






# TransAM™

see your results clearly  
with sensitive, quantitative  
transcription factor ELISAs



TransAM™ Kits are DNA-binding ELISAs that facilitate the study of transcription factor activation in mammalian tissue and cell culture extracts.

## TransAM advantages

-  Highly specific assay provides quantitative results in under 5 hours
-  Eliminates the use of radioactivity and the need to run gels
-  Up to 100-fold more sensitive than gelshift assays
-  No cloning or transfection required
-  Ability to assay tissue samples

TransAM™ Kits are DNA-binding ELISAs\* that facilitate the study of transcription factor activation in mammalian tissue and cell culture extracts. The novel TransAM method is up to 100-fold more sensitive than gelshift and is complete in less than 5 hours. TransAM Kits eliminate the use of radioactivity and the need to run gels, while the high-throughput format enables simultaneous screening of 1 to 96 samples in a single experiment. And unlike reporter assays, TransAM can be used on all sample types, including cell lines and tissues, giving you unsurpassed flexibility.

Understanding and quantifying transcription factors is integral to the study of cellular function in relation to differentiation, brain activity, immune response, inflammation, cancer, and much more. In every cell there are numerous proteins that interact with DNA and each other to form signaling pathways, which regulate cellular function. Families of closely related homo- and heterodimer complexes that bind to DNA (transcription factors) regulate many of the global signaling pathways and are widely studied as disease targets.

Traditionally, transcription factors have been studied using four methods: gelshift, supershift/Electrophoretic Mobility Shift Assays (EMSA), immunoblotting and reporter gene assays. These methods are time-consuming and at best provide only semi-quantitative results. Moreover, they don't support high-throughput methods and tend to lack both sensitivity and reproducibility.

TransAM Kits are highly sensitive ELISA-based assays that are up to 100-fold more sensitive than traditional gelshift techniques. The ELISA format means that TransAM Kits not only eliminate the need for radioactivity, but also provide reproducible results in a high-throughput format.

\* 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 use of TransAM in NFκB-related drug discovery may be covered under U.S. Patent No. 6,350,090 and require a license from Ariad Pharmaceuticals (Cambridge, MA, USA).

## The TransAM method

Each TransAM Kit includes a 96-well plate in which multiple copies of a specific double-stranded oligonucleotide have been immobilized. When nuclear or whole-cell extract is added, the transcription factor of interest binds the oligonucleotide at its consensus-binding site. A primary antibody directed against the transcription factor of interest is

added, followed by a secondary HRP-conjugated antibody and HRP substrate (Figure 1). The colorimetric change is measured with a spectrophotometer and is directly proportional to the quantity of transcription factor present, providing a sensitive, quantitative assay for transcription factor activation.

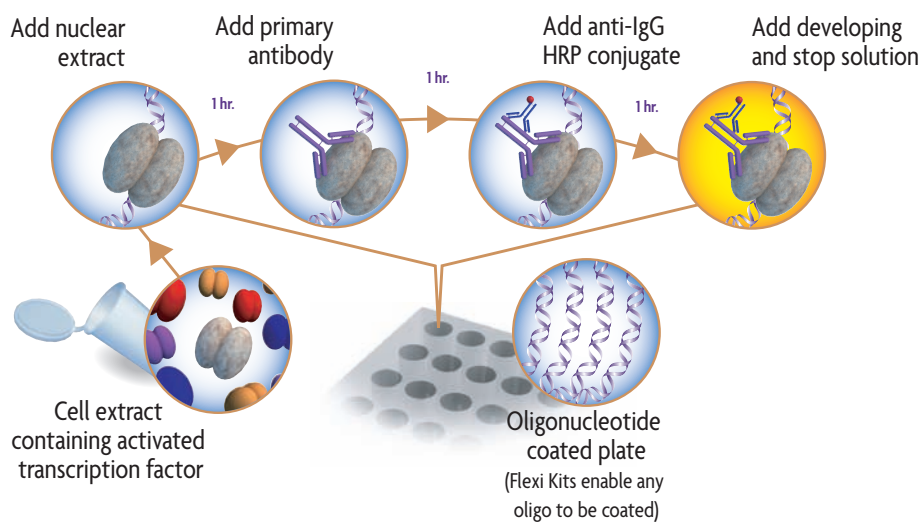


Figure 1: Flow chart of the TransAM procedure.

