibody was anti-TFIIB (lanes 3 and 4) show good amplification of GAPDH DNA after the second ChIP (right panel) indicating co-localization of RNA Pol II and TFIIB at the same region of the GAPDH promoter.

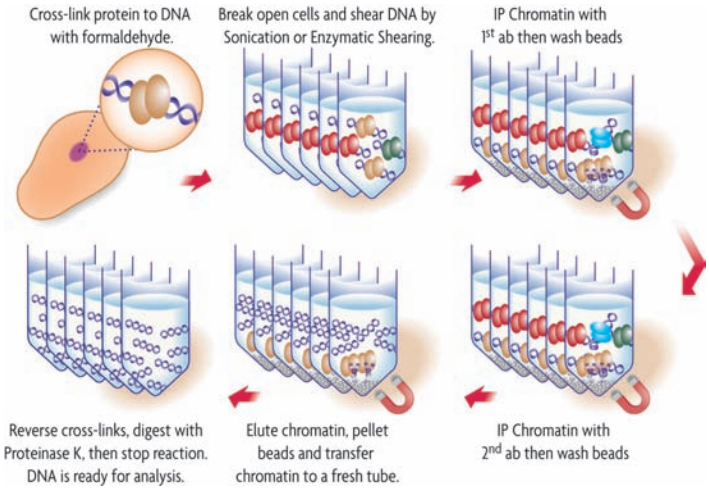

FIGURE 2:
Schematic representation of the Re-ChIP-IT procedure.

Sequential chromatin IP made easy

Re-ChIP-IT uses magnetic beads that have less background than standard agarose beads, so pre-clearing and blocking steps are not needed. Magnetic pull-down occurs in just seconds, and the method's low background has eliminated the need for DNA purification. And, Re-ChIP-IT can be used with chromatin prepared using our sonication or enzymatic shearing kits.

Get started with Re-ChIP

For additional information on the new Re-ChIP-IT Kit go to **www.activemotif.com/rechip**.

Product	Format	Cat. No.
Re-ChIP-IT®	25 rxns	53016

ChIP Accessory Kits and Reagents

maximize your chromatin IP experiments

ChIP & IP enhancer for mouse antibodies

Low antibody binding affinity can make getting good ChIP & IP results difficult. Because mouse IgM and IgG_1 antibodies do not have strong binding affinity for protein G, ChIP and regular immunoprecipitation (IP) can be challenging when using protein G-conjugated beads. The Bridging Antibody for Mouse IgG facilitates improved binding of mouse antibodies, especially IgG_1 and IgM isotypes, to protein G to generate more robust binding and better results in ChIP and standard IP experiments (Figure 1).

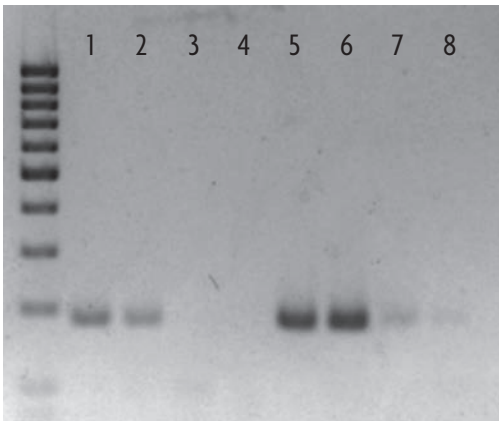

FIGURE 1:

Improvement in chromatin IP using an anti-mouse bridging antibody.
ChIP was performed using chromatin from U-937 cells induced with TNF-α.
PCR was performed with primers corresponding to the human IL-8 promoter.
Lanes 1-4: Beads not pre-incubated with bridging antibody.
Lanes 5-8: Beads pre-incubated with 5 µg bridging antibody.
Lanes 1, 2 & 5, 6: ChIP performed using anti-p65 mouse mAb, 2 µg per IP.
Lanes 3, 4 & 7, 8: ChIP performed using negative control mouse IgG.

Beads, controls and shearing kits

Active Motif offers a broad range of reagents and accessory kits to complement the ChIP-IT® line of ChIP kits. These products will help you prepare chromatin, troubleshoot your ChIP experiments and make them more reproducible.

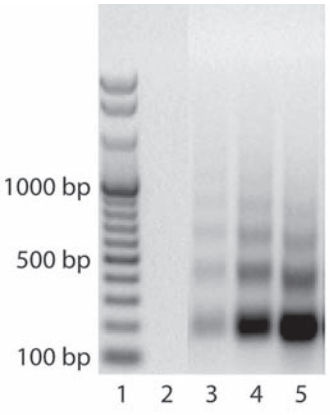

FIGURE 2:

Analysis of DNA sheared using the Enzymatic Shearing Kit.
HeLa cells were fixed for 10 minutes with 1% formaldehyde and then chromatin was prepared using the Enzymatic Shearing Kit protocol. Chromatin was sheared with the Enzymatic Shearing Cocktail for 5, 10 or 15 minutes and the reaction was stopped.

Lane 1: 100 to 1000 bp ladder.
Lane 2: Unsheared HeLa DNA.
Lane 3: HeLa DNA treated for 5 minutes.
Lane 4: HeLa DNA treated for 10 minutes (optimized).
Lane 5: HeLa DNA treated for 15 minutes.

Ready-to-ChIP Chromatin

For your convenience, Active Motif offers Ready-to-ChIP Chromatin from a number of ENCODE cell lines, which have been optimally sheared by sonication and validated in ChIP. As a result, you can more easily validate your own antibodies and primer sets. The chromatin can be used with all of the ChIP-IT Kits and controls, so you can be certain that the only variable in validating a new antibody for ChIP is the antibody itself.

