Signosis
Innovative Plate Assay Solutions

TF Coregulator p300 Interaction Plate Array II

Catalog Number FA-5012 **(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 change and convert the signals into gene expression. Often, TFs regulate gene expression not only through direct binding to their DNA binding site on the target promoter region, but also by interacting with other transcription factors or regulators. Since changes in transcriptional interaction patterns can have dramatic effects on gene expression and cellular responses to stimuli. Monitoring multiple TF/TF and TF/co-regulator interactions simultaneously could yield greater understanding of regulatory mechanisms. Signosis' TF Protein Interaction Plate Array I can monitor TF/TF or TF/co-regulator interactions among 96 different TFs simultaneously in relation to one TF or co-regulator of interest.

Principle of the assay

Signosis' TF Protein Plate Array I can simultaneously profile the interaction of multiple TFs with a TF or co-regulator of interest. In this assay, a series of unique biotin-labeled probes are provided that correspond with the consensus sequences of individual TF DNA-binding sites. Therefore, each probe represents an individual TF. When the probe mix is incubated with nuclear extract, individual probes bind their corresponding TF. The TF or co-regulator of interest is then immunoprecipitated, along with transcriptionally interacting TFs, using a corresponding antibody and protein G or A agarose beads in a column. Unbound probes and proteins are washed away. The bound probes are then detached from the complex and are subsequently denatured. The biotin-labeled DNA strands are hybridized on a white plate and detected with streptavidin-HRP and substrate. The detected signals reflect the interacting TFs with the particular TF or co-regulator. Luminescence is reported as relative light units (RLUs) on a microplate luminometer.

Materials provided with the kit

- One 96-well Hybridization Plate (RT)
- IP Wash Buffer (4 ºC)
- Magnetic beads (4 ºC)
- 5x Binding Buffer (-20 ºC)
- TF Interaction Probe Mix (-20 ºC)
- Elution buffer (RT)
- Streptavidin-HRP conjugate (4ºC)
- Plate hybridization buffer (RT)
- 5x Plate hybridization wash buffer (RT)
- Blocking buffer (4ºC)
- 5x Detection wash buffer (RT)

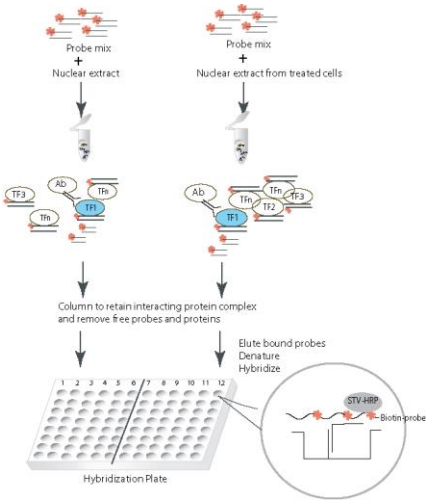

Diagram of Transcriptional Interaction TF Plate Array

Materials provided with the kit (continued)

- Substrate A (4ºC)
- Substrate B (4ºC)
- Substrate dilution buffer (4ºC)
- Foil film
- IP Binding Buffer

Material required but not provided

- Nuclear Extraction Kit from Signosis (SK-0001)
- PCR machine
- Microcentrifuge working at 4ºC
- Hybridization incubator
- Shaker / Rocker
- Plate reader for luminescent detection
- ddH2O (DNase free)
- TF or Co-Regulator Antibody from Signosis (WA-XXXX)

Reagent preparation before starting experiment

- Keep 5x Binding Buffer on ice.
- Keep IP Wash Buffer on ice.
- Warm Plate Hybridization Buffer, Blocking Buffer, and Hybridization Wash buffer at 42ºC before use.
- Dilute 30 ml of 5x Plate Hybridization wash buffer with 120 ml of dH_2O before use.
- Dilute 40 ml of 5x Detection wash buffer with 160 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 and Antibody Complex Formation

1. Mix the following components for each reaction in a tube
   15 µl 5x TF Binding Buffer
   15µl TF Probe mix I
   X µl Nuclear extract (5 µg-15 µg)
   X µl ddH_2O
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   75 µl
2. Incubate at room temperature (20-23°C) for 60 minutes.
3. Add 200 µl of IP Binding Buffer to the mix
4. Add 3-6 µg of antibody
5. Incubate for 1 hour at 4° C on a rocker. This is your TF-Antibody mixture.

