

**Procarta™ Transcription Factor Assay:  
High Throughput, Quantitative Platform for the  
Measurement of Activated Transcription Factors**

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## Abstract

Transcription factors (TFs) play a crucial role in the regulation of gene expression in the human genome and 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 AP1, ER, and p53 function in cancer, NFκB in inflammatory diseases, and PPARγ 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. Here, we describe a commercially available 40-plex Luminex® based assay for the measurement of activated TFs. This assay is designed around the standard 96-well plate format enabling high-throughput, multiplex profiling of the DNA binding activity of TFs in multiple samples with high-sensitivity.

## Introduction

A single signal pathway can activate multiple Transcription Factors, and a single TF can be activated by multiple signal pathways. For example, TNFα can activate NFκB and AP1 as well as other TFs such as E47. NFκB can be activated by the cytokines TNFα, IL1, and the phorbol ester PMA. However, accurate elucidation of these complex, interconnected relationships between signaling molecules requires a technique that can profile the activities of multiple TFs simultaneously. In vitro analysis of TFs has been historically conducted using the electrophoretic mobility shift assay (EMSA) and for in vivo analysis through the use of a luciferase reporter assay. Both of these assays, however, are single plex assays, which detect only one TF per reaction and, therefore, are not suited for high throughput profiling of multiple TFs with multiple samples. At Panomics, we have developed a commercially available, multiplex assay that can analyze up to 40 TFs from a nuclear extract or whole cell lysate sample and up to 96 samples/controls in a microtiterplate based format utilizing the Luminex technology. This 40 plex TF assay is a sensitive and quantitative method for profiling the activation of multiple TFs.

Methods	Format	Number of Target TF per Reaction	Number of Samples per Assay
EMSA	Gel Electrophoresis	1	1 per 3 Lane
ELISA	Antibody Detection	1	96 per Plate
Protein/DNA Array	DNA Based Membrane	50–345	1 per Membrane
Microsphere-based Assay	Multiplexed Microsphere	25–50	96 per Plate

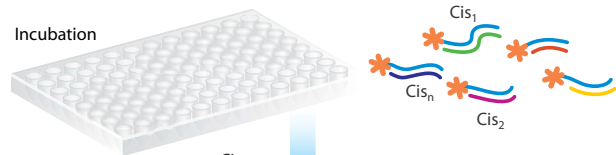
## Assay Overview

The Procarta Transcription Factor Assay is designed to measure the activated transcription factors through the use of double stranded cis-elements (the sense strand is biotinylated). A mixture of up to 40 different cis elements are incubated with individual samples prepared from either whole cell or nuclear extracts. The samples are then transferred to a separation plate and the excess cis elements and unbound proteins are removed through the porous bottom of the separation plate.

Once washed, the activated TF's bound to the cis elements are denatured; the proteins remain bound to the porous membrane and the cis elements are eluted into a PCR plate. The cis elements are denatured at 95°C, chilled on ice, then bound to Luminex beads conjugated to the anti-sense sequence of the TF of interest. The beads are washed and incubated with streptavidin-PE. After incubation, the beads are then washed and read on the Luminex platform.

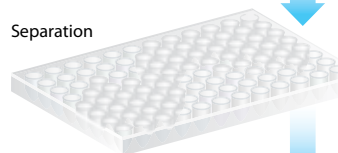
### Step 1:

Incubation of the cis element probes with the nuclear extract or whole cell lysate in 96 well plate.



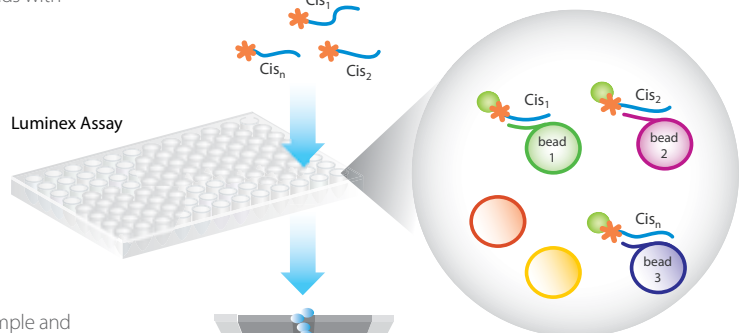
### Step 2:

Transfer probe TF mixture to separation plate and wash TF bound probe mix. Denature cis elements away from TFs.



