



## Oxidative Stress TF Activation Profiling Plate Array

Catalog # FA-1005

(For Research Use Only)

### Introduction

Oxidative Stress occurs when the steady-state balance between pro-oxidant and anti-oxidant is changed. Oxidative stress has been reported to be associated with many diseases such as cancer, Alzheimer's disease, Parkinson's disease, atherosclerosis, and heart failure. Responses to Oxidative Stress are mediated by a large number of transcription factors (TFs), including AP1, ATF4, CBF/NFY, EGR1, ETS, FOXO1, HIF, HSF, IRF, NFAT, NFkB, NRF2/ARE, p53, Smad, SRF and Stat3. Signosis, Inc. has developed the Oxidative Stress TF Activation Profiling Plate Array, which can be used to monitor 16 oxidative stress related TFs simultaneously.

### Principle of the Assay

Signosis, Inc.'s *TF Activation Profiling Plate Array* is used for monitoring the activation of multiple TFs simultaneously. In 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 a plate; 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
- ddH<sub>2</sub>O (DNAase-free)
- 8 and 12 Multi-channel pipettes

### Materials Provided with the Kit

Component	Qty	Store at
96-Well Plate (with aluminum adhesive seal)	1	RT
Isolation Columns	6	RT
Elution Buffer	600 µl	RT
TF Plate Hybridization Buffer	20 ml	RT
5X Plate Hybridization Wash Buffer	30 ml	RT
5X Detection Wash Buffer	40 ml	RT
Blocking Buffer	30 ml	4°C
Filter Wash Buffer	15 ml	4°C
Filter Binding Buffer	1.5 ml	4°C
Substrate A	1 ml	4°C
Substrate B	1 ml	4°C
Streptavidin-HRP Conjugate	20 µl	4°C
Substrate Dilution Buffer	8 ml	4°C
TF Binding Buffer Mix	90 µl	-20°C
TF Oxidative Stress Probe Mix	20 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*, *Blocking Buffer*, and *Hybridization Wash Buffer* **42°C** before use.
3. Aliquot **200 µl** of ddH<sub>2</sub>O in a 1.5 ml microcentrifuge tube (per sample; 3 samples would be 600 µl ddH<sub>2</sub>O) on ice so that it is chilled for the assay (for at least **10 minutes**).
4. Dilute **30 ml** of *5X Plate Hybridization Wash Buffer* with **120 ml** of ddH<sub>2</sub>O before use.
5. Dilute **40 ml** of *5X Detection Wash Buffer* with **160 ml** of ddH<sub>2</sub>O before use.
6. Dilute **20 µl** *Streptavidin-HRP* in **10 ml** Blocking Buffer (1:500 dilution).



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

# Assay Procedure

## TF/ DNA Complex Formation

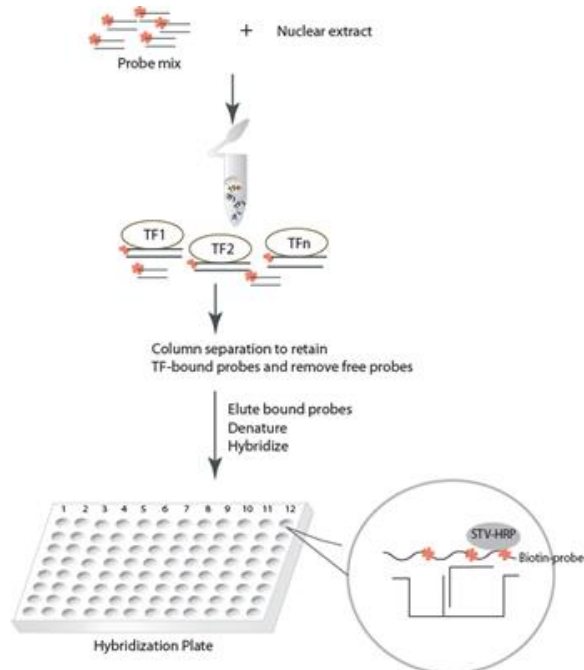
- Mix the following components for each reaction in a tube  
**15  $\mu$ l TF Binding Buffer Mix**  
**3  $\mu$ l TF Probe mix**  
**X  $\mu$ l Nuclear Extract** (5 $\mu$ g-15 $\mu$ g recommended)  
**Y  $\mu$ l ddH<sub>2</sub>O** (add up to final volume)  
**30  $\mu$ l Reaction Mix**
- Incubate the **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  $\mu$ l** pre-chilled *Filter Binding Buffer*. Centrifuge the column with the collection tube at **6,000rpm** for **1 minute** in a microcentrifuge at room temperature.
- Transfer the **30  $\mu$ l 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  $\mu$ l** 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  $\mu$ l** pre-chilled *Filter Wash Buffer* to the *Isolation Column* on ice.
- Centrifuge the *Isolation Column* with the collection tube for **1 minute** at **6,000rpm** in a microcentrifuge at **4°C**. Then discard the flow through.
- Repeat steps 8-9 for an additional **3 times** for a total a 4 washes.

