Signosis
Innovative Plate Assay Solutions

Promoter-Binding TF Profiling Plate Array II

Catalog Number FA-2002 **(For Research Use Only)**

Introduction

To characterize transcription factors (TFs) that binds to a specific promoter or that regulate the expression of a specific gene via its upstream promoter, two common approaches are applied. First is to employ gel shift assay with DNA binding sites of TFs that are silico-identified within the promoter. Second is to remove or knockout the binding site(s) of a specific TF to measure whether the expression of a promoter-linked reporter is increased or decreased. Because many binding sites of one or a few TFs are present within a promoter, it is required to make a series of reporter constructs with the promoter deletions or mutations. Signosis has developed a fast method to facilitate the characterization through a revised TF activation array. This assay will help to test whether a selected 96 TFs bind to the promoter or not.

Principle of the assay

Promoter-binding TF profiling assay is a competition of Signosis' TF activation plate array II. In the TF activation plate array II, if all of 96 targeted transcription factors exist in the assayed samples, they will form 96 types of complexes, each TF with its corresponding biotin-labeled oligo (similar to the complex in the gel shift assay). After a simple spin separation of the complexes from unbound free biotin-labeled oligos with a membrane-based column, TF-bound probes are eluted from the column and used for plate hybridization. The captured probes are then detected with streptavidin-HRP and a chemiluminescent substrate. If any TF is not present, it will not form a complex, leading to no detection of TF in the plate assay. In promoter-binding TF profiling assay, PCR fragment containing the promoter of your interest is mixed with a set of 96 biotin-labeled oligos corresponding to 96 TFs along with an assayed sample. If DNA fragment contains a TF binding sequence, it will compete with the biotin-labeled oligo to bind to the TF in the sample, leading to no or less complex formation and no or lower detection. Through comparison in the presence and absence of the competitor plasmid or DNA fragment, promoter TFs can be identified.

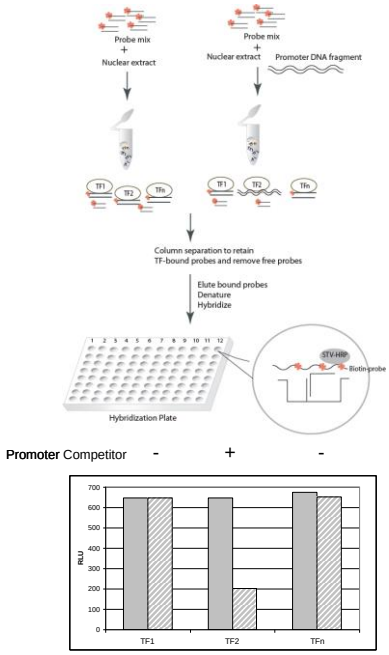


Diagram of Promoter –Binding TF Profiling Assay

Materials provided with the kit

- Four 96-well Hybridization Plate (RT)
- Four isolation columns (RT)
- TF binding buffer mix (-20 ºC)
- TF Probe mix II (-20 ºC)
- Filter binding buffer (4 ºC)
- Filter wash buffer (4 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)
- Substrate A (4ºC)
- Substrate B (4ºC)
- Substrate dilution buffer (RT)
- Foil film

Material required but not provided

- Nuclear Extraction Kit from Signosis (SK-0001)
- DNA PCR product fragment
- PCR machine
- Microcentrifuge working at 4 °C
- Hybridization incubator
- Shaker
- Plate reader for luminescent detection
- ddH2O (RNAase free)

Reagent preparation before starting experiment

- Keep Filter binding buffer and Filter wash buffer on ice
- Warm up Plate Hybridization Buffer, Blocking Buffer, and Hybridization Wash buffer at 42 ºC before use.
- If the Detection Wash Buffer has precipitate, warm up to 42°C until fully dissolved 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 500 times of streptavidin-HRP 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
   15 µl TF binding buffer mix
   5 µl TF Probe mix
   2-5 µl Promoter PCR fragment (0.1-0.5uM)
   X µl Nuclear extract (5µg-15µg)
   X µl ddH2O
   30ul
2. Incubation at room temperature (20-23ºC) for 30 minutes.