Product	Format	Cat. No.
Bridging Antibody for Mouse IgG	500 µg	53017

Product	Format	Cat. No.
ChIP-IT® Protein G Magnetic Beads	25 rxns	53014
ChIP-IT® Control Kit – Human	5 rxns	53010
ChIP-IT® Control Kit – Mouse	5 rxns	53011
ChIP-IT® Control Kit – Rat	5 rxns	53012
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
Ready-to-ChIP Hep G2 Chromatin	10 rxns	53019
Ready-to-ChIP K-562 Chromatin	10 rxns	53020
Ready-to-ChIP NIH/3T3 Chromatin	10 rxns	53021
ChIP-IT® Express Shearing Kit	10 rxns	53032
ChIP-IT® Express Enzymatic Shearing Kit	10 rxns	53035

RapidReporter®

double destabilization improves luciferase assay response and sensitivity

Reporter gene assays are an important tool used for studying signal transduction pathways because of their simplicity and versatility. However, standard reporter assays are limited by the fact that the basal activity of the cloned promoter results in accumulation of luciferase mRNA and protein. The slow clearance rate of these reporter molecules substantially delays and dilutes the measurable response to stimulation or repression. As a result, with standard reporter assays, transient or relatively minor effects are hidden and kinetic assays are inaccurate. To solve this problem, some assays incorporate a protein-destabilizing element in the vector, causing the luciferase to degrade more rapidly. However, this only partly addresses the problem as reporter clearance rates are also dependent on the half-life of the reporter mRNA.

Active Motif's patented RapidReporter®* method overcomes these limitations by utilizing vectors that include both protein and mRNA destabilizing elements. This causes the cell to rapidly degrade both the luciferase protein and mRNA, which lowers background and increases both the magnitude of the response that can be measured after stimulation or repression, and the speed with which the assay can report changes in transcription. In addition, RapidReporter uses a modified *Gaussia* luciferase, the most sensitive luciferase known. The combination of *Gaussia* expression and double destabilization means that RapidReporter enables researchers to detect smaller changes in activity than is possible using non-destabilized reporter gene assays, or those that destabilize the protein only.

The RapidReporter® advantage

RapidReporter vectors utilize a non-secreted form of the extremely bright *Gaussia* luciferase gene, and are available as "empty" vectors with a multiple cloning site (MCS) for insertion of promoters/enhancers, or as pre-made vectors that contain a widely studied promoter such as NFκB, CREB (CRE) or GR. In the RapidReporter method, cells are plated and transiently transfected with the appropriate RapidReporter vector. After an overnight incubation, the stimulator/repressor of transcriptional activity is added for the appropriate time. The cells are lysed and flash luminescence is measured. All vectors are available separately or as a complete assay kit, which includes an EF1α promoter-driven positive control vector, as well as Lysis and Assay Buffers and a substrate optimized for use with the *Gaussia* luciferase, which guarantees the best results possible.

WHY USE RAPIDREPORTER®?

- Stronger fold induction reduces false positives
- Faster response enables detection of transient effects, and drugs that decompose rapidly
- Your choice of stringency – pRR-High is highly destabilized for best response to changes in transcription, while pRR-Low contains fewer destabilizing elements for a stronger basal signal
- Improved performance increases productivity and lead identification
- Offered as "empty" vectors for cloning your own elements or as pre-mades, which contain widely studied promoter sites

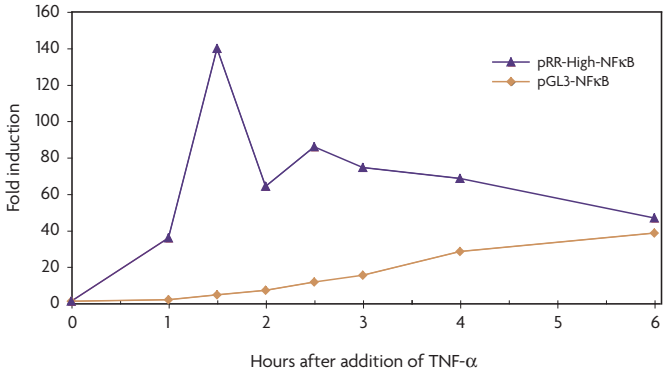

FIGURE 1:
RapidReporter "unmasks" hidden effects.
HeLa cells were transiently transfected with pRR-High-NFκB or pGL3 vector containing NFκB, then plated in 96-well plates. Twenty-four hours post-transfection, the cells were stimulated with 10 ng/ml TNF-α and measured for Gaussia Luciferase (pRR) and firefly luciferase (pGL3) at the indicated time points. Because RapidReporter has double destabilizing elements that reduce background, relatively small & transient events, like the natural oscillation of NFκB from the cytosol to the nucleus during its activation, can be observed.

Your choice of stringency

RapidReporter vectors are offered in two different stringencies, pRR-High and pRR-Low. The pRR-High vector provides the lowest background possible because it contains more and stronger destabilization elements, thereby providing maximum responsiveness. The fold induction of stimulated vs. non-stimulated samples is highest using pRR-High whereas the basal (background) level will be lowest. This makes pRR-High vectors ideal in cases where your stimulation will only produce a weak effect. In contrast, the pRR-Low vector contains fewer and weaker destabilization elements. While this lowers the level of fold-induction observed, it enables weaker basal signals to be detected. pRR-Low vectors are ideal for use when a low signal strength is expected, such as with cells that transfect only at a low efficiency or when testing a promoter with only weak activity in the cells of interest. Regardless of the RapidReporter vector used, the fold induction observed will be higher than with other luciferase reporter systems such as firefly (Figure 1) and *Renilla* (data not shown).

Pre-made reporter vectors

The RapidReporter vectors were designed with the *Gaussia* luciferase reporter and an ampicillin resistance gene for plasmid selection. The multiple cloning site offers maximum flexibility for insertion, whatever cloning method is used. For your convenience, Active Motif also offers a number of pre-made vectors that already contain widely studied promoters. Each pre-made is available in the pRR-High vector (Figure 2) to maximize responsiveness to changes in transcription.

