



# Tools For Transcription Factor Research

## Transcription Factors—Pivotal Proteins in Transcription Regulation

The process by which a DNA sequence is enzymatically copied by RNA polymerase to produce complimentary RNA is known as transcription. In the case of protein-coding DNA for example, transcription is the start of the process that eventually leads to the creation of a functional peptide or protein, via the mRNA intermediate. Transcription Factor proteins play a pivotal role in the process, acting as regulators of gene expression, specifically regulating the activation of transcription in the eukaryotic nucleus. They do this through binding, either by themselves, or by controlling the binding of other proteins to the promoter and enhancer sequence elements of the genes to be transcribed. As such master regulators, Transcription Factors are biologically interesting targets to many researchers.

## Panomics—Delivering Tools for Transcription Factor Research

We have developed a comprehensive suite of tools to aid in Transcription Factor research. Our comprehensive profiling assays, including a unique multiplex assay, allows researchers to monitor the activities of many Transcription Factors simultaneously. Changes in Transcription Factor activity in response to environmental or physiological stimulus can be indicative of changes to specific signaling pathways and can be used as biomarker fingerprints or signatures. Thus, studies of TFs are particularly useful for cancer researchers, physiologists, immunologists and virologists.

Our comprehensive and growing ELISA menu for the most important Transcription Factors are the most sensitive on the market, and all products have the same simple assay workflow.

The use of Stable Cell Lines is becoming important as more contextual cell-based assays are being developed, particularly in the pharmaceutical industry. Our range of stable cell lines help with this paradigm shift in assay development.

We also offer a comprehensive range of EMSA Gel-Shift assays and unique reporter assays.

### Product Portfolio

Stable Cell Lines

Transcription Factor Profiling Assay

Transcription Factor Luminex Assays

Transcription Factor ELISA Kits

Transcription Factor EMSA Gel-shift Assays

Transcription Factor Reporter Assays

## Stable Cell Lines

### NFκB Reporter A549, HeLa, 293, NIH3T3, and C2C12 Stable Cell Lines

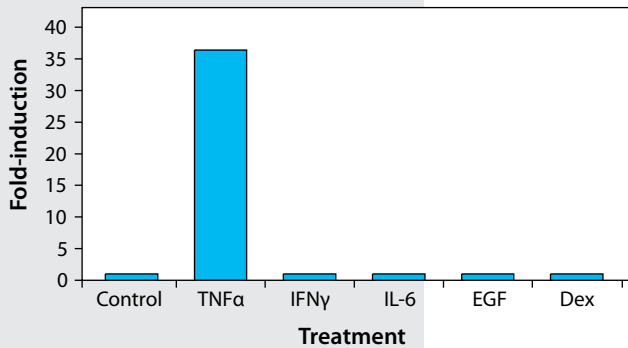
For high-throughput analysis of NFκB activation

- Monitor biochemical changes in NFκB pathway
- Screen compounds that inhibit or activate any pathway components
- No hassle or reporter construct transfection

Panomics offers NFκB, CREB, AP-1, GR, NFAT, STAT, SRF, and HIF Stable Reporter Cell Lines for high-throughput analysis of in vivo drug efficacy and specificity. These stable cell lines also provide a reproducible, ready-to-use platform for performing cell-based assays. Among other applications, they can be used to evaluate uncharacterized growth factors, extracellular stimuli, and upstream events in the NFκB, CREB, AP-1, NFAT, STAT, SRF, and HIF signaling pathways. We also offer custom Stable Reporter Cell Lines, please contact your Panomics representative for further details.

