Signosis
BioSignal Capture

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 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 II is used for monitoring 96 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 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 simple spin column purification method. 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

- Two 96-well Hybridization Plate (RT)
- Two Isolation columns (RT)
- TF binding buffer mix (-20 °C)
- TF probe mix II(-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)
- Blocking buffer (RT)
- 5x Detection wash buffer (RT)
- Substrate A (4°C)
- Substrate B (4°C)
- Substrate dilution buffer (RT)
- Foil film

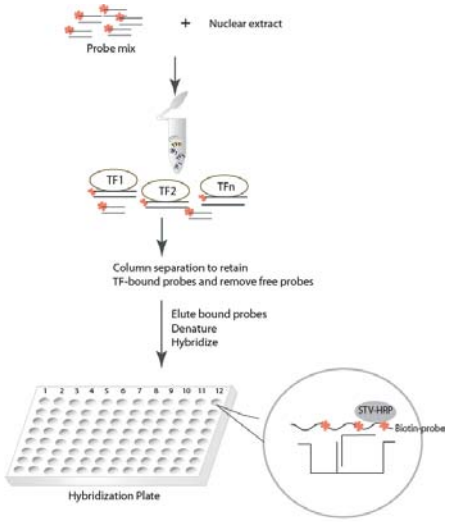

Diagram of TF Activation Profiling Plate Array

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
- ddH2O (DNAase 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 60ml of 5X Plate Hybridization wash buffer with 240 ml of dH_2O before use.
- Dilute 60ml of 5X Detection wash buffer with 240 ml of dH_2O before use.
- Dilute streptavidin-HRP 500 times with blocking buffer before use.

Assay Procedure
Read the procedure carefully before you start

TF DNA Complex Formation

1. Mix the following components for each reaction in a tube or one well of a PCR plate
   15ul TF binding buffer mix
   5ul TF Probe mix II
   Xul nuclear extract (5µg-15µg)
   Xul ddH2O
   30ul
2. Incubation at room temperature (20-23^{o}C) for 30 minutes.

Separation of TF DNA Complex from Free Probes

3. Equilibrate the Isolation Column by adding 200ul cold Filter binding buffer, and centrifuge at 6000 rpm for 1 min in microcentrifuge at room temperature.
4. Transfer the 30ul reaction mix directly onto the filter in the center of the Isolation Column (avoiding bubbles).
5. Incubate on ice for 30 minutes.
   Don't incubate longer than 30 minutes, which results in high background.
6. Add 500ul cold Filter wash buffer to the column, and incubate for 2-3 minutes on ice.
7. Centrifuge at 6000 rpm for 1 min in microcentrifuge at 4^{o}C, and discard the flow through.
8. Wash the column by adding 500ul cold Filter wash buffer to the column on ice.
9. Centrifuge for 1 min at 6000 rpm in microcentrifuge at 4^{o}C, and discard the flow through.
10. Repeat the step 8-9 for additional 3 time washes.

Elution of Bound Probe

11. Add 100ul of Elution buffer onto the center of column, and incubate at room temperature for 5 minutes.
12. Put the column on a 1.5 ml microcentrifuge tube, and centrifuge at 10,000 rpm for 2 minutes at room temperature.
13. Chill 500ul ddH2O (DNAase free) in a 1.5ml microcentrifuge tube on ice for at least 10 minutes, and **keep on ice**.
14. Transfer the eluted probes to a PCR tube and denature the eluted probes at 98 ^{o}C for 5 minutes.
15. **Immediately** transfer the denatured probes to the chilled ddH2O from Step.13 and place **on ice**. The samples are ready for hybridization or store -20 ^{o}C for the future use (the probe must be denatured again before use).

Hybridization of Eluted Probe with Hybridization Plate

16. Remove the sealing film from the plate.
17. Add 10 ml warmed Hybridization buffer to a dispensing reservoir (DNase free) and then add 600ul denatured probes. Mix them together by gently shaking the reservoir.
18. Dispensing 100ul of the mixture into the corresponding wells with 8 or 12 multi-channel pipette **immediately**.
    If a blank well is desired to perform, select one TF well you may not be interested in from the diagram below as a blank well and add 1x Hybridization buffer only without the eluted probe
19. Seal the wells with foil film securely and hybridize at 42 ^{o}C for overnight. Ensure the numbers and letters on the plate are clearly visible from under foil seal by pressing the foil down on every single experimental well.