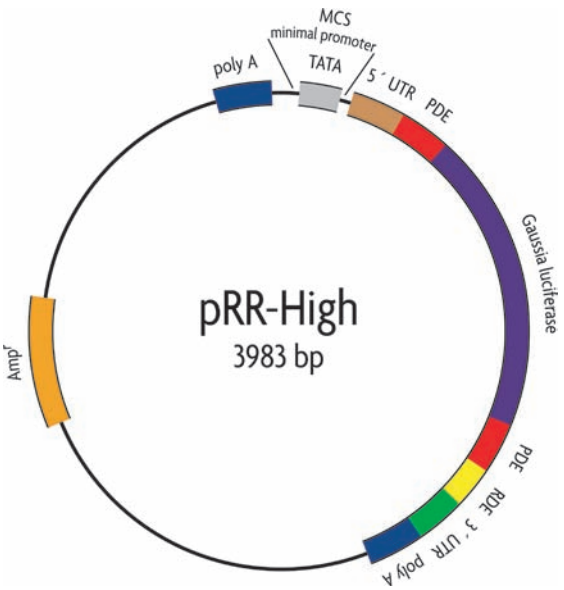

FIGURE 2:

The RapidReporter pRR-High vector.

The pRR-High plasmid contains two strong protein-destabilizing elements (PDE), while pRR-Low contains only one PDE. Each vector also contains an RNA-destabilizing element (RDE); the RDE in the pRR-High vector is strong while the RDE in the pRR-Low vector is weaker. The vectors also contain an intron for improved expression by enhancing RNA processing, and an optimized synthetic poly A signal on the reporter gene to enhance its expression.

Optimized buffers and substrates

Active Motif's *Gaussia* Luciferase Assay Kit contains proprietary Lysis and Assay Buffers and a *Gaussia* Substrate, which are optimized for use with the *Gaussia* luciferase-fusion proteins expressed by the RapidReporter vectors. This enables you to take advantage of the enhanced sensitivity of pRR-High and pRR-Low plasmids. Because the *Gaussia* luciferase gene encoded by pRR-High and -Low vectors has been modified for intracellular expression, it is not suitable for use with standard *Gaussia* assay kits or assay kits designed for use with other luciferases. For your convenience the RapidReporter *Gaussia* Luciferase Assay Kits are available in both 100 and 1000 reaction formats.

* RapidReporter is covered under U.S. Patent No. 7,157,272 and various other patents worldwide, is a registered trademark of, and is sold under license granted by GeneStream Pty Ltd. Purchasers are subject to a Limited-use Label License that allows them to use RapidReporter for a 6-month evaluation period. Commercial entities and all users who wish to perform high-throughput screening will be required to enter into an End User License Agreement with GeneStream following the evaluation period. Please contact Active Motif's Technical Services for details.

Product	Format	Cat. No.
RapidReporter® *Gaussia* Luciferase Assay	100 rxns	33001
	1000 rxns	33002
RapidReporter® pRR-High vector	10 µg	33003
RapidReporter® pRR-High Assay	100 rxns	33004
RapidReporter® pRR-Low vector	10 µg	33005
RapidReporter® pRR-Low Assay	100 rxns	33006
RapidReporter® pRR-High-CRE vector	10 µg	33007
RapidReporter® pRR-High-CRE Assay	100 rxns	33008
RapidReporter® pRR-High-GR vector	10 µg	33011
RapidReporter® pRR-High-GR Assay	100 rxns	33012
RapidReporter® pRR-High-IRF-1 vector	10 µg	33017
RapidReporter® pRR-High-IRF-1 Assay	100 rxns	33018
RapidReporter® pRR-High-NFκB vector	10 µg	33009
RapidReporter® pRR-High-NFκB Assay	100 rxns	33010
RapidReporter® pRR-High-STAT1 vector	10 µg	33015
RapidReporter® pRR-High-STAT1 Assay	100 rxns	33016
RapidReporter® pRR-High-STAT3 vector	10 µg	33013
RapidReporter® pRR-High-STAT3 Assay	100 rxns	33014

Recombinant Proteins

purified proteins for all your research

Active Motif provides an extensive line of recombinant proteins, including transcription factors 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 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 2). The list below is only a few of the over 100 proteins available; visit **www.activemotif.com/proteins** for a complete product listing and more information on the recombinant proteins, including detailed technical data sheets that specify the protein length, the method used for purification, *etc.*

PRODUCT	EXPRESSED IN	FORMAT	Cat. No.
Recombinant c-Fos protein	Baculovirus	5 µg	31115
Recombinant c-Jun protein	Baculovirus	5 µg	31116
Recombinant c-Myc protein	*E. coli*	5 µg	31117
Recombinant NFκB p50 protein	*E. coli*	5 µg	31101
Recombinant NFκB p65 protein	*E. coli*	5 µg	31102
Recombinant p53 protein	*E. coli*	5 µg	31103
Recombinant p300 protein	Baculovirus	4 µg	31124
Purified Sp1 protein	HeLa cells	2 µg	31137
Recombinant STAT3 protein	*E. coli*	10 µg	31140

FunctionELISA™ IκBα

study the phosphorylation state of IκBα

NFκB is regulated by the IκB family of inhibitory proteins. Phosphorylation of IκBα leads, ultimately, to activation of NFκB. Thus, analysis of the phosphorylation state of IκBα provides insights about NFκB and the many genes it regulates (see page 2 for information on TransAM® NFκB Kits). FunctionELISA IκBα uses a Capture Antibody specific for phosphorylated IκBα It utilizes highly sensitive chemiluminescent detection to enable accurate study of the phosphorylation state of IκBα (Figure 1). FunctionELISA IκBα requires the use of a luminometer.

Applications

FunctionELISA™ IκBα Kits can be used to study the phosphorylation state of IκBα and to correlate this information with the activation and translocation of NFκB. Applications include drug screening and/or potency effects on phosphorylation of IκBα and time course experiments to monitor the duration of IκBα phosphorylation.