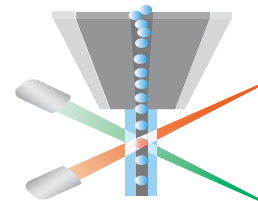
### Step 3:

Denature cis elements by heat and anneal to Luminex beads with anti-sense sequence.



### Step 4:

Add streptavidin PE to sample and read on Luminex machine

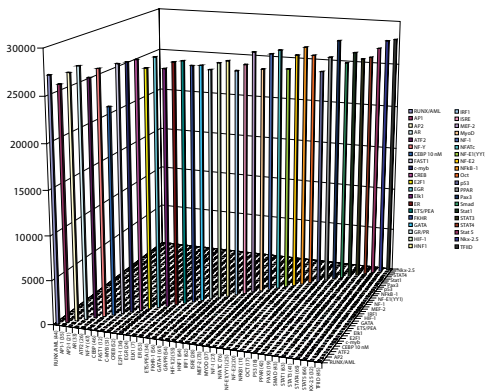


# High Throughput, Quantitative Platform for Transcription Factors

## Material and Methods

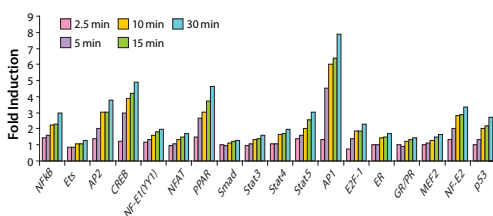
Preparation of nuclear extracts and gel shift assays (EMSA) were performed using kits (Panomics) according to the manufacturer's instructions. For EMSA assays, 5 µg of nuclear extract was used per reaction. The Procarta Transcription Factor assay performed in this study was carried out following the procedure in the Procarta TF Kit User manual (Panomics). For Procarta assays, 2 µg of nuclear extract was used per multiplex reaction. The probes for the EMSA assay were 5'-biotin-labeled and identical in sequences to the probes used in the Procarta TF assay. A table and chart is illustrated below of the possible TF's available for profiling with the Procarta TF assay as well as the specificity of the probes used in this assay.

RUNX/AML	CREB	GR/PR	NFAT	PAX3
AP1	E2F1	HIF-1	NF-E1(Y1)	PAX5
AP2	ELK1	HNF1	NF-E2	PPAR
AR	ER	IRF1	NFκB1	SMAD
ATF2	ETS/PEA	ISRE	NKX-2.5	STAT1
BRN3	FAST1	MEF-2	NF-Y	STAT3
CEBP	FKHR-1	MYOD	OCT	STAT4
C-MYB	GATA-1	NF-1	P53	STAT5

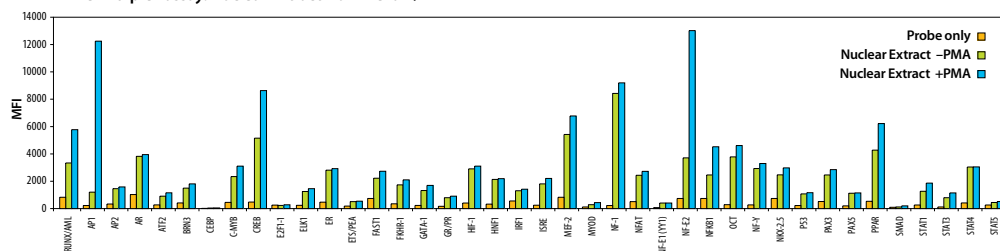


## TNFα Induction

Human HeLa cells were maintained in DMEM containing 10% fetal bovine serum, 100 units/ml penicillin and 0.1 mg/ml streptomycin at 37°C and 5% CO<sub>2</sub>. For nuclear extraction of TNFα-treated HeLa cells, the cells were starved for 16 h with DMEM containing 0.2% FBS, 100 units/ml penicillin and 0.1 mg/ml streptomycin at 37°C and 5% CO<sub>2</sub>, and treated with 10 ng/ml TNFα. Nuclear extractions were prepared at time points of 2.5, 5, 10, 15 and 30 minutes induction. A 19 plex assay was performed on these samples and fold induction was measured by normalizing the treated samples against the untreated sample.