## Elution of Bound Probe

- Add **50  $\mu$ l** of *Elution Buffer* onto the center of *Isolation Column*, and incubate at room temperature for **5 minutes**.
- Place the *Isolation Column* on a new 1.5 ml microcentrifuge tube and centrifuge at **10,000 rpm** for **2 minutes** at room temperature.
- If you have yet to do so, chill **200  $\mu$ l** ddH<sub>2</sub>O (DNAase free) in a 1.5 ml 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 ddH<sub>2</sub>O 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 *96-Well Plate*.
- Aliquot **2 ml** pre-warmed *TF Plate Hybridization Buffer* to a dispensing reservoir (DNase free) and then add **200  $\mu$ l** denatured probes. Mix them together by gently shaking the reservoir.
- Using a 8 multi-channel pipette **100  $\mu$ l** of the mixture from step 18. into the corresponding wells with 8 multi-channel pipette **immediately**.

**Note:** the *96-Well Plate* is divided into 6 sections of two columns each for 6 samples. Two columns are used per sample. **If you wish to have a blank 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  $\mu$ l 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 the *96-Well Plate* 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 *96-Well Plate* over an appropriate container and expel the contents forcibly.
23. Wash the plate 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 plate for **5 minutes** with gentle shaking at room temperature on a plate-shaker. Completely remove at end of 5 minutes by tapping the plate 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 plate over an appropriate container to forcibly remove *Blocking Buffer* from the wells.
27. If you have yet to do so: add **20 µl** of *Streptavidin-HRP Conjugate* in **10 ml Blocking Buffer** (1:500 dilution), enough for the whole plate (6 sections). This is the *diluted Streptavidin-HRP Conjugate*

28. 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.
29. After the **45 minutes** have elapsed, forcibly remove the *96-Well Plate* contents in an appropriate container. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
30. Wash the *96-Well Plate* 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. Decant the liquid from the wells.
31. Repeat step 30. for a total of 3 washes. At the last wash, invert plate on clean paper towels for **1 minute** to remove excessive liquid.
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 the entire 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. Allow plate to sit inside machine for **4 minutes** before reading. Set integration time to **1 second** with no filter position. For the best results, read the plate within **5-20 minutes**.

#### Oxidative Stress TF Activation Profiling Array Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	AP1	IRF	AP1	IRF	AP1	IRF	AP1	IRF	AP1	IRF	AP1	IRF
B	ATF4	NFAT	ATF4	NFAT	ATF4	NFAT	ATF4	NFAT	ATF4	NFAT	ATF4	NFAT
C	CBF/NFY	NFkB	CBF/NFY	NFkB	CBF/NFY	NFkB	CBF/NFY	NFkB	CBF/NFY	NFkB	CBF/NFY	NFkB
D	EGR1	NRF2/ARE	EGR1	NRF2/ARE	EGR1	NRF2/ARE	EGR1	NRF2/ARE	EGR1	NRF2/ARE	EGR1	NRF2/ARE
E	ETS	P53	ETS	P53	ETS	P53	ETS	P53	ETS	P53	ETS	P53
F	FOXO1	Smad	FOXO1	Smad	FOXO1	Smad	FOXO1	Smad	FOXO1	Smad	FOXO1	Smad
G	HIF	SRF	HIF	SRF	HIF	SRF	HIF	SRF	HIF	SRF	HIF	SRF
H	HSF	Stat3	HSF	Stat3	HSF	Stat3	HSF	Stat3	HSF	Stat3	HSF	Stat3

#### Related Products

Catalog #	Product Description
<b>FA-1001</b>	TF Activation Profiling Plate Array I
<b>FA-1002</b>	TF Activation Profiling Plate Array II
<b>FA-1003</b>	Stem Cell TF Activation Profiling Plate Array
<b>FA-1004</b>	Cancer Stem Cell TF Activation Profiling Plate Array
<b>FA-1006</b>	ER (UPR) Stress TF Activation Profiling Plate Array