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

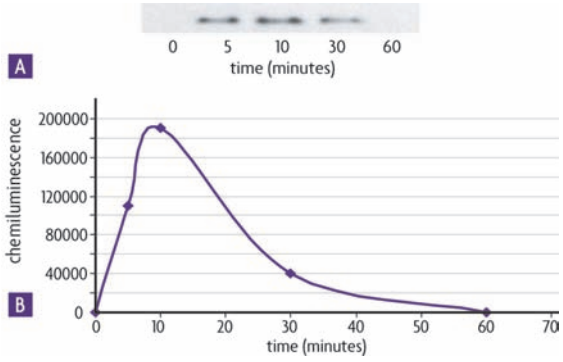

FIGURE 1:
Induction of IκBα phosphorylation.
Jurkat cells were treated with 1 nM TNF-α and harvested at the indicated time points. Whole-cell extracts were assayed in Western blot analysis (A) using IκBα phospho Ser32,36 mAb (Cat. No. 40904) and the FunctionELISA IκBα Kit (B).

Cell Extracts

high-quality extracts ensure reproducible results

Active Motif provides high-quality extracts from a variety of cell types and tissue sources. Our extracts are available in nuclear, cytoplasmic and whole-cell formats, using extraction protocols that have been optimized to produce high yields of cell signaling and transcription factor-related proteins. In addition, many of our extracts are prepared from cells that have been stimulated or treated to specifically induce activation of hard-to-detect transcription factors.

Active Motif's ready-to-use extracts are ideal for use as positive controls in a variety of applications, including TransAM® Kits (see pages 2-5), gelshift and supershift assays, Western blot analysis or as a starting point for transcription factor purification. As the list below is only a small subset of the over 100 extracts available, please visit **www.activemotif.com/extracts** for a complete product listing and more complete information.

EXTRACT	FORMAT	Cat. No.
3T3-L1 nuclear extract	200 µg	36071
COS-7 nuclear extract	200 µg	36079
COS-7 nuclear extract ($CoCl_2$ treated)	200 µg	40600
COS-7 nuclear extract (IFNγ treated)	200 µg	36112
HeLa whole-cell extract	200 µg	40050
HeLa nuclear extract	200 µg	36010
HeLa whole-cell extract (IL-1α stimulated)	200 µg	40100
HeLa whole-cell extract (TNF-α stimulated)	200 µg	40200
HeLa nuclear extract (2 hr serum response)	200 µg	36104
HeLa nuclear extract (4 hr serum response)	200 µg	36086
HeLa nuclear extract (Anisomycin treated)	200 µg	36111
HeLa nuclear extract (TNF-α stimulated)	200 µg	40210
HeLa nuclear extract (TPA stimulated, 10 minutes)	200 µg	36109
HeLa nuclear extract (TPA stimulated, 2 hours)	200 µg	36009
Hep G2 cytoplasmic extract	200 µg	36400
Hep G2 nuclear extract	200 µg	36011
Hep G2 nuclear extract (Acetaldehyde treated)	200 µg	36065
Hep G2 whole-cell extract (IL-6 stimulated, 10 ng/ml)	200 µg	36413
Hep G2 nuclear extract (IL-6 stimulated, 10 ng/ml)	200 µg	36092
Hep G2 nuclear extract (IL-6 stimulated, 100 ng/ml)	200 µg	36107
HL-60 nuclear extract	200 µg	36072
Jurkat cytoplasmic extract	200 µg	36402

EXTRACT	FORMAT	Cat. No.
Jurkat nuclear extract	200 µg	36014
Jurkat nuclear extract (Heat Shock)	200 µg	36069
Jurkat nuclear extract (PHA stimulated)	200 µg	36108
Jurkat nuclear extract (1 day growth)	200 µg	36110
Jurkat nuclear extract (TPA + CI stimulated)	200 µg	36013
MCF-7 nuclear extract	200 µg	36017
MCF-7 nuclear extract (Estradiol treated)	200 µg	36016
MCF-7 nuclear extract (H_2O_2-treated)	200 µg	40800
MCF-7 nuclear extract (H_2O_2 post-treated)	200 µg	40810
Nb2 nuclear extract	200 µg	36105
Nb2 nuclear extract (prolactin stimulated)	200 µg	36106
NIH/3T3 cytoplasmic extract	200 µg	36403
NIH/3T3 nuclear extract	200 µg	36020
NIH/3T3 whole-cell extract (TGF-β1 treated)	200 µg	36414
NIH/3T3 whole-cell extract (TPA stimulated)	200 µg	36415
U-937 cytoplasmic extract	200 µg	36410
U-937 nuclear extract	200 µg	36030
U-937 nuclear extract (IFNα treated)	200 µg	36077
U-937 nuclear extract (TPA stimulated)	200 µg	36029
WI-38 nuclear extract	200 µg	40310
WI-38 nuclear extract (Forskolin stimulated)	200 µg	40300
WI-38 nuclear extract (TPA + CI stimulated)	200 µg	40500

Antibodies

highly characterized to meet your needs

Active Motif offers 100's of antibodies directed against a variety of transcription factor, histone and cell signaling-related proteins. These antibodies are ideal for performing ChIP, Western blots, ELISAs, immunoprecipitation and supershift/electrophoretic mobility shift assays (EMSA). Please visit **www.activemotif.com/abs** for a complete product listing and more detailed information.