Separation of TF and Antibody Complex from Free Probes

6. Wash 10µl Protein A/G Magnetic beads in 500ul of IP Binding Buffer in a new tube by placing the tube on a magnetic stand for 30 seconds and then discard the buffer.
7. Transfer the TF-Antibody mixture to beads and suspend the beads in the solution gently.
8. Incubate on a rocker for 1 hour at 4°C.
9. Wash the TF-Antibody and bead mixture with 500 µl of IP Wash Buffer by placing the tube on a magnetic stand for 30 seconds and then discard the buffer.
10. Repeat washing step for two more times.

Elution of Bound Probe

11. Place the magnetic stand in the ice to pre-chill the stand.
12. Add 80 µl of Elution buffer and suspend the beads and incubate at room temperature for 10 minutes.
13. Heat the tube at 98°C for 5 minutes and transfer the tube to the magnetic stand surrounding by ice. Incubate for at least 5 minutes. The eluted probes are in the solution sand ready for use. Keep the tube on ice until use or store at -20 °C for the future use (the probe must be denatured again before use).

Hybridization of Denatured, Eluted Probe with Plate

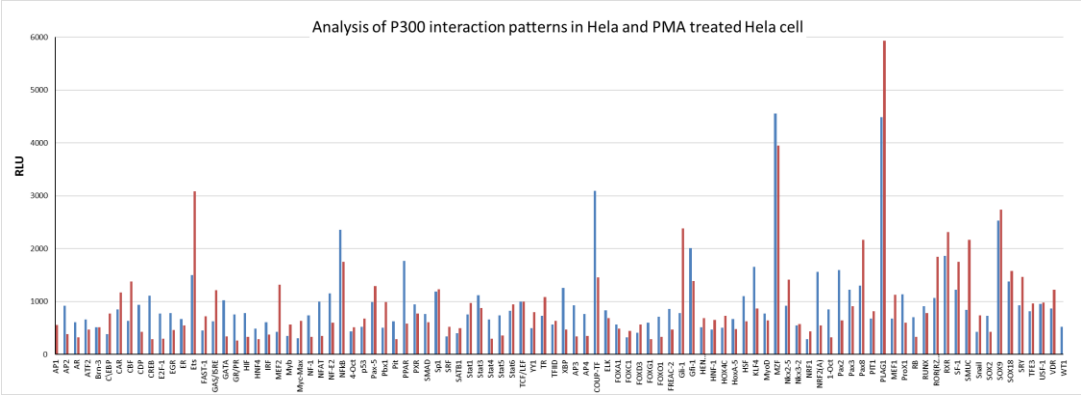
14. Remove the sealing film from the plate.
15. Add 70-80 µl denatured probes (directly form ice) to 5.5 ml warmed Hybridization buffer in a dispensing reservoir (DNase free). Mix by gently shaking the reservoir.
16. Immediately dispense 100 µl of the mixture into the corresponding wells by column with an 8 multi-channel pipette.
    Note: The 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.
    If a blank well is desired, add 1x Hybridization Buffer without the eluted probe to a TF well that you are not interested in.
17. Seal the wells with foil film securely and hybridize at 42°C 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