- NFκB Reporter A549, HeLa, 293, NIH3T3 and C2C12 Stable Cell Lines
- NFκB p50 Knockdown Stable Cell Line
- CREB Reporter 293 and CHO Stable Cell Lines
- AP-1 Reporter 293 HeLa Stable Cell Line
- GR Reporter 293 and HeLa Stable Cell Line
- NFAT Reporter HeLa and K562 Stable Cell Lines
- STAT-1 Reporter HeLa Stable Cell Line
- STAT-3 Reporter HeLa Stable Cell Line
- SRF Reporter HeLa Stable Cell Line
- HIF Reporter NIH3T3 Stable Cell Line
- NFκB p50 Knockdown Stable Cell Line

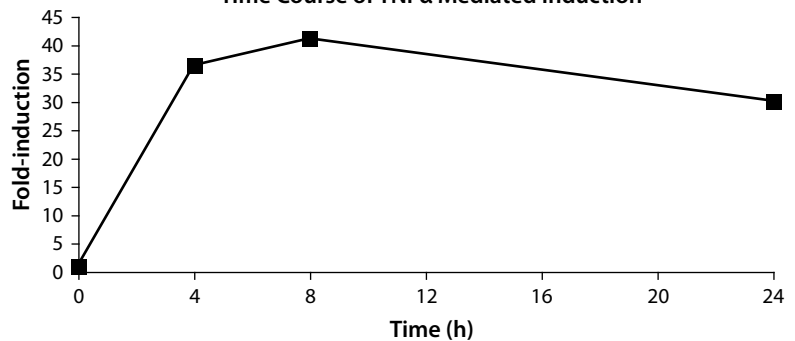
**Induction of Luciferase Activity in NIH3T3/NFκB-luc Stable Reporter Cell Line**



#### Induction of Luciferase Activity by Different Stimuli

NIH3T3/NFκB-luc cells were treated with different factors for 8 hours. Induction of luciferase activity is very specific with cells only responding to TNFα, but not interferon-γ (IFNγ), interleukin-6 (IL-6), Epidermal Growth Factor (EGF) or dexamethasone (Dex).

**Time Course of TNFα Mediated Induction**

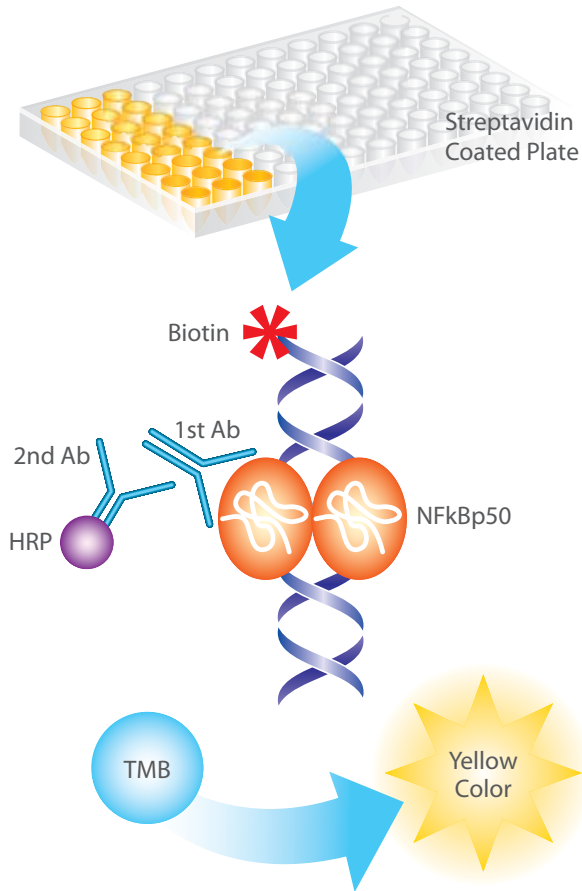


#### Time Course of TNFα induction

NIH3T3/NFκB-luc cells were treated with 20 ng/ml TNFα over a 24 hour time course. Luciferase activity is induced after 4 hours and maintained for at least 24 hours with TNFα.

