



Combo TF Activation Profiling Plate Array

Catalog Number: FA-1016

(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 changes and convert signals into gene expression. Often, a specific cellular signal pathway can activate multiple TFs. The expression of a specific gene can also be under the control of multiple TFs. Thus, monitoring the activation of multiple TFs simultaneously is critical to understanding the molecular mechanism of cellular regulation underlying cell signaling and gene expression. **Signosis, Inc.**'s *Combo TF Activation Profiling Plate Array* is used for monitoring 192 different TFs simultaneously from one sample.

Principle of the assay

Signosis, Inc.'s *Combo TF Activation Profiling Plate Array* is used for monitoring the activation of multiple TFs simultaneously. With this technology a series of biotin-labeled probes are made based on the consensus sequences of TF DNA-binding sites. When the probe mix incubates with nuclear extracts, individual probes will find its corresponding TF and form TF-probe complexes, which can be easily separated from free probes through a spin-column purification. The bound probes are detached from the complex and analyzed through **hybridization** with the *96-Well Plates*. Each well is specifically pre-coated with complementary sequences of the probes. The captured DNA probe is further detected with *Streptavidin-HRP Conjugate*. Luminescence is reported as relative light units (RLUs) on a microplate luminometer

Materials Required but Not Provided

- Nuclear Extraction Kit from Signosis (SK-0001)
- PCR machine and PCR tubes
- Microcentrifuge working at 4 °C
- Hybridization incubator at 42°C
- Plate-Shaker
- Plate reader for luminescent detection
- ddH₂O (DNAase-free)
- 12 Multi-channel pipettes

Materials Provided with the Kit

Component	Qty	Store at
96-Well Plates (with aluminum adhesive seal)	4	RT
Isolation Columns	2	RT
Elution Buffer	400 µL	RT
TF Plate Hybridization Buffer	40 mL	RT
5X Plate Hybridization Wash Buffer	60 mL	RT
	x2	
5X Detection Wash Buffer	60 mL	RT
	x2	
Blocking Buffer	60 mL	RT
	x2	
Filter Wash Buffer	10 mL	4°C
Filter Binding Buffer	1 mL	4°C
Substrate A	4 mL	4°C
Substrate B	4 mL	4°C
Streptavidin-HRP Conjugate	80 µL	4°C
Substrate Dilution Buffer	32 mL	4°C
TF Binding Buffer Mix	120 µL	-20°C
Combo TF Probe Mix	16 µL	-20°C

Before Starting the Experiment Prepare the Following:

1. Place *Filter Binding Buffer* and *Filter Wash Buffer* on **ice** so they are chilled for the assay (for at least **10 minutes**).
2. Warm up *TF Plate Hybridization Buffer* and *Hybridization Wash Buffer* to **42°C** before use.
3. Aliquot **500 µL** of ddH₂O in a 1.5mL microcentrifuge tube **per sample** on ice so that it is chilled for the assay (for at least **10 minutes**).
4. Dilute **120 mL** of *5X Plate Hybridization Wash Buffer* with **480 mL** of ddH₂O before use.
5. Dilute **120 mL** of *5X Detection Wash Buffer* with **480 mL** of ddH₂O before use.
6. **Prepare only when needed for Step 27**, Dilute **80 µL** *Streptavidin-HRP* in **40 mL** Blocking Buffer (1:500 dilution).