Separation of TF DNA Complex from Free Probes

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

Elution of Bound Probe

11. Add 100 µl 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 500 µl ddH2O (DNAase free) in a 1.5 ml microcentrifuge tube on ice for at least 10 minutes and **keep on ice**.
14. Transfer the eluted probe to a PCR tube and denature the eluted probes at 98 ºC 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 ºC 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 600 µl denatured probes. Mix them together by gently shaking the reservoir.
18. Dispensing 100 µl of the mixture into the corresponding wells with 8 multi-channel pipette **immediately**.
    Note: **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 42ºC 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. Remove the foil film from the experimental wells with a blade. Keep the unused well sealed.
21. Invert the Hybridization Plate over an appropriate container and expel the contents forcibly and wash the plate 3 times by adding 200 µl of pre-warmed 1x Plate hybridization wash buffer to each well. At each wash, incubate the plate for 5 minutes with gently shaking at room temperature.
22. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
23. Add 200 µl of Blocking Buffer to each well and incubate for 15 minutes at room temperature with gently shaking.
24. Invert the plate over an appropriate container to remove block buffer.

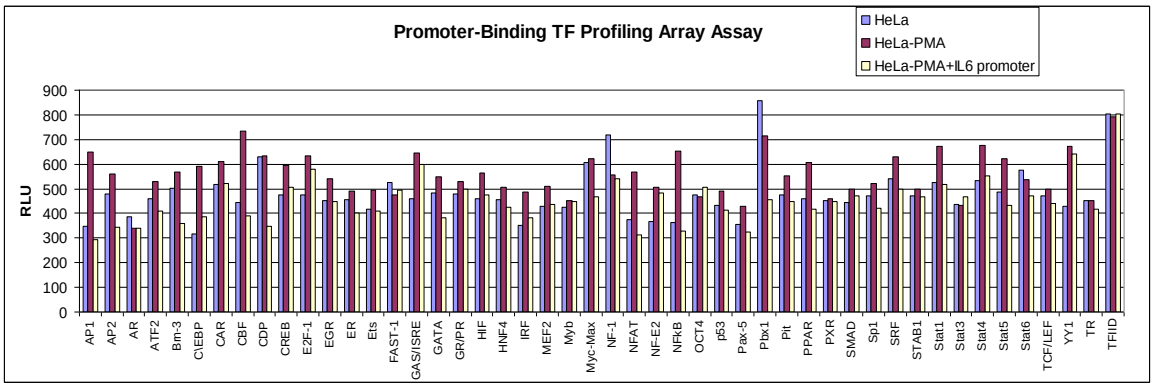
25. Add 40 µl of streptavidin-HRP conjugate in 20 ml blocking buffer (1:500) dilution, enough for two plates. Add 95 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gently shaking.
26. Wash the plate 3 times by adding 200ul 1X Detection wash buffer to each well, At each wash, incubate the plate for 10 minutes gently shaking at room temperature.
27. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels. At the last wash, invert plate on clean paper towels for 1-2 min to remove excessive liquid.
28. Freshly prepare the substrate solution:
    For the whole plate:
    1 ml Substrate A
    1 ml Substrate B
    8 ml Substrate dilution buffer
29. Add 95 µl substrate solution to each well and incubate for 1 min.
30. Place the plate in the luminometer. Allow plate to sit inside machine for 5 min 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 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

Notes: TFIID can be used to normalize the readings for comparison between two samples if the promoter of interest doesn't contain TFIID binding site, TATA box. The TATA box has the core DNA sequence 5'-TATAAA-3' or a variant, which is usually followed by three or more adenine bases. It is usually located 25 base pairs upstream of the transcription sites

Data Example



AP1
TGCTGAGTCACTAATAAAAGAAAAAAAGAAAGTAAAGGAAGAGTGGTTCTGCTTCTTAGCGCTAGCCTCAATGACGAC
C/EBP
CTAAGCTGCACTTTTCCCCCTAGTTGTGTCTTGCCATGCTAAAGGACGTCACATTGCACAATCTTAATAAGGTTTCCAAT
NFkB
CAGCCCCACCCGCTCTGGCCCCACCCTCACCCTCCAACAAAGATTTATCAAATGTGGGATTTTCCCATGAGTCTCAATA
TTAGAGTCTCA

Figure. Promoter-Binding TF Profiling Assay I. Promoter-Binding TF Profiling Assay: HeLa cells were treated with or without PMA. PMA was used to active TFs including AP1 and NFkB. Nuclear extracts were prepared and incubated with TF binding oligo probe mix: control HeLa cells without PMA treatment with the probe mix (blue); PMA-treated HeLa cells with the probe mix alone (red) and the probe mix plus IL6 promoter DNA fragment (yellow).

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	RUNX	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 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	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