# Panomics ELISA Assays—When Sensitivity Matters

**Transcription Factor ELISA Kits:** NFkBp50 • NFkBp65 • HIF • ER • AP-1 • p53 • GR • STAT-1 • MEF-2 • Sp-1 • GATA-1 • PPAR $\gamma$  • PPAR $\alpha$  • FKHR • SMAD-4 • SMAD-2 • EGR-1 • HNF-3 • NFAT • E2F-1



## Workflow

- Incubate biotin labeled TF-consensus DNA complex to the streptavidin coated plate
- Add primary targeted TF antibody for specificity
- Add secondary detection antibody, conjugated to horseradish peroxidase (HRP), for quantitation

## Advantages

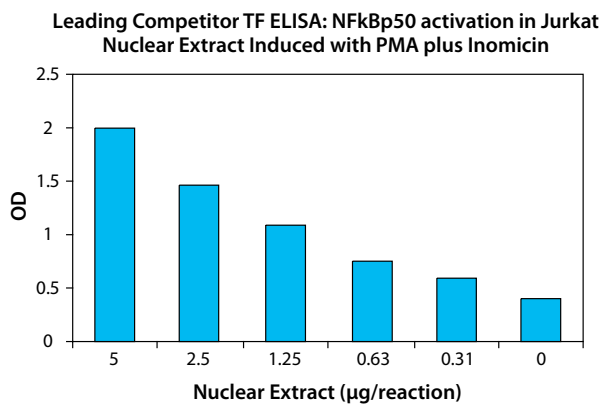
**Specific:** quantify transcription factor activation

**Sensitive:** detect activation with as little as 0.5  $\mu$ g cellular extract

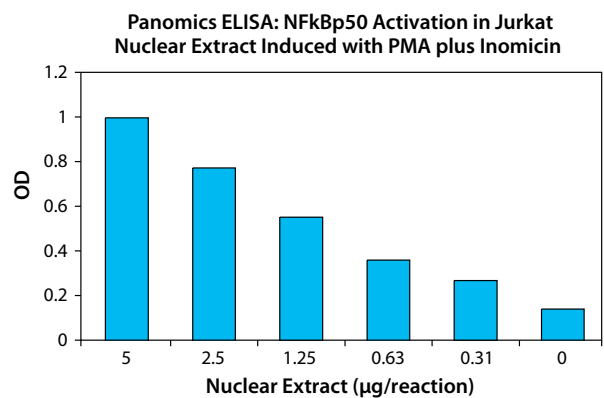
**Fast:** assay completed in less than 4 hours

**User Friendly:** all assays formatted to same workflow

## High-Sensitivity: Comparison to a Leading Competitor



Leading Competitor NFkBp50 activation in Jurkat cells  
Sensitivity 0.63  $\mu$ g/reaction



Panomics NFkB activation in Jurkat cells  
Sensitivity 0.31  $\mu$ g/reaction

# Profiling Assays for Global Analysis of Transcription Factors

## TF Membrane Array Solutions

### Protein/DNA Arrays

With these Arrays, you can profile the activities of multiple TFs simultaneously, allowing the study of TF activation in a variety of biological processes, including cell proliferation, differentiation, transformation, and apoptosis.

### Function-Specific Protein/DNA Arrays

Function-Specific Protein/DNA Arrays are designed for researchers who need to focus on TFs involved in specific biological processes. We currently offer three function-specific arrays:

- cAMP/Calcium Protein/DNA Array
- Nuclear Receptor Protein/DNA Array
- Cell Growth Protein/DNA Array

### TF – TF Interaction Arrays

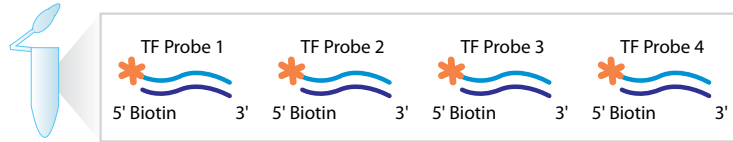
TF-Specific-TF Interaction Arrays enable you to determine how NFκB, PPAR, GR, p300, and SRC-1 interact with multiple TFs—all in one experiment.

### TF Protein Arrays

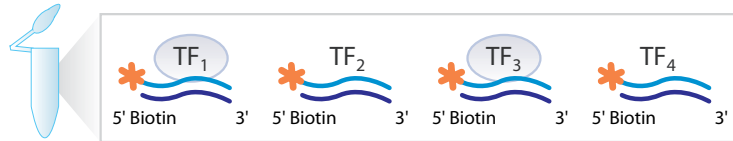
With Panomics' TF Protein Array, you can determine how a particular protein interacts with multiple transcription factor proteins or analyze which transcription factors regulate gene expression.