**Please Read the
Assay Procedure
Before You Begin**

Assay Procedure

TF/ DNA Complex Formation

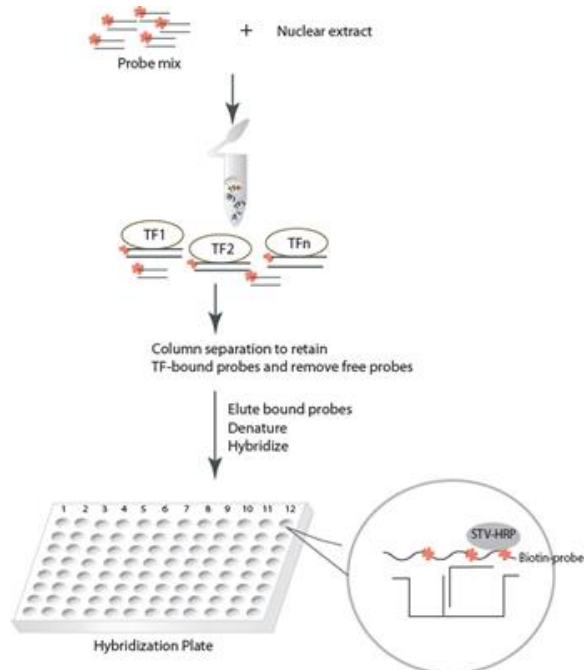
- Mix the following components for **each sample** in a tube
15 μL TF Binding Buffer Mix
5 μL Combo TF Probe Mix
X μL Nuclear Extract (5 μg -15 μg recommended)
X μL ddH₂O (add up to final volume)
30 μL Reaction Mix [final volume]
- Incubate the **Reaction Mix** at room temperature (20-23°C) for **30 minutes**.

Separation of TF DNA Complex from Free Probes

- Equilibrate an *Isolation Column* by adding **200 μL** pre-chilled *Filter Binding Buffer*. Centrifuge the column with the collection tube at **6,000 rpm** for **1 minute** in a microcentrifuge at room temperature.
- Transfer the **30 μL Reaction Mix** directly onto the filter in the center of the *Isolation Column* (avoiding bubbles).
- Incubate on ice for **30 minutes**. **DO NOT** incubate longer than 30 minutes; this will result in high background.
- Add **500 μL** pre-chilled *Filter Wash Buffer* to the *Isolation Column* and incubate for **3 minutes** on ice.
- Centrifuge the *Isolation Column* with the collection tube at **6,000 rpm** for **1 minute** in a microcentrifuge at **4°C**. Discard the flow through from the collection tube.
- Wash the column by adding **500 μL** pre-chilled *Filter Wash Buffer* to the *Isolation Column* on ice.
- Centrifuge the *Isolation Column* with the collection tube for **1 minute** at **6,000 rpm** in a microcentrifuge at **4°C**. Then discard the flow through.
- Repeat steps 8-9 for an additional **3 times** for a total of 4 washes.

Elution of Bound Probe

- Place the *Isolation Column* in a new 1.5mL microcentrifuge tube. Add **100 μL** of *Elution Buffer* onto the center of *Isolation Column* and incubate at room temperature for **5 minutes**.
- Centrifuge the microcentrifuge tube with the *Isolation Column* at **10,000 rpm** for **2 minutes** at room temperature.
- If you have yet to do so, chill **500 μL** ddH₂O (DNAase free) in a 1.5mL microcentrifuge tube on ice for at least **10 minutes** and **keep on ice**.
- Transfer the eluted probe to a PCR tube and denature the eluted probes at **98°C** for **5 minutes**.
- Immediately** transfer the denatured probes to the chilled ddH₂O from Step 13 and place **on ice**.



The samples are ready for the hybridization phase of the assay. You can store the sample at **-20°C** for future use. If you decided to store your sample, go to **step 16**. before proceeding to the hybridization phase.

16. Skip this step if you did not freeze your sample for future use.

- Thaw your sample back to an aqueous phase at room temperature.
- Redistribute the sample into PCR tubes to be reheated at **98°C** for **5 minutes**.
- Afterwards, **immediately** place the PCR tubes on ice.
- You may now proceed to Step 17.

