



TF Activation Profiling Plate Array II

Catalog Number: FA-1002

(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 sensors to monitor cellular changes and convert signals into gene expression. Often, a specific cellular signal pathway can activate multiple TFs. The expression of a specific gene can also be under the control of multiple TFs. Thus, monitoring the activation of multiple TFs simultaneously is critical to understanding the molecular mechanism of cellular regulation underlying cell signaling and gene expression. **Signosis, Inc.'s TF Activation Profiling Plate Array II** is used for monitoring 96 different TFs simultaneously from one sample.

Principle of the assay

Signosis, Inc.'s TF Activation Profiling Plate Array II is used for monitoring the activation of multiple TFs simultaneously. With this technology a series of biotin-labeled probes are made based on the consensus sequences of TF DNA-binding sites. When the probe mix incubates with nuclear extracts, individual probes will find its corresponding TF and form TF-probe complexes, which can be easily separated from free probes through a spin-column purification. The bound probes are detached from the complex and analyzed through **hybridization** with the *96-Well Plate*. Each well is specifically pre-coated with complementary sequences of the probes. The captured DNA probe is further detected with *Streptavidin-HRP Conjugate*. Luminescence is reported as relative light units (RLUs) on a microplate luminometer

Materials Required but Not Provided

- Nuclear Extraction Kit from Signosis (SK-0001)
- PCR machine and PCR tubes
- Microcentrifuge working at 4 °C
- Hybridization incubator at 42°C
- Plate-Shaker
- Plate reader for luminescent detection
- ddH₂O (DNAase-free)
- 8 and 12 Multi-channel pipettes

Materials Provided with the Kit

Component	Qty	Store at
96-Well Plates (with aluminum adhesive seal)	2	RT
Isolation Columns	2	RT
Elution Buffer	400 µL	RT
TF Plate Hybridization Buffer	20 mL	RT
5X Plate Hybridization Wash Buffer	60 mL	RT
5X Detection Wash Buffer	60 mL	RT
Blocking Buffer	60 mL	4°C
Filter Wash Buffer	5 mL	4°C
Filter Binding Buffer	1 mL	4°C
Substrate A	2 mL	4°C
Substrate B	2 mL	4°C
Streptavidin-HRP Conjugate	40 µL	4°C
Substrate Dilution Buffer	16 mL	4°C
TF Binding Buffer Mix	60 µL	-20°C
TF Probe Mix II	20 µL	-20°C

Before Starting the Experiment Prepare the Following:

1. Place *Filter Binding Buffer* and *Filter Wash Buffer* on **ice** so they are chilled for the assay (for at least **10 minutes**).
2. Warm up *TF Plate Hybridization Buffer*, *Blocking Buffer*, and *Hybridization Wash Buffer* to **42°C** before use.
3. Aliquot **500 µL** of ddH₂O in a 1.5mL microcentrifuge tube **per sample** on ice so that it is chilled for the assay (for at least **10 minutes**).
4. Dilute **60 mL** of *5X Plate Hybridization Wash Buffer* with **240 mL** of ddH₂O before use.
5. Dilute **60 mL** of *5X Detection Wash Buffer* with **240 mL** of ddH₂O before use.
6. Dilute **40 µL** *Streptavidin-HRP* in **20 mL** *Blocking Buffer* (1:500 dilution).