ANTIBODY	IMMUNOGEN	REACTIVITY	APPLICATIONS	FORMAT	Cat. No.
AML-1/Runx1 pAb	Raised against amino acids 231-245 of human AML-1/Runx1.	H, M	WB	100 µl	39000
AML-2/Runx3 pAb	Raised against full-length human AML-2/Runx3.	H	SS, WB	100 µl	39301
AML-3/Runx2 pAb	Raised against amino acids 333-348 of human AML-3/Runx2.	H	SS	100 µl	39302
c-Fos pAb	Raised against amino acids 358-372 of human c-Fos.	H, M	WB	100 µl	39008
c-Fos pAb	Raised against amino acids 358-372 of human c-Fos.	H, M	SS	100 µl	39308
c-Jun pAb	Raised against amino acids 20-37 of human c-Jun.	H, M	WB	100 µl	39009
c-Jun pAb	Raised against amino acids 20-37 of human c-Jun.	H, M	ChIP, IF, SS	100 µg	39309
c-Myc mAb (61 kDa form)	Raised against amino acids 408-420 of human c-Myc; detects 61 kDa form.	H	WB	100 µl	39502
c-Myc pAb (61 kDa form)	Raised against amino acids 9-22 of human c-Myc; detects 65 kDa form.	H, M	WB	100 µl	39011
c-Myc pAb (65 kDa form)	Raised against amino acids 9-22 of human c-Myc.	H, M, MK	WB	100 µl	39012
c-Rel pAb	Raised against amino acids 507-524 of human c-Rel.	H	SS, WB	100 µg	39311
C/EBPα pAb	Raised against amino acids 1-14 of human C/EBPα.	H, R	ChIP, IF, SS, WB	100 µg	39306
C/EBPβ pAb	Raised against amino acids 228-242 of human C/EBPβ.	H, R	SS, WB	100 µl	39307
CREB mAb	Raised against a synthetic peptide to human CREB.	H	WB	100 µg	40961
CREB-1 pAb	Raised against amino acids 6-23 of human CREB-1.	H, R	WB	100 µl	39013
CREB-1 pAb	Raised against amino acids 6-23 of human CREB-1.	H, R	SS	17 rxns	39310
E2F-1 mAb	Raised against full-length human E2F-1.	H	SS	17 rxns	39601
E2F-1 pAb	Raised against amino acids 309-323 of human E2F-1.	H, M	ChIP, SS	17 rxns	39313
E2F-3 mAb	Raised against full-length human E2F-3.	H	WB	100 µl	39507
E2F-3 mAb	Raised against full-length human E2F-3.	H	SS	17 rxns	39602
E2F-4 mAb	Raised against full-length human E2F-4.	H	WB	100 µl	39508
E2F-4 mAb	Raised against full-length human E2F-4.	H	SS	17 rxns	39603
E2F-6 mAb	Raised against full-length human E2F-6.	H	ChIP, WB	100 µl	39509
ERα mAb	Raised against full-length human ERα.	H	WB	100 µl	39510
ERα pAb	Raised against amino acids 145-159 of human ERα.	H	WB	100 µl	39021
ERα pAb	Raised against amino acids 145-159 of human ERα.	H, M	SS	17 rxns	39316
FosB mAb	Raised against amino acids 2-14 of human FosB.	H	WB	100 µg	40960
FosB pAb	Raised against amino acids 2-14 of human FosB.	H, M	WB	100 µl	39022
FosB pAb	Raised against amino acids 2-14 of human FosB.	H, M	SS	17 rxns	39318
FosB pAb	Raised against amino acids 2-14 of human FosB.	H	WB	100 µg	40959
Fra-2 pAb	Raised against amino acids 289-305 of human Fra-2.	H, M	WB	100 µl	39023
HDAC1 pAb	Raised against amino acids 1-5, 433-448 & 467-482 of hHDAC1.	H	WB	100 µg	40967
HDAC3 pAb	Raised against amino acids 2-17 of human HDAC3.	H	ChIP, WB	100 µg	40968
HDAC4 pAb	Raised against amino acids 194-209 of human HDAC4.	H	ChIP, WB	100 µg	40969
HDAC5 pAb	Raised against amino acids 572-587 of human HDAC5.	H, M	ChIP, WB	100 µg	40970
HDAC6 pAb	Raised against amino acids 1-16 of human HDAC6.	H, M	ChIP, WB	100 µg	40971
HDAC11 pAb	Raised against amino acids 238-251 of human HDAC11.	H, M	WB	200 µl	39208
HNF-1α pAb	Raised against amino acids 540-555 of human HNF-1α.	H, M, R	WB	100 µl	39030
HNF-1α pAb	Raised against amino acids 540-555 of human HNF-1α.	H, M, R	SS	17 rxns	39321
HNF-3α pAb	Raised against amino acids 150-165 of human HNF-3α.	H, R	SS	17 rxns	39322
HNF-3β pAb	Raised against amino acids 295-311 of human HNF-3β.	H	WB	100 µl	39096
HNF-3γ pAb	Raised against amino acids 287-299 of human HNF-3γ.	H, R	WB	100 µl	39031

REACTIVITY KEY: **B** = Bovine; **H** = Human; **M** = Mouse; **MK** = Monkey; **R** = Rat