Hybridization of Eluted Probe with Hybridization Plate

- Remove the clear adhesive film sealing from the provided *TF Hybridization I Plate* and *TF Hybridization II Plate*.
- Aliquot **20 mL** pre-warmed *TF Plate Hybridization Buffer* to a dispensing reservoir (DNase free) and then add **600 μL** denatured probes. Mix them together by gently shaking the reservoir.
- Using a 12 multi-channel pipette **100 μL** of the mixture from step 18 into the corresponding wells with 8 multi-channel pipette **immediately**. Do this for one set of *TF Hybridization I Plate* and *TF Hybridization II Plate*. For a total of two plates per sample.

Note: If you wish to have a blank to compare your wells against, select one TF you are not interested in and determine its location on the plate by using the diagram on the third page. Add **100 µL TF Plate Hybridization Buffer** only **without** the eluted probe.

20. Firmly seal the wells with the aluminum adhesive seal to secure well contents. Press the foil over the letters and numbers on the plate to help orient well designations. Hybridize the well contents to the plate by placing both plates in an incubator set at **42°C** overnight.

Detection of Bound Probe

21. Remove the aluminum adhesive seal from the experimental wells with a razor blade. Keep the unused wells sealed.
22. Invert the **two 96-Well Plates** over an appropriate container and expel the contents forcibly.
23. Wash the plates by adding **200 µL** of pre-warmed *IX Plate Hybridization Wash Buffer* to each well by **row** with a **12 multi-channel pipette**. Incubate the plates for **5 minutes** with gentle shaking at room temperature on a plate-shaker. Completely remove at end of 5 minutes by tapping the plates against clean paper towels.
24. Repeat step 23. two more times for a total of three washes.
25. 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 gentle shaking on a plate-shaker.
26. Invert the plates over an appropriate container to forcibly remove *Blocking Buffer* from the wells.
27. Add **95 µL** of *diluted Streptavidin-HRP Conjugate* to each well by **row** with a **12 multi-channel pipette** and incubate for **45 minutes** at room temperature on a plate-shaker with gentle shaking.

28. After the **45 minutes** have elapsed, forcibly remove the two *96-Well Plates* contents in an appropriate container. Complete the removal of all liquid at each wash by firmly tapping the plate against clean paper towels.
29. Wash the two *96-Well Plates* by adding **200 µL IX Detection Wash Buffer** to each well by **row** with a **12 multi-channel pipette**. Incubate the plate for **5 minutes** with gentle shaking on a plate-shaker at room temperature.
30. Repeat step 28. two more times. At the third and final wash, invert plates on clean paper towels for **1 minute** to remove excessive liquid.

Luminescence Reading

Note: Complete steps 32-35 for one plate before continuing with other plates. Do not proceed with other plates until first plate is finished with the luminometer reading. *Substrate Solution* is time sensitive.

32. Freshly prepare the *Substrate Solution* in the following ratio:

1 part **Substrate A** / 1 part **Substrate B** /
8 parts **Substrate Dilution Buffer**.

For example, for one 96-Well Plate:

1 mL Substrate A
1 mL Substrate B
8 mL Substrate Dilution Buffer
10 mL Substrate Solution

33. Add **95 µL Substrate Solution** to each well by **row** with a **12 multi-channel pipette** and incubate the solution in the wells for **1 minute** at room temperature.
34. Place the plate in the luminometer. Set integration time to **1 second** with no filter position. For the best results, read the plate **immediately**, or incubate for no longer than 5 minutes. Read each plate in the same manner for accurate comparison.
35. Repeat steps 33-35 for each plate **one at a time**.