*\*These DNA consensus sequences are regions in the promoters of the genes the TF targets regulate—also known as cis-elements. These cis-regulatory elements are often binding sites of one or more trans-acting factors. A cis-element may be located in the promoter region 5' to the gene it controls, in the intron, or in the 3' region.*

### 1. Mixture of pre-labeled TF probes



### 2. Incubate with nuclear extract



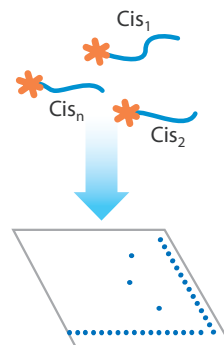
### 3. Separation of protein/DNA complexes

#### Spin column



1. Mix biotin labeled DNA binding probes with a nuclear extract or cell lysate, this allows the formation of "DNA/Protein" complexes.
2. After incubation, these complexes are added to a spin column which allows the separation of unbound probes.
3. After elution of the DNA/Protein complexes, they are denatured to liberate the free probe.
4. Hybridize the free probe to the membrane, which contains an array of Transcription Factor consensus binding sequences.\*

### 4. Hybridization



**Efficient**—Profile multiple transcription factors simultaneously

**Easy**—All-in-one system; no additional equipment needed

**Safe**—No radioactivity required

**High Sensitivity**—Improved HRP-based chemiluminescence

**No Antibodies**—Removes lot to lot variations

**Multiple Arrays**—Choose from a variety of arrays

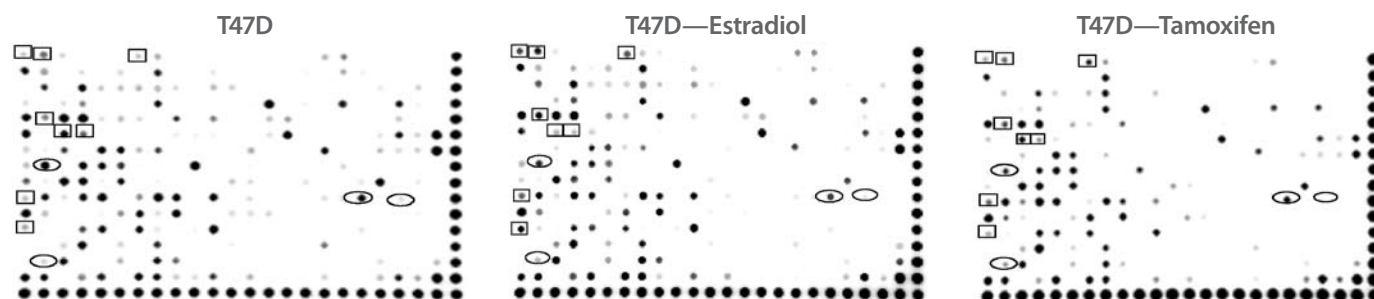
## Applications

Protein/DNA (PD) arrays can be used to monitor TF expression levels as cells (experimental against a control) are perturbed by different stimuli or as a result of being subjected to different physiological states. This approach can aid in the understanding of signaling pathways and can also be used to generate biomarker fingerprints or signatures. PD arrays can also be used

