



TF-Coregulator Interaction Plate Array II

Catalog Number FA-4002

(For Research Use Only)

Introduction

Transcriptional co-regulators interact specifically and non-covalently with one or multiple DNA-binding transcription factors (TFs) to either activate or repress the transcription of specific genes. Dysregulated expression of coregulator, such as SRC-1/NcoA-1, p300, CBP and HADC, has a significant effect on the regulation of a TF in the recruitment of other transcription factors and chromatin remodeling. For example, the upregulation of SRC-1 has known roles in mediating steroid receptor transcription in androgen receptor activity as well as prostate cancer progression.

Signosis has developed TF-Coregulator Interaction Plate Array II allowing for high throughput studying of co-regulator interaction networks with 96 different TFs.

Principle of the assay

Signosis' TF-Coregulator Interaction Plate Array can simultaneously profile the transcriptional interaction of multiple TFs with a 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 to their corresponding TF. The 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 tube. 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 pre-coat plate and detected with streptavidin-HRP and substrate. The detected signals reflect the interacting TFs with the particular co-regulator of interest. Luminescence is reported as relative light units (RLUs) on a microplate luminometer.

Materials provided with the kit

- Two 96-well Hybridization Plate (RT)
- IP Wash Buffer (4 °C)
- Protein A/G Magnetic Beads (4 °C)
- 5x TF Binding Buffer (-20 °C)
- TF Coregulator Interaction Probe Mix II (-20 °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)

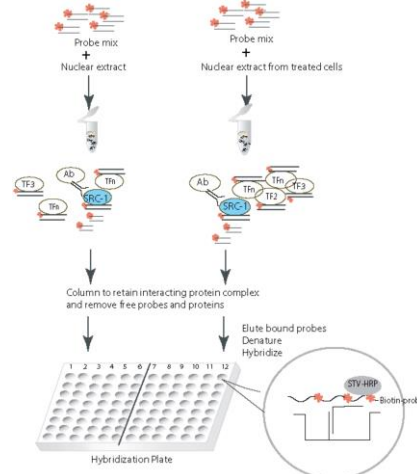


Diagram of Transcriptional Interaction TF Plate Array

Materials provided with the kit (continued)

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

Material required but not provided

- Nuclear Extraction Kit from Signosis (SK-0001)
- PCR machine
- Microcentrifuge working at 4°C
- 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 and Hybridization Wash buffer at 42°C before use.
- Dilute 60 ml of 5x Plate Hybridization wash buffer with 240 ml of dH₂O before use.
- Dilute 60 ml of 5x Detection wash buffer with 240 ml of dH₂O 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

- Mix the following components for each reaction in a tube
15 μ l 5x TF Binding Buffer
15 μ l TF Probe mix II
X μ l Nuclear extract (5 μ g-15 μ g)
X μ l ddH₂O

75 μ l
- Incubate at room temperature (20-23°C) for 60 minutes.
- Add 200 μ l of IP Binding Buffer to the mix
- Add 3-6 μ g of antibody
- 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

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

Elution of Bound Probe

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

Hybridization of Denatured, Eluted Probe with Plate

- Remove the sealing film from the plate.

- Add 70-80 μ l denatured probes (directly from ice) to 10 ml warmed Hybridization buffer in a dispensing reservoir (DNase free). Mix by gently shaking the reservoir.
- Immediately dispense 100 μ l of the mixture into the corresponding wells by column with an 8 multi-channel pipette.
Note: If a blank well is desired, add 1x Hybridization Buffer without the eluted probe to a TF well that you are not interested in.
- 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

- Remove the foil film from the experimental wells with a blade. Keep any unused wells sealed.
- Invert the Hybridization Plate over an appropriate container and expel the contents forcibly by firmly tapping the plate against clean paper towels.
- Wash the plate 3 times by adding 200 μ l 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.
- Completely remove the liquid from the wells by firmly tapping the plate against clean paper towels.
- Add 200 μ l 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.
- Invert the plate over an appropriate container to remove the Blocking Buffer.
- If you have yet to do so: Add 20 μ l of Streptavidin-HRP conjugate in 10 ml Blocking Buffer (1:500) dilution; this will be enough for all 96 wells. Add 95 μ l 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.
- Wash the plate 5 times by adding 200 μ l 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.
- 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.

TF-Coregulator 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	Bmi-3	ER	IRF	OCT4	Sp1	TCF/LEF	ELK	Gli-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

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