



TF-Coregulator p300 Interaction Plate Array I

Catalog Number FA-4011

(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. The transcriptional co-activator p300 is a histone acetyltransferase that is typically recruited to transcriptional enhancer and interacts with transcription factors and regulates gene expression.

Signosis has developed TF-Coregulator p300 Interaction Plate Array Assay I, allowing for high throughput studying of co-regulator interaction networks with 48 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 p300. Luminescence is reported as relative light units (RLUs) on a microplate luminometer.

Materials provided with the kit

- One 96-well Hybridization Plate (RT)
- p300 antibody
- IP Wash Buffer (4 °C)
- Magnetic beads (4 °C)
- 5x Binding Buffer (-20 °C)
- TF Interaction Probe Mix (-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)

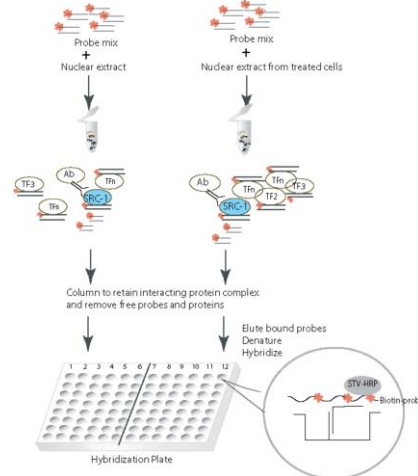


Diagram of Transcriptional Interaction TF Plate Array

Materials provided with the kit (continued)

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

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)

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 30 ml of 5x Plate Hybridization wash buffer with 120 ml of dH₂O before use.
- Dilute 40 ml of 5x Detection wash buffer with 160 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 I
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 20 μ l p300 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-p300 complex to beads and suspend the beads in the solution gently.
- Incubate on a rocker for 1 hour at 4°C.
- Wash the TF-p300 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 transfer the tube to the magnetic stand surrounding by ice. Incubate for at least 5 minutes. The eluted probes are in the solution and ready for use. Immediately keep the tube on ice until using or store at -20 °C for the future use (If stored for future use, the probe must be denatured again at 98°C for 5 minutes 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 5.5 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: 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.

- 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.
- 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 I

	1	2	3	4	5	6	7	8	9	10	11	12
A	AP1	CDP	GATA	NF-1	Pit	Stat3	AP1	CDP	GATA	NF-1	Pit	Stat3
B	AP2	CREB	GR/PR	NFAT	PPAR	Stat4	AP2	CREB	GR/PR	NFAT	PPAR	Stat4
C	AR	E2F-1	HIF	NF-E2	PXR	Stat5	AR	E2F-1	HIF	NF-E2	PXR	Stat5
D	ATF2	EGR	HNF4	NFkB	SMAD	Stat6	ATF2	EGR	HNF4	NFkB	SMAD	Stat6
E	Brn-3	ER	IRF	4-Oct	Sp1	TCF/LEF	Brn-3	ER	IRF	4-Oct	Sp1	TCF/LEF
F	C\EBP	Ets	MEF2	p53	SRF	YY1	C\EBP	Ets	MEF2	p53	SRF	YY1
G	CAR	FAST-1	Myb	Pax-5	SATB1	TR	CAR	FAST-1	Myb	Pax-5	SATB1	TR
H	CBF	GAS/ISRE	Myc-Max	Pbx1	Stat1	TFIID	CBF	GAS/ISRE	Myc-Max	Pbx1	Stat1	TFIID

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
Bm-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 1, 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