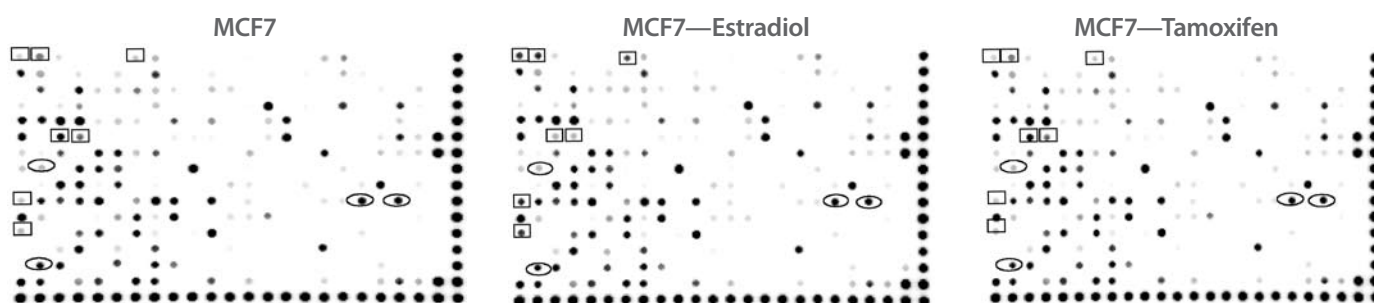
as tools for developing cell-based assays. Compounds that inhibits receptors, kinases, or other signaling proteins will lead to changes in activation of TFs. PD arrays can quantify changes in activation. This information can be used to guide development of TF Reporter Stable Cell Lines.

## Array Analysis to Identify Biomarkers of Breast Cancer Cell Lines

### A) T47D: ER+ TAM Resistant



### MCF7: ER+ TAM Resistant



B)

	AP-1	STAT-1	EGR-1	ER	HiF	p53	NF-1	VDR	RXR	MBP-1	PBGD
T47D	±	±	±	+	±	±	++	++	+++	++	±
T47D-estradiol	+++	+++	++	+++	++	±	++	±	±	++	±
T47D-tamoxifen	±	±	++	+	++	±	++	++	++	++	±
MCF7	±	+	±	+	±	++	±	+++	++	+++	+++
MCF7-estradiol	+++	+++	+++	+++	++	++	±	+	±	+++	+++
MCF7-tamoxifen	±	±	±	+	±	++	±	++	+++	+++	+++

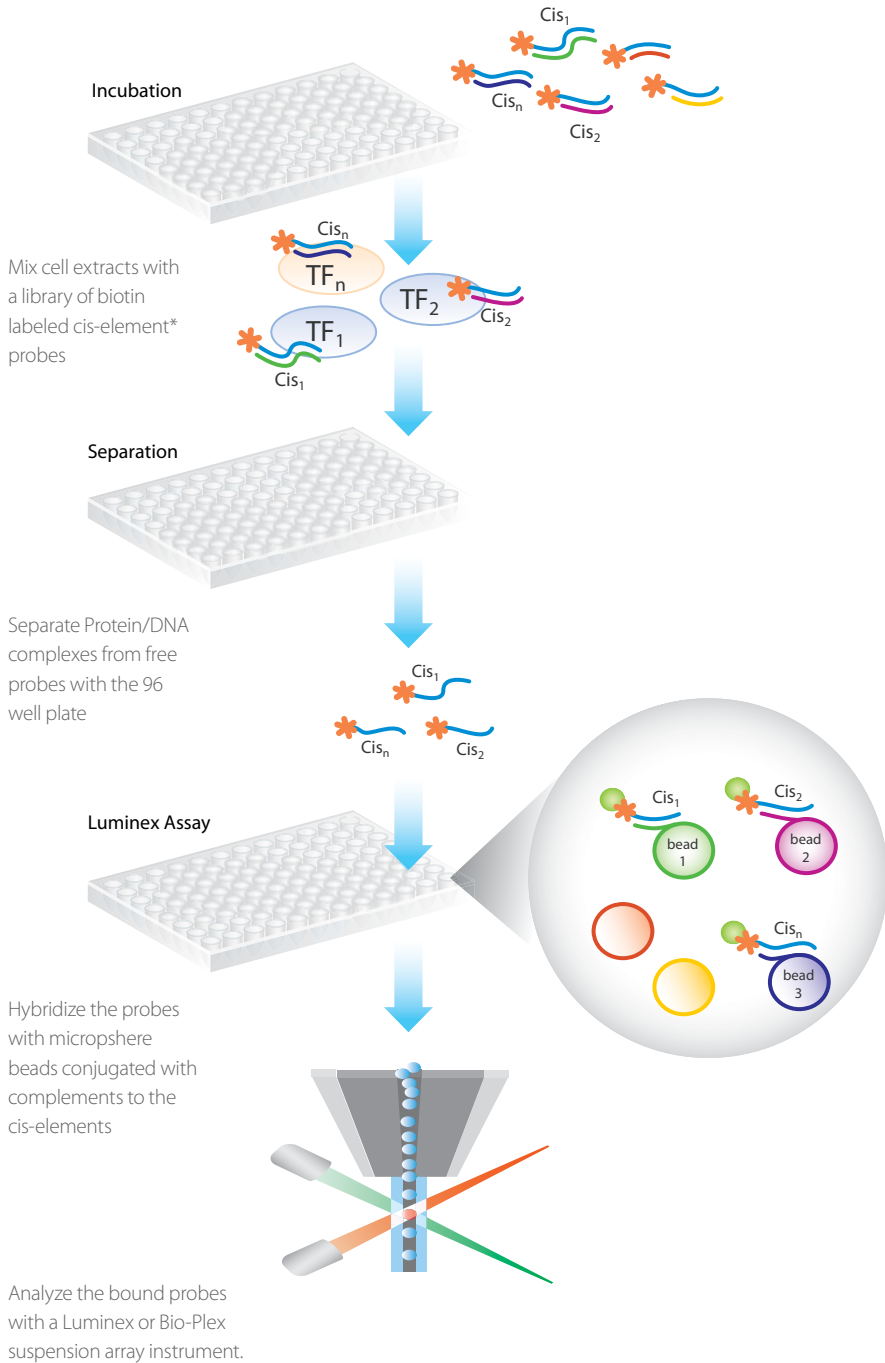
Two cancer cell lines; T47D and MCF7, were examined for TF activity. Comparisons were made following treatment with estradiol and tamoxifen. A number of unique TF activity patterns were identified for each cell line, which allowed a potential correlation between estrogen and tamoxifen treatment to be established.