## Easy and safe – non-radioactive, colorimetric assay

Gelshift/EMSA assays utilize the DNA binding capability of transcription factors to bind to radiolabeled oligonucleotide probes prior to electrophoresis. This can be time consuming and hazardous work to perform. While TransAM Kits also utilize the DNA binding properties of transcription factors, unlike traditional methods, they detect activated pro-

tein using antibodies rather than radioactivity. This means that you can safely assay for a specific transcription factor without the need for radioactivity or the hassle of running gels. The colorimetric change is easily measured using a spectrophotometer, further improving the ease of using TransAM Kits.

## Proven specificity – for improved accuracy

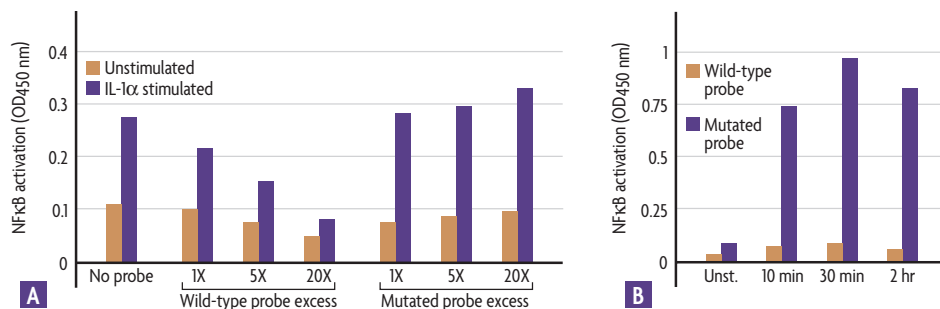
To accurately study transcription factor activation, it is vital to be able to determine which isoform of the transcription factor of interest is involved in pathway regulation. TransAM Kits are tested for specificity by assaying in the presence of an excess of oligonucleotide containing a wild-type or mutated consensus binding site (Figure 2). This competitive assay proves that the transcription factor detected is binding specifically to the probe that has been immobilized to the TransAM plate. Our antibodies are also assayed for cross-reactivity with other closely related family members to ensure that you are detecting the isoform of interest. With TransAM Kits you are guaranteed to detect only the transcription factor that you want.

## Fast, quantitative results

Western blotting and reporter assays commonly require long incubation periods either following electrophoresis or DNA transfection, which can be frustrating and inconvenient to perform. With TransAM Kits you can go from sample to quantitative results in under 5 hours. Why waste time waiting when you can have your results in the same day?

## Optimized sample preparation

To get the best results possible from TransAM, be sure to use our Nuclear Extract Kit to prepare your nuclear, cytoplasmic and whole-cell extracts. This kit contains buffers that are optimized for use with TransAM, which can help to reduce inconsistencies that may arise from using homemade buffers. The Nuclear Extract Kit is suitable for use with both cell and tissue samples.



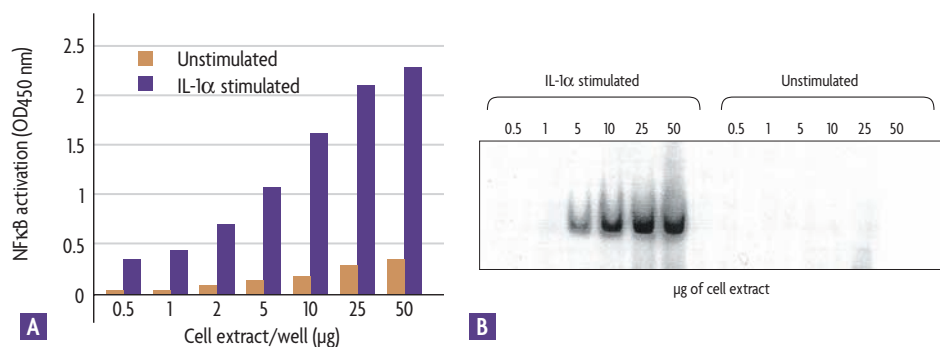
**Figure 2: Specificity of TransAM Kits.**

TransAM NFκB p50 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 and 30 minutes, and 2 hours (B).