Detection of Bound Probe

20. 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 to each well.
21. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
22. Add 200µl of Blocking buffer incubate for 15 minutes at room temperature with gently shaking.
23. Invert the plate over an appropriate container to remove block buffer.
24. Add 20 µl of streptavidin-HRP conjugate in 10ml blocking buffer (1:500) dilution, enough for two plates. Add 95 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gently shaking.
25. 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.
26. Repeat step 25 for additional 2 time washes.
27. Freshly prepare the substrate solution:
    For the whole plate:
    1ml Substrate A
    1ml Substrate B
    8ml Substrate dilution buffer
28. Add 95µl substrate solution to each well and incubate for 1 min.
    Notes: Substrate solution can be added to one plate first. After the measurement of the first plate is done, the substrate solution can be then added to the second plate.
29. Place the plate in the luminometer. Allow plate to sit inside machine for 5min before reading. Set integration time to 1 second with no filter position. For the best results, read the plate within 5-20 minutes.

Data Example

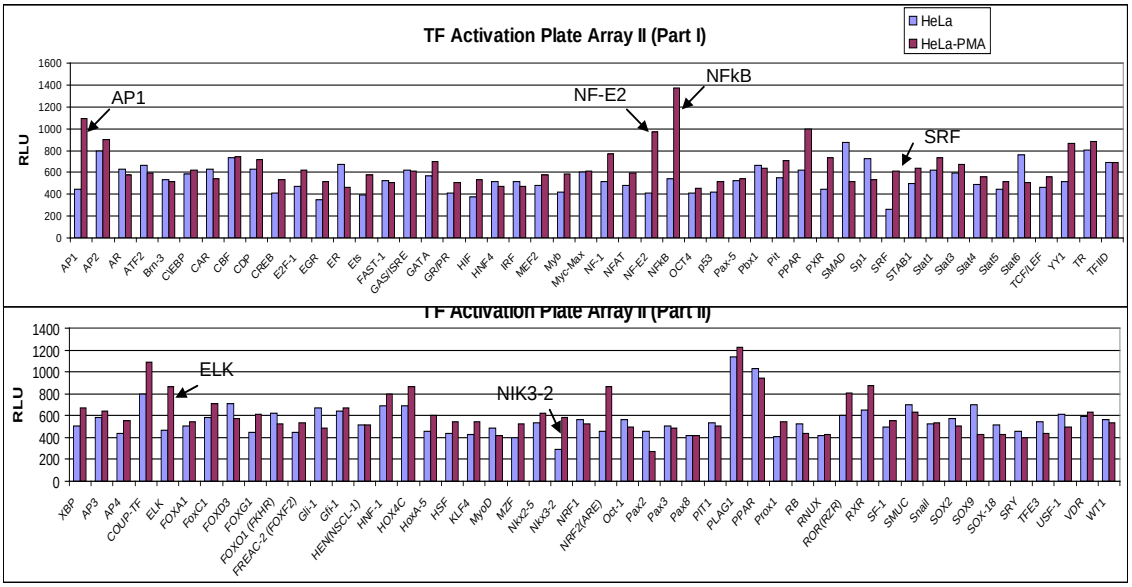

Figure: TF Activation Plate Array II Assay. HeLa cells were treated with and without PMA. Nuclear extracts were prepared and subjected to TF activation Plate array II assay.

TF Activation Plate Array II Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	AP1	CDP	GATA	NF-1	Pit	Stat3	XBP	FOXG1	HoxA-5	NRF2(A	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	RNUX	SOX18
D	ATF2	EGR	HNF4	NFkB	SMAD	Stat6	COUP-TF	Gli-1	MyoD	Pax3	ROR(RZR)	SRY
E	Brn-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/ISR	Myc-Ma	Pbx1	Stat1	TFIID	FOXD3	HOX4C	NRF1	MEF1	Snail	WT1

Data analysis notes:

1. TFIID can be used to normalize the readings for comparison between two samples in most cases.
2. The TF readings within blank reading ±10%blank reading are considered to be too low for analysis.
3. The changes in reading between two samples need to be over 2 fold (increase or decrease) to be significant.

TF 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	RNUX	SL3-3 enhancer 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 18
Stat4	Signal transducer and activator of transcription 4	SOX9	SOX protein 2
Stat5	Signal transducer and activator of transcription 5	SOX-18	SOX protein 9
Stat6	Signal transducer and activator of transcription 6	SRY	sex determining region Y
TCF/LEF	Runt-related transcription factor 2	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 suppresor protein1