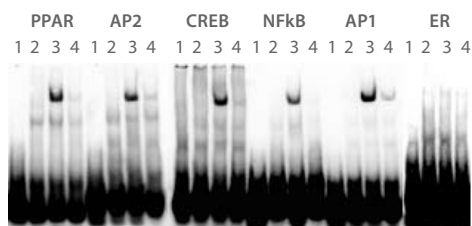


TF Plex 40-plex assay: Nuclear Extract from HeLa +/- PMA



## EMSA Confirmation

The Procarta TF assay was confirmed using the electrophoretic mobility shift assay of selected transcription factors. Results from EMSA gave similar fold induction compared to Procarta TF assay. Lane 1 is probe only, Lane 2 is untreated, Lane 3 is treated at 30 min and Lane 4 is cold and labeled probes

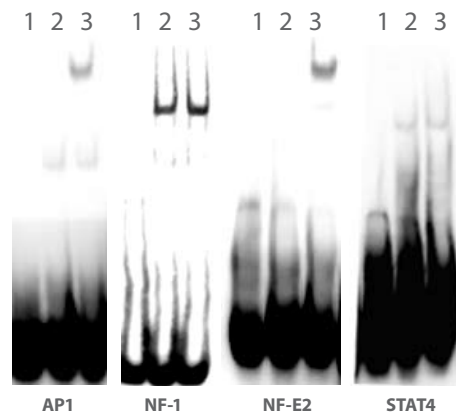


## PMA Induction

Nuclear extracts from HeLa cells were prepared as previously described but induced with PMA at 10 ng/ml. The median fluorescent intensity was measured of treated and untreated samples and displayed in the graph above.

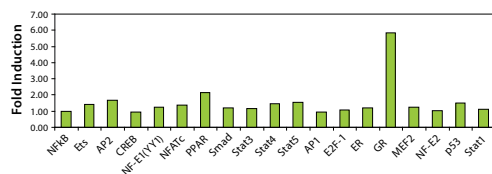
## EMSA Confirmation

Confirmation of the the PMA treated HeLa cells measured by the Procarta TF assay was confirmed using the electrophoretic mobility shift assay of selected transcription factors. Results from EMSA gave similar fold induction compared to Procarta TF assay. Lane 1 is probe only, Lane 2 is untreated and Lane 3 is PMA treated.



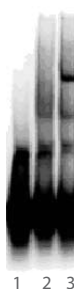
## GR Detection

Cos-1 cells were maintained in DMEM containing 10% fetal bovine serum, 100 units/ml penicillin and 0.1 mg/ml streptomycin at 37°C and 5% CO<sub>2</sub>. Cos-1 cells were transfected with pCMV-GRα expression vector (Panomics) and the cells were seeded without antibiotics sixteen hours prior to transfection. Transfection was performed using LipofectAMINE 2000 reagent. Cos-1 cells were plated in 10 cm culture dishes and transfected. After 16 h, the cells were treated with or without dexamethasone for one hour and then the nuclear extracts were prepared. A 19 plex assay was performed on the treated and untreated samples. Fold induction was measured by normalizing the treated samples against the untreated sample.



## GR EMSA Confirmation

Nuclear extracts of transfected and non-transfected Cos-1 cells were prepared as previously described and measured for the GR transcription factor. Lane 1 is probe only, Lane 2 is untransfected, and Lane 3 is transfected. Results match change of fold induction to that of Procarta TF Plex assay.



## Summary

The Procarta TF assay is a high throughput assay platform for the quantitative measurement of up to 40 different activated TF's from a single sample. The throughput and ease of use of this assay lends itself to being an exceptional profiling tool for biomarker discovery and validation.

- **Insight:** More information to better understand signaling pathways
- **High throughput:** 96 samples with 40 TF's per sample
- **Accurate:** Quantitation utilizing Luminex bead technology
- **Sensitive:** Assay uses 2 µg of nuclear extract
- **Easy to Use:** ELISA like workflow and data under 4 hours

## Abstract

*Transcription factors (TFs) play a crucial role in the regulation of gene expression in the human genome and 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 AP1, ER, and p53 function in cancer, NFκB in inflammatory diseases, and PPARγ 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. Here, we describe a commercially available 40-plex Luminex® based assay for the measurement of activated TFs. This assay is designed around the standard 96-well plate format enabling high-throughput, multiplex profiling of the DNA binding activity of TFs in multiple samples with high-sensitivity.*



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