Signosis
Innovative Plate Assay Solutions

TF-Coregulator CBP Interaction Plate Array II

Catalog Number FA-5032 **(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. CBP is a transcriptional coactivator that physically interacts with diverse transcription factors and regulate gene expression.
Signosis has developed TF-Coregulator CBP Interaction Plate Array Assay II, allowing for high throughput studying of co-regulator interaction networks with 96 different TFs.

Principle of the assay

Signosis' TF-Coregulator CBP 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 CBP. Luminescence is reported as relative light units (RLUs) on a microplate luminometer.

Materials provided with the kit

- Two 96-well Hybridization Plate (RT)
- Two Filter Columns (RT)
- CBP antibody
- 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 (RT)
- 5x Detection wash buffer (RT)

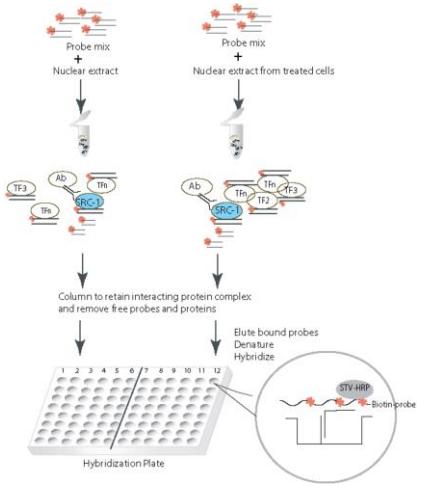

Diagram of Transcriptional Interaction TF Plate Array

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)

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 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 II
   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 20 µl CBP 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-CBP complex to A/G Magnetic beads and suspend the beads in the solution gently.
8. Incubate on a rocker for 1 hour at 4°C.
9. Wash the TF-CBP 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. Immediately keep the tube on ice until use or store at -20°C for the future use (If stored for future usage, the probe must be denatured again at 98°C for 5 minutes 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 10ml 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 row with a 12 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.**
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.

TF-Coregulator Interaction 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(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

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 suppresor protein1

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