ANTIBODY	IMMUNOGEN	REACTIVITY	APPLICATIONS	FORMAT	Cat. No.
HNF-3γ pAb	Raised against amino acids 287-299 of human HNF-3γ.	H	SS	17 rxns	39323
IκBα phospho Ser 32, 36 mAb	Raised against a peptide with phospho Ser32 & Ser36 on human IκBα.	H	WB	100 µg	40904
IKKα mAb	Raised against full-length human IKKα.	H, M	IP, WB	100 µg	40905
IKKβ mAb (Clone 10AG2)	Raised against full-length human IKKβ.	H	WB	100 µg	40906
IKKβ mAb (Clone 10A9B6)	Raised against full-length human IKKβ.	H	IP, WB	100 µg	40907
IKKγ mAb	Raised against full-length human IKKγ.	H	WB	100 µg	40908
IRF-1 pAb	Raised against amino acids 126-140 of human IRF-1.	H	SS	17 rxns	39325
IRF-3 pAb	Raised against amino acids 306-312 of human IRF-3.	H	WB	200 µl	39371
IRF-3 pAb	Raised against full-length human IRF-3.	B, H	ChIP, WB	100 µl	39033
IRF-6 pAb	Raised against amino acids 358-372 of human IRF-6.	H	WB	100 µg	39080
IRF-7 pAb	Raised against amino acids 463-477 of human IRF-7.	H	WB	100 µg	39081
JunB pAb	Raised against amino acids 28-42 of human JunB.	H	WB	200 µl	39549
JunB pAb	Raised against amino acids 3-17 of human JunB.	H, M	WB	100 µl	39034
JunB pAb	Raised against amino acids 3-17 of human JunB.	H	ChIP, SS	17 rxns	39326
JunD pAb	Raised against amino acids 231-245 of human JunD.	H	WB	100 µg	39082
JunD pAb	Raised against amino acids 143-157 of human JunD.	H	ChIP, SS	100 µl	39328
NAK1/Nur77 pAb	Raised against amino acids 251-266 of human NAK1/Nur77.	H, M	WB	200 µl	40982
NF-1A pAb	Raised against amino acids 478-492 of human NF-1A.	H	WB	200 µl	39397
NF-1A pAb	Raised against amino acids 478-492 of human NF-1A.	H, R	WB	100 µl	39036
NF-1A pAb	Raised against amino acids 478-492 of human NF-1A.	H, R	SS	100 µl	39329
NFκB p50 pAb	Raised against amino acids 363-376 of human NFκB p50.	H, M	WB	100 µl	39037
NFκB p50 pAb	Raised against amino acids 363-376 of human NFκB p50.	H, M, R	SS	17 rxns	39330
NFκB p65 pAb	Raised against C-terminal of human NFκB p65.	H	WB	200 µl	39369
NFκB p65 pAb	Raised against amino acids 2-17 of human NFκB p65.	H, M, R	WB	100 µl	39038
NFκB p65 pAb	Raised against amino acids 501-515 of human NFκB p65.	H, R	WB	100 µl	39039
NFκB p65 mAb	Raised against amino acids 526-539 of human NFκB p65.	H, M	FC, WB	100 µg	40916
Oct-2 mAb	Raised against full-length human Oct-2.	H	WB	100 µl	39517
Oct-2 mAb	Raised against full-length human Oct-2.	H	SS	17 rxns	39604
p53 pAb (Clone DO1)	Raised against full-length human p53.	H	IP, WB	200 µl	39553
p53 pAb	Raised against amino acids 367-381 of human p53.	H, M	WB	100 µl	39041
p53 pAb	Raised against amino acids 367-381 of human p53.	H	ChIP, SS	17 rxns	39334
Pax-5 pAb	Raised against amino acids 1-15 of human Pax-5.	H	WB	100 µl	39043
Pax-5 pAb	Raised against amino acids 1-15 of human Pax-5.	H	SS	17 rxns	39336
RelB pAb	Raised against amino acids 563-579 of human RelB.	H	WB	100 µl	39053
RelB pAb	Raised against amino acids 476-490 of human RelB.	H	SS	17 rxns	39339
RNA pol II CTD phospho Ser2 pAb	Raised against RNA pol II CTD sequence phosphorylated at serine 2.	H	WB	200 µl	39563
RNA pol II mAb	Raised against a synthetic peptide YSPTSpPS of human RNA pol II.	H, M, R	ChIP, ELISA, IF, IP, WB	200 µl	39097
Sp1 pAb	Raised against full-length human Sp1.	H	WB	100 µl	39057
Sp1 pAb	Raised against amino acids 520-534 of human Sp1.	H, M, R	WB	100 µl	39058
Sp3 pAb	Raised against full-length human Sp3.	H, M	SS	17 rxns	39341
STAT1α pAb	Raised against amino acids 712-750 of human STAT1α.	H	WB	100 µl	39059
STAT2 pAb	Raised against amino acids 830-849 of human STAT2.	H, M	WB	100 µg	39060
STAT3 pAb	Raised against full-length human STAT3.	H	WB	100 µl	39061
STAT4 pAb	Raised against amino acids 731-748 of mouse STAT4.	H, M, R	WB	100 µl	39062
STAT5A pAb	Raised against full-length human STAT5A.	H	WB	100 µl	39063
STAT5B pAb	Raised against amino acids 777-787 of human STAT5B.	H	WB	100 µl	39064
STAT6 pAb	Raised against amino acids 808-826 of human STAT6.	H	WB	100 µl	39065
TRF2 pAb	Raised against full-length human TRF2.	H, M, R	ChIP, IP, WB	100 µg	39223
VDR pAb	Raised against amino acids 5-19 of human VDR.	H, R	WB	100 µl	39069
VDR pAb	Raised against amino acids 5-19 of human VDR.	H, R	SS	17 rxns	39344
YY1 pAb	Raised against amino acids 109-123 of human YY1.	H, M, MK, R	WB	100 µl	39071
YY1 pAb	Raised against amino acids 109-123 of human YY1.	H	SS	17 rxns	39345

APPLICATIONS KEY: **ChIP** = Chromatin Immunoprecipitation; **FC** = Flow Cytometry; **IF** = Immunofluorescence; **IP** = Immunoprecipitation; **SS** = Supershift; **WB** = Western Blot

Nuclear Extract Kit

high yields of specifically segregated extracts

The Nuclear Extract Kit is ideal for the preparation of nuclear, whole-cell and cytoplasmic extracts from mammalian cells and tissues. The resultant high-quality extracts may be used with Active Motif's TransAM® Kits (see page 2) or in gelshift assays, Western blots, DNA footprinting or as a starting point for transcription factor purification.

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

The Nuclear Extract Kit advantage

In the Nuclear Extract Kit, cells are collected in ice-cold PBS in the presence of phosphatase inhibitors to limit further protein modifications. Next, the cells are resuspended in hypotonic buffer to swell the cell membrane. Addition of detergent causes leakage of the cytoplasmic proteins into the supernatant. After collection of the cytoplasmic fraction, the nuclei are lysed and the nuclear proteins are solubilized in lysis buffer in the presence of the protease inhibitors. Whole-cell extracts can also be prepared by collecting the cells in the PBS/phosphatase inhibitors solution and lysing in lysis buffer. Solubilized proteins are separated from the cell debris by centrifugation. The concentration of protein in the cell extract is then measured by Active Motif's ProStain Protein Quantification Kit (Cat. No. 15001) or with a Bradford-based assay.