Combo TF Activation Profiling Plate Array Diagram

Combo Array												
TF Hybridization I Plate												
	1	2	3	4	5	6	7	8	9	10	11	12
A	AP1	CDP	GATA	NF-1	Pit	Stat3	XBP	FOXP1	HoxA-5	NRF2	ProX1	SOX2
B	AP-2	CREB	GRE	NFATc	PPAR	Stat4	AP3	FOXO1	HSF	OCT1	RB	SOX9
C	ARE	E2F-1	HIF	NF-E2	PXR	Stat5	AP4	FREAC-2	KLF4	Pax2	RUNX	SOX18
D	ATF2	EGR	HNF4	NFkB	SMAD	Stat6	COUP-TF	Gli-1	MyoD	Pax3	ROR	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	Nkx2-5	PIT1	SF-1	USF-1
G	CAR	FAST-1	Myb	Pax5	SATB1	TR	FOXC1	HNF-1	Nkx3-2	PLAG1	SMUC	VDR
H	CBF/CEBP	GAS/ISRE	Myc-Max	Pbx1	Stat1	TFIID	FOXO3	HOXA4	NRF1	MEF1	Snail	WT1
TF Hybridization II Plate												
	1	2	3	4	5	6	7	8	9	10	11	12
A	AHR	Evi-1	INSM1	MNF1	PEA3	SREBP1	AML1	ELF1	HAND	MAFA	Nkx2.5	Six6
B	ATF3	FXR	ISGF	MTF	PITX2	TBET	ARID3B	ERR	Hmx1	MAFB	Nkx6.1	SKOR1
C	ATF6	GBX2	KRF1	Nanog	PRDM14	TBX3	ATF5	FLI1	IRF8	MAZ	NOR1	TBX2
D	CHOP	GKLF	LRH-1	Nhlh1	PROP1	Tead	BATF	FOXA3	Islet1	MEF3	NR1D1(TH)	TBX5
E	E12/E47	HES1	LXR	NoTCH	pu.1	TFEB	CHF1	FoxH1	KLF2	MR	NURR-1	TEAD1
F	EBF1	HFH1	MAF	NUR77	REST	TGIF	Clock	FOXL2	KLF6	MYT1	Pax4	TFAM
G	EBP-80	HLF	MIBP	OLIG1	Slug	Twist1	DACH	FOXM1	KLF14	Neurod1	pdx-1	Tfcp2l1
H	EKLF	HOXA4	MITF	Pax6	Sperm1	VAX2	EBF4	FTF	KLF15	Nkx2.2	SALL4	VSX1

Data analysis notes:

1. TF readings within $\pm 10\%$ of a blank reading are 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.

Related Products

<i>Catalog #</i>	<i>Product Description</i>
FA-1001	TF Activation Profiling Plate Array I
FA-1003	Stem Cell TF Activation Profiling Plate Array
FA-1004	Cancer Stem Cell TF Activation Profiling Plate Array
FA-1005	Oxidative Stress TF Activation Profiling Plate Array
FA-1006	ER (UPR) Stress TF Activation Profiling Plate Array