## Sensitive – for improved transcription factor monitoring

Small changes in transcription factor levels can have a significant impact on cellular function. Therefore, it is vital to use a sensitive assay when studying transcription factor activation. TransAM Kits are 10-fold more sensitive than gelshift assays (Figure 3). And, TransAM Chemi Kits are even better, with

more than 100-fold increased sensitivity as compared to gelshift assays. Not only does this enable you to monitor small changes in activated transcription factor levels, but you can subtract the level of endogenous factor present in your untreated samples, further improving your results.



**Figure 3: TransAM NFκB is 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 complete solution for the future

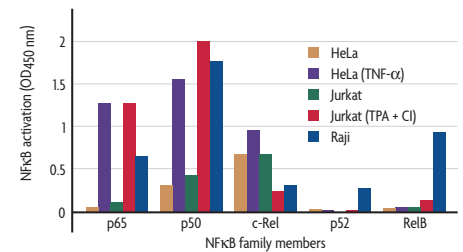
Reporter constructs expressed transiently or from a stable cell line are commonly used to monitor transcription factor activation. However, while such systems are convenient, it can be difficult to relate this data to actual *in vivo* experiments, which can limit more

relevant, downstream research. TransAM Kits provide a complete solution that is well suited for both model systems and *in vivo* studies. Don't limit tomorrow's experiments with reporter assays, when you can use TransAM today.

## TransAM Family Kits – NFκB, AP-1, STAT & GATA

Transcription factor families consist of various groups of homo- and heterodimer subunits that are differentially regulated within the cell. Therefore, being able to profile which members of a transcription factor family are involved in cell regulation can help identify potential disease targets. However, profiling an entire transcription factor family using gelshift, Westerns or reporter assays is time consuming and expensive. TransAM Family Kits enable you to profile activation of various transcription factor family members

in less than 5 hours using one simple kit. The TransAM NFκB Family Kit includes antibodies specific for detection of activated p65, p50, p52, c-Rel and RelB (Figure 4). The TransAM AP-1 Family Kit includes antibodies specific for active c-Fos, FosB, Fra-1, Fra-2, c-Jun, JunB and JunD. The TransAM STAT Family Kit detects phosphorylated STATα, STAT3, STAT5A and STAT5B. The TransAM GATA Family Kit detects GATA-1, GATA-2 and GATA-3. For details, visit our website at [www.activemotif.com/transam](http://www.activemotif.com/transam).

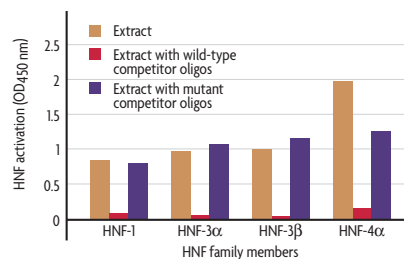


**Figure 4: Profiling NFκB family DNA binding activation.** Nuclear extracts were prepared from HeLa, HeLa treated with TNF-α, Jurkat, Jurkat treated with TPA and calcium ionophore, & Raji cells, and then assayed at 10 μg/well.

## TransAM Multiple-oligo Family Kits – MAPK & HNF

Some transcription families have members that do not share a single consensus-binding site, making EMSA and reporter assays difficult. To overcome this, Active Motif has developed TransAM Family Kits that have multiple binding sites in each well. TransAM HNF Family Kits contain a mixture of oligonucleotides with the HNF-1, -3 and -4 binding sites. HNF dimers contained in nuclear extracts bind specifically to these oligonucleotides and are detected using antibodies against HNF-1, -3α, -3β and -4α (Figure 5). Similarly, the TransAM MAPK Family Kit contains a mixture of oligos and antibodies that enable detection of activated ATF-2, c-Jun,

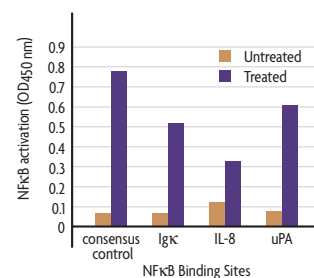
c-Myc, MEF2 and STATα. By immobilizing a mixture of binding sites, each well in these kits can be used to assay any family member.