A. Panomics' Protein/DNA Combo Array analysis of two breast cancer cell lines, MCF7, T47D untreated, estradiol-treated and tamoxifen treated samples were analyzed respectively. TFs whose activities were changed in response to treatments were surrounded by boxes. TFs with distinct activities were surrounded by ovals.

B. A summary table lists the changes in activities of several important TFs. ± represents a density reading below 10; + represents a density reading between 10-20; ++ represents a density reading between 20-40, and +++ represents a density reading greater than 40.

## Procarta™ Transcription Factor Luminex Assays

Our novel, Procarta TF assay allows for the profiling of multiple TF's from a variety of sample types including cell lysates and nuclear extracts. Up to 40 TF's can be analyzed in one well.



## Procarta Transcription Factor Profiling Assays

Transcription factors (TFs), which play crucial roles in the regulation of gene expression in the human genome, are highly regulated by a variety of mechanisms. A single extracellular stimulus can trigger multiple signaling pathways, and these in turn can activate multiple TFs to mediate the inducible expression of target genes. Alterations in the activities of TFs are often associated with human diseases, such as altered activating factor 1, estrogen receptor, and p53 function in cancer, nuclear factor kB in inflammatory diseases, and peroxisome proliferator-activated receptor gamma in obesity. A systematic assay for profiling the activation of TFs will aid in elucidating the mechanisms of TF activation, reveal altered TFs associated with human diseases, and aid in developing assays for drug discovery. We have developed a 40-plex fluorescent microsphere-based TF activation assay system with a 96-well plate format. The assay system enables high-throughput profiling of the DNA binding activity of TFs in multiple samples with high sensitivity.