Gene Description Plate I

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

Gene Description Plate II

TF Names	Gene Description	TF Names	Gene Description
AHR	Aryl Hydrocarbon Receptor	AML1	Acute Myeloid Leukemia 1 Protein
ATF3	Activating Transcription Factor 3	ARID3B	AT-Rich Interactive Domain Protein 3B
ATF6	Activating Transcription Factor 6	ATF5	Activating Transcription Factor 5
CHOP	Growth Arrest and DNA Damage-Inducible Protein	BATF	Basic Leucine Zipper ATF-Like
E12/E47	E2A Immunoglobulin Enhancer-Binding Factor	CHF1	Cardiovascular Helix-Loop-Helix Factor 1
EBF1	Early B-Cell Factor 1	Clock	Circadian Locomotor Output Cycles Protein Kaput
EBP-80	Emopamil binding protein 80	DACH	Dachshund Homolog 1
EKLF	Erythroid Kruppel-like factor	EBF4	Early B Cell Factor 4
Evi-1	Ecotropic Virus Integration Site 1 Protein Homolog	ELF1	E74 Like ETS Transcription Factor 1
FXR	Nuclear receptor subfamily 1 group H member 4	ERR	Estrogen Related Receptor Alpha
GBX2	Gastrulation Brain Homeobox 2	FLI1	Fli-1 Proto-Oncogene, ETS Transcription Factor
GKLF	Gut-Enriched Kruppel like factor 4	FOXA3	Forkhead Box A3
HES1	Hes Family BHLH Transcription Factor 1	FoxH1	Forkhead Activin Signal Transducer-1
HFH1	Hepatocyte Nuclear Factor 3 Forkhead Homolog 1	FOXL2	Forkhead Box Protein L2
HLF	hepatic leukemia factor	FOXM1	Forkhead Box Protein M1
HOXA4	Homeobox Protein Hox-A4	FTF	Alpha-1-fetoprotein transcription factor
INSM1	INSM transcriptional repressor 1	HAND	Heart And Neural Crest Derivatives Expressed 1
ISGF	Interferon-Stimulated Gene Factor	Hmx1	H6 Family Homeobox 1
KRF1	keratinocyte-specific transcription factor	IRF8	Interferon Regulatory Factor 8
LRH-1	liver receptor homolog 1	Islet1	ISL LIM homeobox 1
LXR	Liver X receptors (LXRs)	KLF2	Kruppel Like Factor 2
MAF	V-Maf Avian Musculoaponeurotic Fibrosarcoma Oncogene	KLF6	Kruppel Like Factor 6
MIBP	c-myc intron binding protein 1	KLF14	Kruppel Like Factor 14
MITF	Microphthalmia-Associated Transcription Factor	KLF15	Kruppel Like Factor 15
MNF1	Mitochondrial nucleoid factor 1	MAFA	V-Maf Musculoaponeurotic Fibrosarcoma Oncogene Homolog A
MTF	Myelin Transcription Factor	MAFB	V-Maf Musculoaponeurotic Fibrosarcoma Oncogene Homolog B
Nanog	Nanog homeobox	MAZ	Myc-Associated Zinc Finger Protein
Nhlh1	nescient helix loop helix 1	MEF3	Pentatricopeptide repeat (PPR) superfamily protein
NoTCH	Notch Receptor 1	MR	Mineralocorticoid Receptor
NUR77	nerve growth factor IB	MYT1	Myelin Transcription Factor 1
OLIG1	oligodendrocyte transcription factor 1	Neurod1	Neuronal Differentiation 1
Pax6	Paired Box Gene 6	Nkx2.2	NK2 Homeobox 2
PEA3	ETS variant transcription factor 4	Nkx2.5	NK2 Homeobox 5
PITX2	Paired Like Homeodomain 2	Nkx6.1	NK6 Homeobox 1
PRDM14	PR/SET Domain 14	NOR1	Neuron-Derived Orphan Receptor 1
PRO1	Homeobox Protein Prophet of Pit-1	NR1D1	Nuclear Receptor Subfamily 1 Group D Member 1
pu.1	ETS-domain binding to a purine-rich sequence.	NURR-1	Nuclear Receptor Related 1
REST	repressor element 1 silencing transcription factor	Pax4	Paired Box 4
Slug	snail family transcriptional repressor 2	pdx-1	Pancreatic And Duodenal Homeobox 1
Sperm1	Sperm-related protein	SALL4	Spalt Like Transcription Factor 4
SREBP1	Sterol regulatory element-binding protein 1	Six6	Sine Oculis Homeobox Homolog 6
TBET	T-cell-specific T-box transcription factor	SKOR1	SKI Family Transcriptional Corepressor 1
TBX3	T-box transcription factor 3	TBX2	T-Box Transcription Factor 2
Tead	Transcriptional enhancer factor TEF-1	TBX5	T-Box Transcription Factor 5
TFEB	T-Cell Transcription Factor EB	TEAD1	TEA Domain Transcription Factor 1
TGIF	myeloid ecotropic viral integration site 1	TFAM	Transcription Factor A, Mitochondrial
Twist1	Twist Basic Helix-Loop-Helix Transcription Factor 1	Tfcp2L1	Transcription Factor CP2-Like Protein 1
VAX2	Ventral Anterior Homeobox 2	VSX1	Visual System Homeobox 1