**Figure 5: Specific assay for HNF family members.** Nuclear extracts from unstimulated Hep G2 cells were assayed at 5 μg/well using the TransAM HNF Family Kit in the absence or presence of 20 pmol of a mixture of competitor oligonucleotides.

## TransAM Flexi Kits

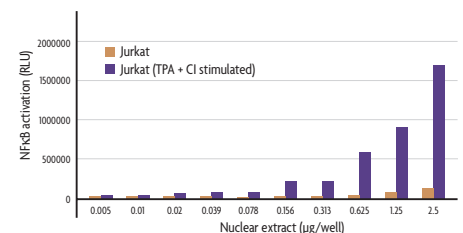
TransAM Flexi Kits enable efficient study of transcription factor binding at any DNA-binding site. With TransAM Flexi, you can study variant transcription factor-binding sites, analyze native promoters (Figure 7), confirm chromatin immunoprecipitation (ChIP) results and determine isoform-binding affinity. To measure your site of interest, first design appropriate biotinylated oligos. Each oligo is incubated with nuclear extract and transferred to a 96-well streptavidin-coated plate. Primary antibody for the factor of interest is added, followed by secondary antibody and developing reagent.



**Figure 7: Testing natural NFκB binding sites.** Five μg of nuclear extract from untreated and TNF-α-treated HeLa cells were used to assay for NFκB binding affinity to four different 50-mer oligonucleotides. Three oligos were synthesized to represent the natural binding sites on various promoters regulated by NFκB: Igκ, IL-8 and uPA.

## TransAM Chemi Kits

Researchers who require maximum sensitivity or with a limited amount of sample will love TransAM Chemi Kits. With chemiluminescent detection, transcription factor activation can be assayed using as little as 40 ng of cell extract (Figure 6). This makes TransAM Chemi Kits over 100-fold more sensitive than gelshift assays. Plus, you get the added convenience of TransAM's non-radioactive, 96-stripwell format. Don't waste your precious sample or be forced to pool together multiple samples; assay your samples with TransAM Chemi Kits.



**Figure 6: TransAM NFκB p50 Chemi sensitivity.** Nuclear extracts from Jurkat cells and Jurkat cells stimulated with TPA and calcium ionophore were assayed from 0.005 to 2.5 μg/well for NFκB p50 activation using the TransAM NFκB p50 Chemi Kit.

## Latest additions

We are continually developing new TransAM Kits. Plus, we also now offer functionally active recombinant proteins. To learn more, please go to our website to download more complete, up-to-date information.

## TransAM – Ordering information

Product	Format	Catalog No.
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
TransAM™ AP-1 Family <sup>1</sup>	2 x 96-well plates	44296
	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
TransAM™ ATF-2	1 x 96-well plate	42396
	5 x 96-well plates	42896
TransAM™ c-Myc	1 x 96-well plate	43396
	5 x 96-well plates	43896
Recombinant c-Myc protein	5 µg	31117
TransAM™ C/EBP $\alpha/\beta$	1 x 96-well plate	44196
	5 x 96-well plates	44696
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
Recombinant CREB protein	10 µg	31107
TransAM™ Elk-1	1 x 96-well plate	44396
	5 x 96-well plates	44896
TransAM™ ER	1 x 96-well plate	41396
	5 x 96-well plates	41996
TransAM™ FKHR (FOXO1)	1 x 96-well plate	46396
	5 x 96-well plates	46896
TransAM™ GATA Family <sup>2</sup>	2 x 96-well plates	48296
	1 x 96-well plate	46496
TransAM™ GATA-4	1 x 96-well plate	46496
	5 x 96-well plates	46996
TransAM™ GR	1 x 96-well plate	45496
	5 x 96-well plates	45996
TransAM™ HIF-1	1 x 96-well plate	47096
	5 x 96-well plates	47596
TransAM™ HNF Family <sup>3</sup>	2 x 96-well plates	46296
	1 x 96-well plate	46196
TransAM™ HNF-1	1 x 96-well plate	46196
	5 x 96-well plates	46696
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
TransAM™ IRF-7	1 x 96-well plate	50196
	5 x 96-well plates	50696
TransAM™ MAPK Family <sup>4</sup>	2 x 96-well plates	47296
	1 x 96-well plate	43196
TransAM™ MEF2	1 x 96-well plate	43196
	5 x 96-well plates	43696
TransAM™ MyoD	1 x 96-well plate	47196
	5 x 96-well plates	47696