### Procarta TF Menu

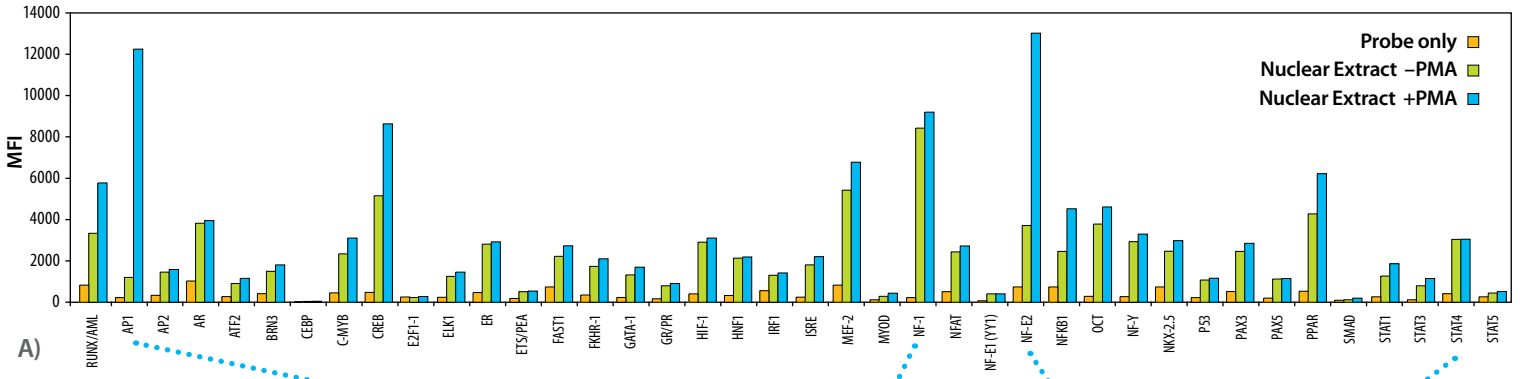
RUNX/AML	ELK-1	ISRE	OCT
AP-1	ER	MEF-2	p53
AP-2	ETS/PEA	MYOD	PAX-3
AR	FAST-1	NF-1	PAX-5
ATF-2	FKHR-1	NFAT	PPAR
BRN-3	GATA-1	NF-E1/YY1	SMAD
CEBP	GR/PR	NF-E2	STAT-1
C-MYB	HIF-1	NFkB	STAT-3
CREB	HNF1	NKX-2.5	STAT-4
E2F-1	IRF-1	NF-Y	STAT-5

Visit our website to see the most current list.

## Monitoring PMA-Mediated Induction in Nuclear Extracts

Panomics Procarta Transcription Factor Assay was used to simultaneously measure the activity of 40 TFs in a single assay well. HeLa cells were serum starved overnight and subsequently stimulated PMA or a vehicle control for 4 hours.

### TF Plex 40-plex assay: Nuclear Extract from HeLa +/- PMA



### Sample Inductions Confirmed by EMSA

To confirm the inductions observed in the Procarta TF assay, EMSA Gel Shift assays were run.

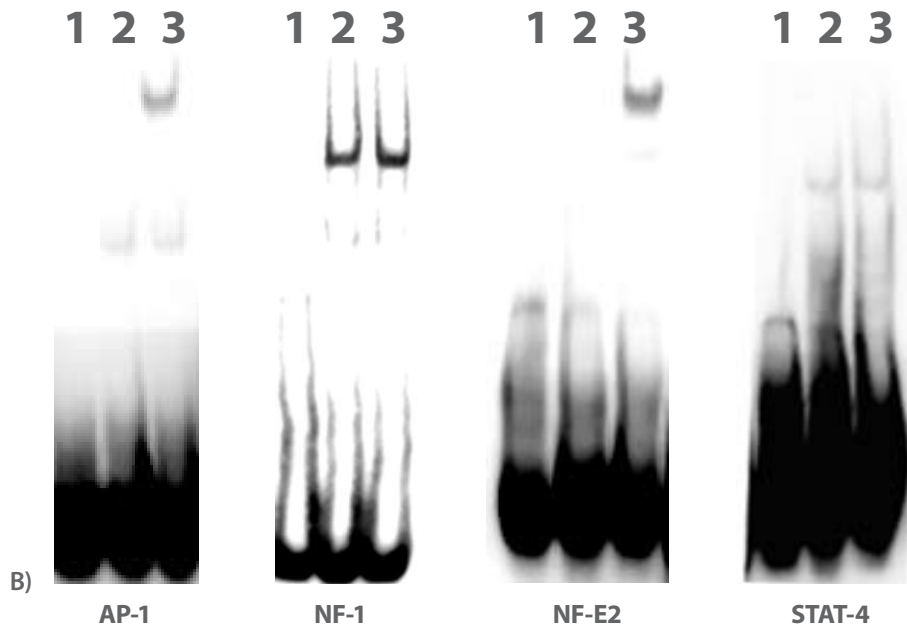
A) As expected, PMA induced AP1 and NF-E2 activity and the activity of NF-1 and STAT4 were unchanged.