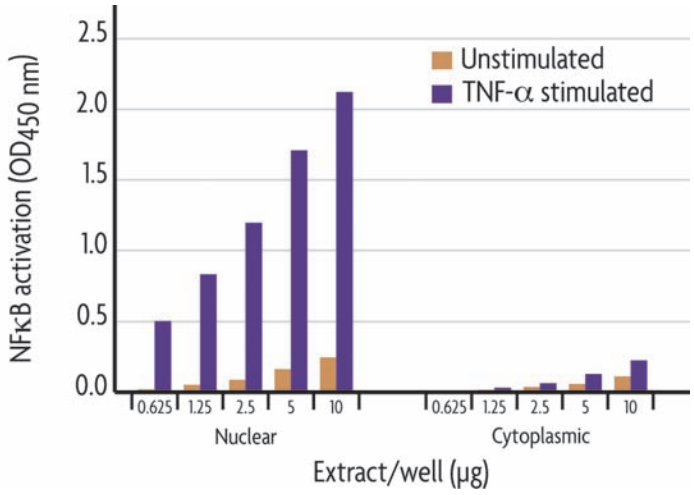

FIGURE 1:
Specific extraction of nuclear and cytoplasmic extracts.
Nuclear and cytoplasmic extracts were prepared using the Nuclear Extract Kit from HeLa samples unstimulated or stimulated with TNF-α for 30 minutes and assayed using the TransAM® NFκB p50 Kit. Because activated NFκB translocates to the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

WHY USE THE NUCLEAR EXTRACT KIT?

- Quality-controlled reagents ensure reproducibility
- No need to optimize your own procedure
- Complete kit contains all required reagents
- Prepare nuclear, cytoplasmic or whole-cell extracts with one kit
- Ability to prepare extracts from both cultured cells and tissue samples

Product	Format	Cat. No.
Nuclear Extract Kit	100 rxns	40010
	400 rxns	40410

Nuclear Receptor ELISAs

two quantitative methods to study nuclear receptors

Active Motif offers two innovative kit formats for studying nuclear receptors (NR). The NR Peptide ELISA kits enable quantitative measurement of ligand-activated nuclear receptor proteins, while the NR Sandwich ELISA kits provide a quick and easy method to measure the total amount of a nuclear receptor in a given sample. Both methods combine a fast, user-friendly format in a highly sensitive ELISA-based format.

Nuclear receptors belong to a superfamily of structurally related ligand-inducible transcription factors whose members include receptors for estrogen (ER), glucocorticoid (GR), androgen (AR), thyroid hormone (TR), progesterone (PR) and vitamin D (VDR). Inappropriate signaling by these receptors is associated with numerous disease states, including cancer, asthma, infertility, osteoporosis and arthritis, making them drug targets.

WHY USE NR PEPTIDE & SANDWICH ELISAS?

- NR Peptide measures ligand-activated NR
- Quantitative results in just hours
- ELISA format eliminates the need to run, blot and develop gels
- NR Sandwich measures total NR
- Analyze multiple samples in low volume, high throughput
- Ability to assay both cellular extracts and recombinant proteins

NR Peptide ELISA method

Active Motif's NR Peptide ELISAs are specifically designed to capture ligand-activated nuclear receptor. Each NR Peptide Kit provides a 96-well plate coated with Capture Peptide that includes the consensus-binding motif of the nuclear receptor's co-activator. Addition of your sample results in binding of the activated nuclear receptor to the immobilized Capture Peptide. Each well is then incubated with a specific primary antibody. Subsequent incubation with HRP-conjugated antibody and developing solution provides an easily quantified readout.

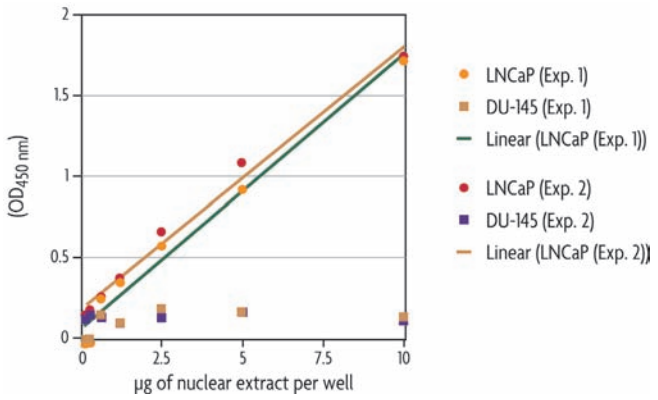

FIGURE 1:

Reproducibility of NR Sandwich ELISAs.

Increasing amounts of nuclear extract from two different prostate cancer cell lines, LNCaP and DU-145, were assayed for levels of androgen receptor using the NR Sandwich AR Kit. Each dilution was assayed in duplicate and the experiment was repeated two times. The results confirm that LNCaP cells are positive for AR, while DU-145 cells are AR negative; it also exhibits very low inter- and intra-plate variation, which demonstrates the high reproducibility of the NR Sandwich AR Kit.

NR Sandwich ELISA method

To measure total levels of a given nuclear receptor within a sample, Active Motif's NR Sandwich Kits provide a simple, rapid and quantitative alternative to traditional Western blots, EMSA and reporter assays. The NR Sandwich ELISA kits use two antibodies that recognize different epitopes on the protein of interest. The Capture Antibody, which is bound to an ELISA plate, binds the nuclear receptor protein when the sample is added. The Detecting Antibody then binds to the captured nuclear receptor protein. Subsequent incubation with a HRP-conjugated secondary antibody provides an easily quantified readout of total nuclear receptor protein levels. Because the response of nuclear receptors to a single ligand can vary between cell types, nuclear receptor assays need to be highly reproducible. Therefore, our NR Sandwich ELISAs are rigorously tested to ensure the highest level of reproducibility (Figure 1).