Product	Format	Catalog No.
TransAM™ NF-YA	1 x 96-well plate	40396
	5 x 96-well plates	40896
TransAM™ NFATc1	1 x 96-well plate	40296
	5 x 96-well plates	40796
TransAM™ Flexi NF $\kappa$ B Family <sup>5</sup>	2 x 96-well plates	43298
	1 x 96-well plate	41098
	1 x 96-well plate	40098
TransAM™ NF $\kappa$ B Family <sup>5</sup>	2 x 96-well plates	43296
	1 x 96-well plate	41096
TransAM™ NF $\kappa$ B p50	1 x 96-well plate	41096
	5 x 96-well plates	41596
	1 x 96-well plate	41097
TransAM™ NF $\kappa$ B p50 Chemi	1 x 96-well plate	41097
	5 x 96-well plates	41597
Recombinant NF $\kappa$ B p50 protein	5 µg	31101
TransAM™ NF $\kappa$ B p52	1 x 96-well plate	48196
	5 x 96-well plates	48696
TransAM™ NF $\kappa$ B p52 Chemi	1 x 96-well plate	48197
	5 x 96-well plates	48697
TransAM™ NF $\kappa$ B p65	1 x 96-well plate	40096
	5 x 96-well plates	40596
TransAM™ NF $\kappa$ B p65 Chemi	1 x 96-well plate	40097
	5 x 96-well plates	40597
Recombinant NF $\kappa$ B p65 protein	5 µg	31102
TransAM™ Nrf2	1 x 96-well plate	50296
	5 x 96-well plates	50796
TransAM™ Oct-4	1 x 96-well plate	42496
	5 x 96-well plates	42996
TransAM™ p53	1 x 96-well plate	41196
	5 x 96-well plates	41696
Recombinant p53 protein	5 µg	31103
TransAM™ PPAR $\gamma$	1 x 96-well plate	40196
	5 x 96-well plates	40696
TransAM™ Sp1	1 x 96-well plate	41296
	5 x 96-well plates	41796
TransAM™ Sp1/Sp3	1 x 96-well plate	40496
	5 x 96-well plates	40996
Recombinant Sp1 protein	5 µg	31136
TransAM™ STAT Family <sup>6</sup>	2 x 96-well plates	42296
	1 x 96-well plate	45196
TransAM™ STAT3	1 x 96-well plate	45196
	5 x 96-well plates	45696
TransAM™ T-bet	1 x 96-well plate	40396
	5 x 96-well plates	40896
Nuclear Extract Kit	100 rxns	51396
	400 rxns	51896
ProStain™ Protein Quantification	1000 rxns	15001

1. AP-1 Family includes: c-Fos, FosB, Fra-1, Fra-2, c-Jun, JunB & JunD
2. GATA Family includes: GATA-1, GATA-2 & GATA-3
3. HNF Family includes: HNF-1, HNF-3 $\alpha$ , HNF-3 $\beta$  & HNF-4 $\alpha$
4. MAPK Family includes: ATF-2, c-Jun, c-Myc, MEF2 & STAT1 $\alpha$
5. NF $\kappa$ B Family includes: p50, p52, p65, c-Rel & RelB
6. STAT Family includes: STAT1 $\alpha$ , STAT3, STAT5A & STAT5B

See Contents tab on each kit's page at [www.activemotif.com/transam](http://www.activemotif.com/transam) for kit-specific contents and storage information.