**Please Read the
Assay Procedure
Before You Begin**

Assay Procedure

TF/ DNA Complex Formation

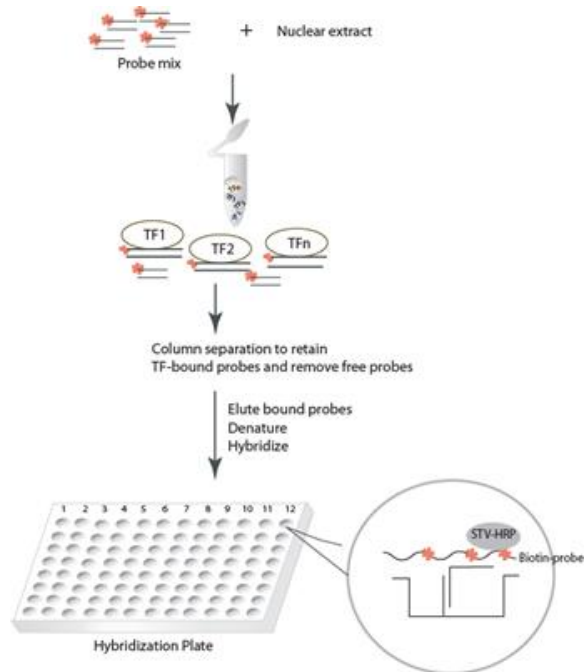
- Mix the following components for each reaction in a tube
15 μ L TF Binding Buffer Mix
5 μ L TF Probe Mix II
X μ L Nuclear Extract (5 μ g-15 μ g recommended)
X μ L ddH₂O (add up to final volume)
30 μ L Reaction Mix [final volume]
- Incubate the **Reaction Mix** at room temperature (20-23°C) for **30 minutes**.

Separation of TF DNA Complex from Free Probes

- Equilibrate an *Isolation Column* by adding **200 μ L** pre-chilled *Filter Binding Buffer*. Centrifuge the column with the collection tube at **6,000 rpm** for **1 minute** in a microcentrifuge at room temperature.
- Transfer the **30 μ L Reaction Mix** directly onto the filter in the center of the *Isolation Column* (avoiding bubbles).
- Incubate on ice for **30 minutes**. **DO NOT** incubate longer than 30 minutes; this will result in high background.
- Add **500 μ L** pre-chilled *Filter Wash Buffer* to the *Isolation Column* and incubate for **3 minutes** on ice.
- Centrifuge the *Isolation Column* with the collection tube at **6,000 rpm** for **1 minute** in a microcentrifuge at **4°C**. Discard the flow through from the collection tube.
- Wash the column by adding **500 μ L** pre-chilled *Filter Wash Buffer* to the *Isolation Column* on ice.
- Centrifuge the *Isolation Column* with the collection tube for **1 minute** at **6,000 rpm** in a microcentrifuge at **4°C**. Then discard the flow through.
- Repeat steps 8-9 for an additional **3 times** for a total of 4 washes.

Elution of Bound Probe

- Place the *Isolation Column* in a new 1.5mL microcentrifuge tube. Add **100 μ L** of *Elution Buffer* onto the center of *Isolation Column* and incubate at room temperature for **5 minutes**.
- Centrifuge the microcentrifuge tube with the *Isolation Column* at **10,000 rpm** for **2 minutes** at room temperature.
- If you have yet to do so, chill **500 μ L** ddH₂O (DNAase free) in a 1.5mL microcentrifuge tube on ice for at least **10 minutes** and **keep on ice**.
- Transfer the eluted probe to a PCR tube and denature the eluted probes at **98°C** for **5 minutes**.
- Immediately** transfer the denatured probes to the chilled ddH₂O from Step 13 and place **on ice**. The samples are ready for the hybridization phase of the assay. You can store the sample at **-20°C**



for future use. If you decided to store your sample, go to **step 16**. before proceeding to the hybridization phase.

16. Skip this step if you did not freeze your sample for future use.

- Thaw your sample back to an aqueous phase at room temperature.
- Redistribute the sample into PCR tubes to be reheated at **98°C** for **5 minutes**.
- Afterwards, **immediately** place the PCR tubes on ice.
- You may now proceed to Step 17.

Hybridization of Eluted Probe with Hybridization Plate

- Remove the clear adhesive film sealing from the provided *96-Well Plate*.
- Aliquot **10 mL** pre-warmed *TF Plate Hybridization Buffer* to a dispensing reservoir (DNase free) and then add **600 μ L** denatured probes. Mix them together by gently shaking the reservoir.
- Using a 12 multi-channel pipette **100 μ L** of the mixture from step 18. into the corresponding wells with 8 multi-channel pipette **immediately**.

Note: If you wish to have a blank to compare your wells against, select one TF you are not interested in and determine its location on the plate by using the diagram on the third page. Add **100 μ L** *TF Plate Hybridization Buffer* only **without** the eluted probe.

- Firmly seal the wells with the aluminum adhesive seal to secure well contents. Press the foil over the letters and numbers on the plate to help orient well designations. Hybridize the well contents to the plate by placing the *96-Well Plate* in an incubator set at **42°C** overnight.

