



## TF Activation Profiling Plate Array I

Catalog Number FA-1001

(For Research Use Only)

### Introduction

Transcription factors (TFs) are a group of cellular proteins that play essential roles in regulating gene expression. They act as a sensor to monitor cellular change and convert the signals into gene expression. Often, a specific cellular signal pathway can activate multiple TFs and the expression of a specific gene is under the control of multiple TFs. Hence, monitoring the activation of multiple TFs simultaneously is critical to understanding the molecular mechanism of cellular regulation underlying cell signaling and gene expression. Signosis' TF activation profiling plate array I is used for monitoring 48 different TFs simultaneously in one sample.

### Principle of the assay

Signosis' TF activation profiling plate array is used for monitoring the activation of multiple TFs simultaneously. In the technology, a series of biotin-labeled probes are made based on the consensus sequences of TF DNA-binding sites. When the probe mix incubates nuclear extracts, individual probes will find its corresponding TF and form TF/probe complexes, which can be easily separated from free probes through a simple spin column purification. The bound probes are detached from the complex and analyzed through hybridization with a plate; each well is specifically pre-coated with complementary sequences of the probes. The captured DNA probe is further detected with streptavidin-HRP. Luminescence is reported as relative light units (RLUs) on a microplate luminometer.

### Materials provided with the kit

- One 96-well Hybridization Plate (RT)
- Two Isolation columns (RT)
- TF binding buffer mix (-20 °C)
- TF Probe mix I (-20 °C)
- Filter binding buffer (4 °C)
- Filter wash buffer (4 °C)
- Elution buffer (RT)
- Streptavidin-HRP conjugate (4 °C)
- Plate hybridization buffer (RT)
- 5x Plate hybridization wash buffer (RT)
- Block buffer (RT)
- 5x Detection wash buffer (RT)
- Substrate A (4 °C)
- Substrate B (4 °C)
- Substrate dilution buffer (RT)
- Foil film

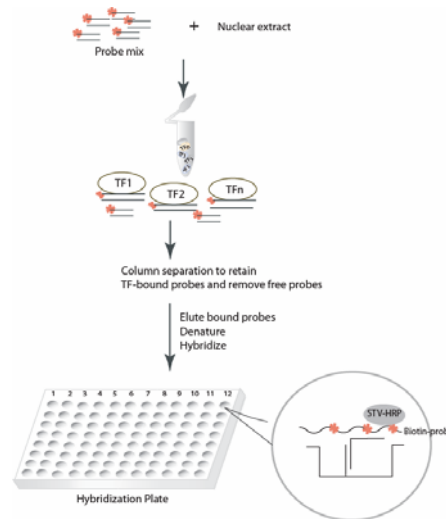


Diagram of TF Activation Profiling Plate Array

### Material required but not provided

- Nuclear Extraction Kit from Signosis (SK-0001)
- PCR machine
- Microcentrifuge working at 4 °C
- Hybridization incubator
- Shaker
- Plate reader for luminescent detection
- ddH<sub>2</sub>O (RNAase free)

### Reagent preparation before starting experiment

- Keep Filter binding buffer and Filter wash buffer on ice
- Warm up Plate Hybridization Buffer and Hybridization Wash buffer at 42 °C before use.
- Dilute 30ml of 5x Plate Hybridization wash buffer with 120 ml of dH<sub>2</sub>O before use.
- Dilute 40ml of 5x Detection wash buffer with 160 ml of dH<sub>2</sub>O before use.
- Dilute 1000 times of streptavidin-HRP with block buffer before use.

## Assay Procedure

### TF DNA Complex Formation

- Mix the following components for each reaction in a tube or one well of a PCR plate
  - 10ul TF binding buffer mix
  - 2ul TF Probe mix I
  - Xul nuclear extract (5µg-15µg)
  - Xul ddH2O
  - 20ul
- Incubation at 16°C for 30 minutes in a PCR machine

### Separation of TF DNA Complex from Free Probes

- Equilibrate the Isolation Column by adding add 500ul cold Filter binding buffer, and centrifuge at 6000 rpm for 1 min in microcentrifuge at room temperature.
- Add 20ul cold Filter binding buffer to 20ul TF-Probe complex from step2. Transfer the entire mix onto the filter in the center of the Isolation Column.
- Incubate on ice for 30 minutes.  
**Don't incubate longer than 30 minutes, which results in high background.**
- Add 500ul cold Filter wash buffer to the column, and incubate for 2-3 minutes on ice.
- Centrifuge at 6000 rpm for 1 min in microcentrifuge at 4°C, and discard the flow through.
- Wash the column by adding 500ul cold Filter wash buffer to the column on ice.
- Centrifuge for 1 min at 6000 rpm in microcentrifuge at 4°C, and discard the flow through.
- Repeat the step 8-9 for additional 3 time washes.