Product	Format	Cat. No.
NR Peptide ERα	1 x 96-well plate 5 x 96-well plates	49096 49596
NR Peptide ERα Chemi	1 x 96-well plate 5 x 96-well plates	49097 49597
NR Sandwich AR	1 x 96-well plate 5 x 96-well plates	49196 49696
NR Sandwich ERα	1 x 96-well plate 5 x 96-well plates	49296 49796
Recombinant ER protein	4,000 units	31119
NR Sandwich PR	1 x 96-well plate 5 x 96-well plates	49396 49896

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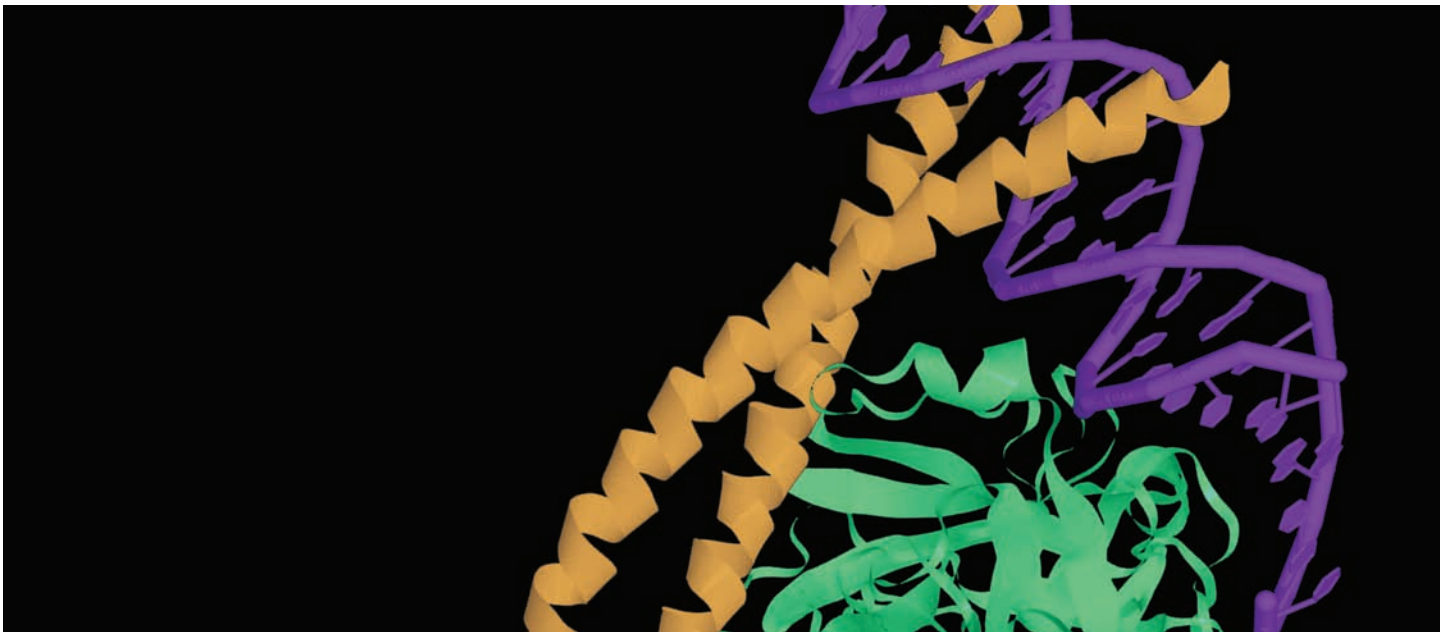

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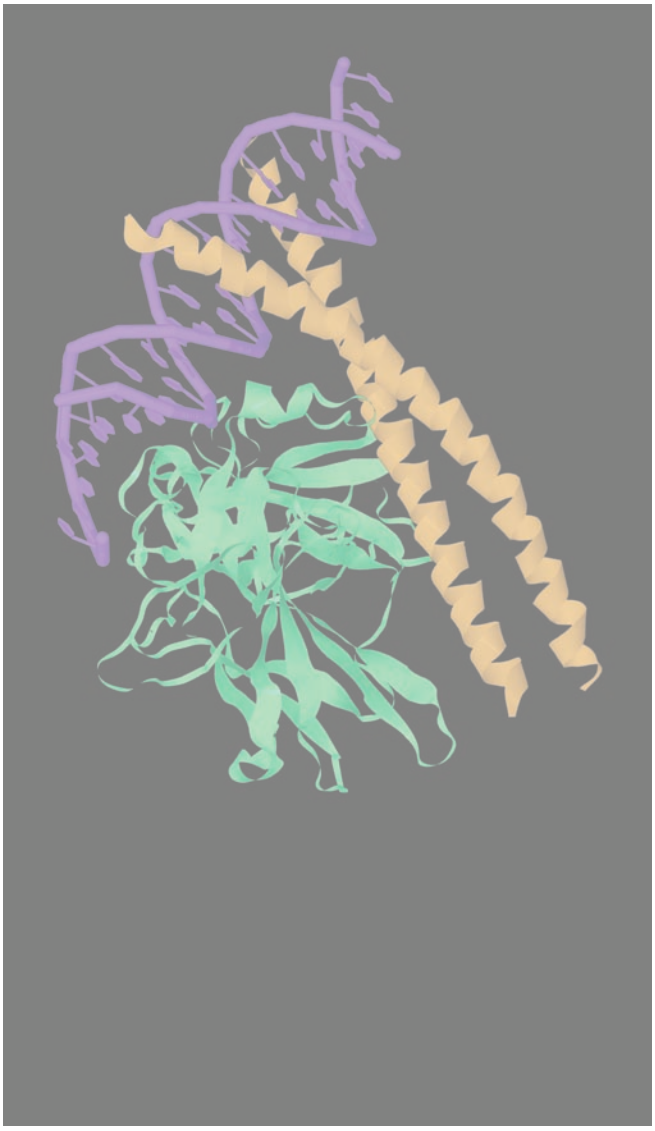

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