Detection of Bound Probe

- Remove the aluminum adhesive seal from the experimental wells with a razor blade. Keep the unused wells sealed.
- Invert the *96-Well Plate* over an appropriate container and expel the contents forcibly.
- Wash the plate by adding **200 µL** of pre-warmed *IX Plate Hybridization Wash Buffer* to each well by **row** with a **12 multi-channel pipette**. Incubate the plate for **5 minutes** with gentle shaking at room temperature on a plate-shaker. Completely remove at end of 5 minutes by tapping the plate against clean paper towels.
- Repeat step 23. two more times for a total of three washes.
- Add **200 µL** of *Blocking Buffer* to each well by **row** with a **12 multi-channel pipette** and incubate for **5 minutes** at room temperature with gentle shaking on a plate-shaker.
- Invert the plate over an appropriate container to forcibly remove *Blocking Buffer* from the wells.
- If you have yet to do so: add **40 µL** of *Streptavidin-HRP Conjugate* in **20 mL Blocking Buffer** (1:500 dilution), enough for the whole plate (6 sections). This is the *diluted Streptavidin-HRP Conjugate*

- Add **95 µL** of *diluted Streptavidin-HRP Conjugate* to each well by **row** with a **12 multi-channel pipette** and incubate for **45 minutes** at room temperature on a plate-shaker with gentle shaking.
- After the **45 minutes** have elapsed, forcibly remove the *96-Well Plate* contents in an appropriate container. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
- Wash the *96-Well Plate* by adding **200 µL IX Detection Wash Buffer** to each well by **row** with a **12 multi-channel pipette**. Incubate the plate for **5 minutes** with gentle shaking on a plate-shaker at room temperature.
- Repeat step 28. two more times. At the third and final wash, invert plate on clean paper towels for **1 minute** to remove excessive liquid.
- Freshly prepare the *Substrate Solution* in the following ratio:
1 part **Substrate A** / 1 part **Substrate B** / 8 parts **Substrate Dilution Buffer**.
For example, for the entire *96-Well Plate*:
1 mL Substrate A
1 mL Substrate B
8 mL Substrate Dilution Buffer
10 mL Substrate Solution
- Add **95 µL Substrate Solution** to each well by **row** with a **12 multi-channel pipette** and incubate the solution in the wells for **1 minute** at room temperature.
- Place the plate in the luminometer. Allow plate to sit inside machine for **4 minutes** before reading. Set integration time to **1 second** with no filter position. For the best results, read the plate within **5-20 minutes**.

TF Activation Profiling Array II Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	AP1	CDP	GATA	NF-1	Pit	Stat3	XBP	FOXP1	HoxA-5	NRF2(ARE)	Prox1	SOX2
B	AP2	CREB	GR/PR	NFAT	PPAR	Stat4	AP3	FOXO1(FKHR)	HSF	Oct-1	RB	SOX9
C	AR	E2F-1	HIF	NF-E2	PXR	Stat5	AP4	FREAC2 (FOXF2)	KLF4	Pax2	RUNX	SOX18
D	ATF2	EGR	HNF4	NFkB	SMAD	Stat6	COUP-TF	Gli-1	MyoD	Pax3	ROR(RZR)	SRY
E	Bm-3	ER	IRF	OCT4	Sp1	TCF/LEF	ELK	Gfi-1	MZF	Pax8	RXR	TFE3
F	C/EBP	Ets	MEF2	p53	SRF	YY1	FOXA1	HEN (NSCL-1)	Nkx2-5	PIT1	SF-1	USF-1
G	CAR	FAST-1	Myb	Pax-5	SATB1	TR	FoxC1	HNF-1	Nkx3-2	PLAG1	SMUC	VDR
H	CBF	GAS/ISRE	Myc-Max	Pbx1	Stat1	TFIID	FOXD3	HOX4C	NRF1	MEF1	Snail	WT1

Data analysis notes:

- TF readings within $\pm 10\%$ of a blank reading are considered to be too low for analysis.
- The changes in reading between two samples need to be over 2-fold (increase or decrease) to be significant.