B) Sample inductions were confirmed by EMSA Gel Shift assays.

Lane 1 = free probe

Lane 2 = HeLa nuclear extract

Lane 3 = PMA induced HeLa nuclear extract



For pricing and more information visit our website at [www.panomics.com](http://www.panomics.com) or call us at 1.877.726.6642.

## EMSA Gel-Shift Assays

Greater than 424 EMSA kits are available covering most widely studied transcription factors.

**Easy**—identify DNA-binding proteins

**Economical**—all-in-one system

**Highly Sensitive**—HRP-based detection system

**Safe**—no radioactivity required

Panomics' EMSA (Electrophoretic Mobility Shift Assay) Kits are useful tools for identifying proteins that interact with DNA. This rapid technique is based on the different electrophoretic mobilities of free DNA and DNA/protein complexes in native (non-denaturing) polyacrylamide gels. The procedure is simple:

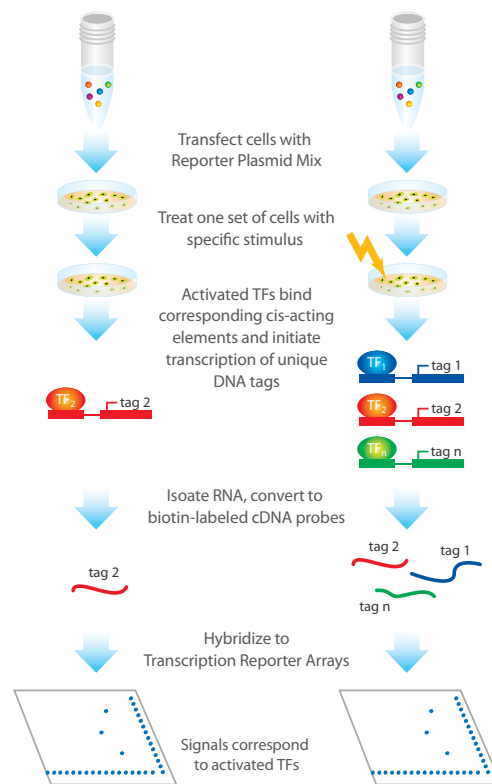
1. Incubate the biotin-labeled DNA probe with your protein(s) of interest (purified or crude extract).
2. Separate the mixture on a non-denaturing polyacrylamide gel.
3. Visualize shifted bands that correspond to the protein/DNA complexes.

Unlike other commercially available EMSA kits, Panomics' Kits include specific transcription factor probes. We currently offer 345 kits, which correspond to the transcription factors included on the Protein/DNA Arrays I, II, III, IV, and V. Additionally, we offer a combination kit, which includes any three probe sets. Probe sets can also be purchased separately.

For more details visit: <http://www.panomics.com/EMSAkit.cfm>

## Transcription Factor Reporter Assay

With Transcription Reporter Arrays, you can simultaneously monitor the activation of multiple transcription factors in signal transduction pathways *in vivo*. You can also obtain information about cross-talk between pathways.



**The procedure is simple:**  
A library of reporter constructs, each of which contains a cis-element and a unique tag sequence, is transfected into host cells.

The expression of the reporter tags will be induced only if their corresponding TFs are activated and bound to the cis-elements.

Then the expressed tags can be determined with the Transcription Reporter Array. Activated TFs will show up as positive signals on the array membrane.

For more details visit: <http://www.panomics.com/MA4010.cfm>



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