18. Remove the foil film from the experimental wells with a blade. Keep any unused wells sealed.
19. Invert the Hybridization Plate over an appropriate container and expel the contents forcibly by firmly tapping the plate against clean paper towels.
20. Wash the plate 3 times by adding 200 µl of pre-warmed 1x Plate Hybridization Wash Buffer to each well by **row** with a **12 multi-channel pipette**. At each wash, incubate the plate for 5 minutes with gently shaking at room temperature.
21. Completely remove the liquid from the wells by firmly tapping the plate against clean paper towels.
22. 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 gently shaking.
23. Invert the plate over an appropriate container to remove the Blocking Buffer.
25. Add 20 µl of Streptavidin-HRP conjugate in 10 ml Blocking Buffer (1:500) dilution; this will be enough for all 96 wells. Add 95 µl of diluted Streptavidin-HRP conjugate to each well by **row** with a **12 multi-channel pipette** and incubate for 45 minutes gently shaking at room temperature.
26. Wash the plate 5 times by adding 200 µl 1x Detection Wash Buffer to each well by **row** with a **12 multi-channel pipette**. At each wash, incubate the plate for 10 minutes with gently shaking at room temperature.
27. Completely remove the liquid at each wash by firmly tapping the plate against clean paper towels. At the last wash, leave the plate inverted on a clean paper towel for 1-2 minutes to remove any excess liquid.

28. Prepare fresh substrate solution:
    For 96 wells:
    1ml Substrate A
    1ml Substrate B
    8ml Substrate dilution buffer
29. Add 95µl substrate solution to each well by **row** with a **12 multi-channel pipette** and incubate for 1 minute.
30. Place the plate in the luminometer. Allow plate to sit inside machine for 5 minutes 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



Analysis of p300 interaction patterns in HeLa and PMA-treated HeLa.
The cells were treated with and without PMA for 1 hour. The nuclear extracts were prepared and incubated with cis-element probe mix and antibody against p300. The p300 interacting TFs were pulled down and the bound probes were eluted and hybridized to the plate.

TF Protein Interaction Plate Array II

	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(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	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/ISRE	Myc-Max	Pbx1	Stat1	TFIID	FOXD3	HOX4C	NRF1	MEF1	Snail	WT1

Data analysis notes:

1. The TF readings within blank reading ±10% blank reading is considered to be too low for analysis.
2. The changes in reading between two samples need to be over 2-fold (increase or decrease) to be significant.

Gene Description

TF	Gene Description	TF	Gene Description
AP1	Activator protein 1 (JUN/FOS)	NF-1	Nuclear factor 1
AP2	Activator protein 2	NFAT	Nuclear factor of activated T-cells
AR	Androgen receptor	NF-E2	Nuclear factor (erythroid-derived 2)
ATF2	activating transcription factor 2	NFkB	nuclear factor of kappa light polypeptide
Brn-3	POU domain, class 4, transcription factor 1	OCT4	POU class 5 homeobox 1
C/EBP	CCAAT/enhancer binding protein (C/EBP),alpha	p53	Tumor protein p53
CAR	nuclear receptor subfamily 1, group I, member 3	Pax-5	Paired box 5
CBF	CCAAT/enhancer binding protein (C/EBP), zeta	Pbx1	Pre-B cell leukemia transcription factor-1
CDP	cut-like homeobox 1; CCAAT displacement protein	Pit	Pituitary specific transcription factor 1
CREB	cAMP responsive element binding protein 1	PPAR	Peroxisome proliferator-activated receptor
E2F-1	E2F transcription factor 1	PXR	Pregnane X Receptor
EGR	Early growth response	SMAD	SMAD family
ER	Estrogen receptor	Sp1	SP1 transcription factor
Ets	v-ets erythroblastosis virus E26 oncogene homolog 1	SRF	Serum response factor
FAST-	Forkhead box H1	SATB1	Special AT-rich sequence binding protein 1
GAS/ISRE	IFN-stimulated response element	Stat1	Signal transducer and activator of
GATA	GATA transcription factor	Stat3	Signal transducer and activator of
GR/PR	Glucocorticoid receptor/Progesterone receptor	Stat4	Signal transducer and activator of
HIF	Hypoxia inducible factor	Stat5	Signal transducer and activator of
HNF4	Hepatocyte nuclear factor 4	Stat6	Signal transducer and activator of
IRF	Interferon regulatory factor	TCF/LEF	Runt-related transcription factor 2
MEF2	Myocyte enhancer factor 2	YY1	YY1 transcription factor
Myb	v-myb myeloblastosis viral oncogene homolog	TR	Thyroid hormone receptor
Myc-Max	v-myc myelocytomatosis viral oncogene homolog (avian)	TFIID	TATA box binding protein