Data Example

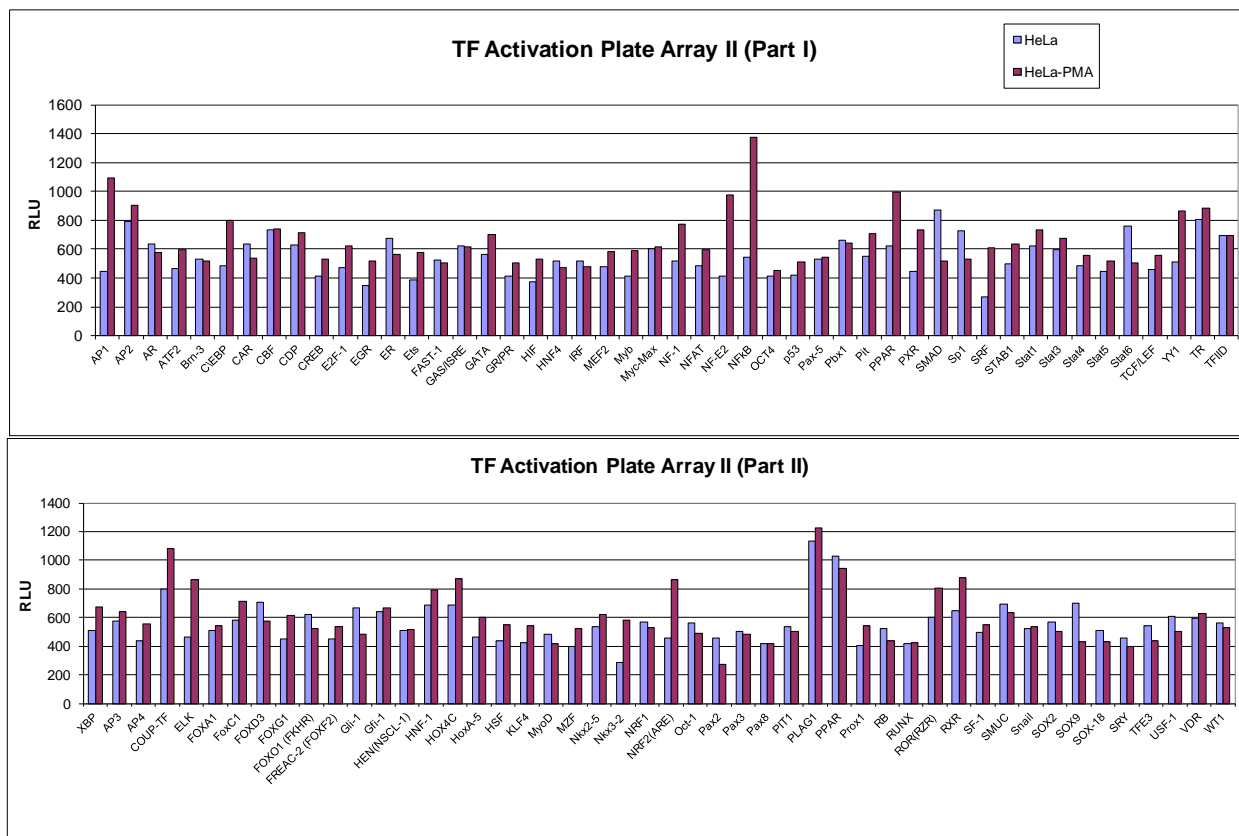


Figure: TF Activation Profiling Plate Array Assay acquired RLUs. HeLa cells were treated with and without PMA. Nuclear Extracts prepared and subjected to the TF Profiling Assay I.

Related Products

<i>Catalog #</i>	<i>Product Description</i>
FA-1001	TF Activation Profiling Plate Array I
FA-1003	Stem Cell TF Activation Profiling Plate Array
FA-1004	Cancer Stem Cell TF Activation Profiling Plate Array
FA-1005	Oxidative Stress TF Activation Profiling Plate Array
FA-1006	ER (UPR) Stress TF Activation Profiling Plate Array