### Elution of Bound Probe

- Add 100ul of Elution buffer onto the center of column, and incubate at room temperature for 5 minutes.
- Put the column on a clean 1.5 ml microcentrifuge tube, and centrifuge at 10,000 rpm for 2 minutes at room temperature.
- Denature the eluted samples at 98 °C for 5 minutes, and transfer on ice immediately. The samples are ready for hybridization or store -20 °C for the future use (the probe must be denatured again before use).

### Hybridization of Eluted Probe with Hybridization Plate

- Remove the sealing film from the plate.
- Add 5ml warmed Hybridization buffer to a dispensing reservoir (DNase free) and then add 100ul denatured probes. Mix them together by gently shaking the reservoir.

- Dispensing 95ul of the mixture into the corresponding wells with 8 channel pipette **immediately**.  
**Note:** 96 well hybridization plate is divided into two sections. Section one (Column 1-6 ) for one sample and Section two (Column 7-12 ) for another sample.
- Seal the wells with foil film securely and hybridize at 42 °C for overnight. Ensure the numbers of letters are clearly visible from under foil seal.

### Detection of Bound Probe

- Invert the Hybridization Plate over an appropriate container and expel the contents forcibly, and wash the plate 3 times by adding 200µl of pre-warmed 1x Plate hybridization wash buffer.
- Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
- Add 200µl of Block buffer incubate for 15 minutes at room temperature with gently shaking.
- Invert the plate over an appropriate container to remove block buffer.
- Add 10 µl of streptavidin-HRP conjugate in 10ml block buffer (1:1000 dilution). Add 95 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gently shaking.
- Wash the plate with 200ul 1X Detection wash buffer for 5 minutes. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
- Repeat step 23 for additional 2 time washes.
- Freshly prepare the substrate solution:  
For the whole plate:  
1ml Substrate A  
1ml Substrate B  
8ml Substrate dilution buffer
- Add 95µl substrate solution to each well and incubate for 1 min.
- Determine the chemiluminescence of each well with a microplate reader within 5 minutes.

### Data Example

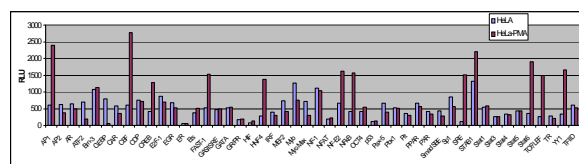


Figure. TF activation profiling plate array assay. HeLa cells were treated with or without PMA. Nuclear extracts were prepared and subjected to TF profiling assay.

	1	2	3	4	5	6	7	8	9	10	11	12
A	AP1	CDP	GATA	NF-1	Pit	Stat3	AP1	CDP	GATA	NF-1	Pit	Stat3
B	AP2	CREB	GR/PR	NFAT	PPAR	Stat4	AP2	CREB	GR/PR	NFAT	PPAR	Stat4
C	AR	E2F-1	HIF	NF-E2	PXR	Stat5	AR	E2F-1	HIF	NF-E2	PXR	Stat5
D	ATF2	EGR	HNF4	NFkB	SMAD	Stat6	ATF2	EGR	HNF4	NFkB	SMAD	Stat6
E	Brn-3	ER	IRF	OCT4	Sp1	TCF/LEF	Brn-3	ER	IRF	OCT4	Sp1	TCF/LEF
F	C/EBP	Ets	MEF2	p53	SRE	YY1	C/EBP	Ets	MEF2	p53	SRE	YY1
G	CAR	FAST-1	Myb	Pax-5	STAB1	TR	CAR	FAST-1	Myb	Pax-5	STAB1	TR
H	CBF	GAS/ISR	Myc-Max	Pbx1	Stat1	TFIID	CBF	GAS/ISR	Myc-Max	Pbx1	Stat1	TFIID