Gene Description

TF names	Gene Description	TF names	Gene Description
AP1	Activator protein 1 (JUN/FOS)	XBP-1	X-box binding protein 1
AP2	Activator protein 2	AP3	AP3 protein
AR	Androgen receptor	AP4	AP4 protein
ATF2	activating transcription factor 2	COUP-TF	nuclear receptor subfamily 2, group F,
Brn-3	POU domain, class 4, transcription factor 1	ELK	ETS domain-containing protein Elk-1
C/EBP	CCAAT/enhancer binding protein (C/EBP),alpha	FOXA1	homeobox A1
CAR	nuclear receptor subfamily 1, group I, member 3	FoxC1	homeobox C1
CBF	CCAAT/enhancer binding protein (C/EBP), zeta	FOXD3	forkhead box D3
CDP	cut-like homeobox 1; CCAAT displacement protein	FOXG1	FOXbox G1
CREB	cAMP responsive element binding protein 1	FOXO1 (FKHR)	FOXbox O1
E2F-1	E2F transcription factor 1	FREAC-2	Forkhead-related activator 2
EGR	Early growth response	Gfi-1	growth factor independent 1 transcription
ER	Estrogen receptor	Gli-1	GLI zinc finger transcription factor
Ets	v-ets erythroblastosis virus E26 oncogene homolog 1	HEN(NSCL-1)	helix-loop-helix protein
FAST-1(FOXH1)	Forkhead box H1	HNF-1	Hepatocyte Nuclear Factor 1
GAS/ISRE	IFN-stimulated response element	HOX4C	HOX4c homobox
GATA	GATA transcription factor	HoxA-5	homeobox A5
GR/PR	Glucocorticoid receptor/Progesterone receptor	HSF	heat shock transcription factor 1
HIF	Hypoxia inducible factor	KLF4	Kruppel-like factor 4
HNF4	Hepatocyte nuclear factor 4	MyoD	myogenic differentiation 1 protein
IRF	Interferon regulatory factor	MZF	zinc finger type transcription factor MZF
MEF2	Myocyte enhancer factor 2	Nkx2-5	Homeobox protein Nkx-2.5
Myb	v-myb myeloblastosis viral oncogene homolog	Nkx3-2	Homeobox protein Nkx-3.2
Myc-Max	v-myc myelocytomatosis viral oncogene homolog	NRF1	nuclear respiratory factor 1
NF-1	Nuclear factor 1	NRF2(ARE)	NRF2-related antioxidant responsive
NFAT	Nuclear factor of activated T-cells	Oct-1	POU domain, class 2, transcription factor
NF-E2	Nuclear factor (erythroid-derived 2)	Pax2	Pair box-2 protein
NFkB	nuclear factor of kappa light polypeptide gene	Pax 3	Pair box-3 protein
OCT4	POU class 5 homeobox 1	Pax8	Pair box-8 protein
p53	Tumor protein p53	PIT1	POU class 1 homeobox 1
Pax-5	Paired box 5	PLAG1	pleiomorphic adenoma gene 1
Pbx1	Pre-B cell leukemia transcription factor-1	MEF1	Myocyte enhancer factor 1
Pit	Pituitary specific transcription factor 1	Prox1	Prospero homeobox protein 1
PPAR	Peroxisome proliferator-activated receptor	RB	Retinoblastoma control element
PXR	Pregnane X Receptor	RUNX	Runt-related transcription factor 1
SMAD (MADH)	SMAD family	ROR(RZR)	retinoic acid receptor-related orphan
Sp1	SP1 transcription factor	RXR	retinoid X receptor
SRF	Serum response factor	SF-1	Steroidogenic factor 1
SATB1	Special AT-rich sequence binding protein 1	SMUC	snail-related transcription factor Smuc
Stat1	Signal transducer and activator of transcription 1	Snail	Snail 1 zinc finger protein
Stat3	Signal transducer and activator of transcription 3	SOX2	SOX protein 2
Stat4	Signal transducer and activator of transcription 4	SOX9	SOX protein 9
Stat5	Signal transducer and activator of transcription 5	SOX-18	SOX protein 18
Stat6	Signal transducer and activator of transcription 6	SRY	sex determining region Y
TCF/LEF	T cell factor / Lymphoid enhancer factor proteins	TFE3	transcription factor binding to IGHM
YY1	YY1 transcription factor	USF-1	upstream transcription factor 1
TR	Thyroid hormone receptor	VDR	vitamin D (1,25- dihydroxyvitamin D3)
TFIID	TATA box binding protein	WT1	Wilms Tumor 1 suppressor protein1