



## Insulin Resistance TF Activation Profiling Plate Array

Catalog # FA-1014

(For Research Use Only)

### Introduction

Insulin resistance and central adiposity are strong risk indicators for type 2 diabetes and coronary heart disease. Insulin resistance is a pathological condition in which cells fail to respond normally to the hormone insulin, leading to high blood sugar. Many transcription factors are related to the regulation of adipocyte metabolism, by helping glucose uptake and lipid accumulation, meanwhile reducing insulin sensitivity. Signosis has developed Insulin Resistant Transcription Factor Activation Plate Array to analyze the activities of 16 Insulin Resistant-related TFs simultaneously in mammalian samples, including AP2, CREB, EGR-1, FOXO1, HNF-1, HSF-1, LXR, MAFA, NEUROD, NKX2.2, NKX6.1, PAX4, PAX6, PDX-1, PPAR, SREBP1.

### 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 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	600µL	RT
TF Plate Hybridization Buffer	12mL	RT
5X Plate Hybridization Wash Buffer	30mL	RT
5X Detection Wash Buffer	40mL	RT
Blocking Buffer	30mL	4°C
Filter Wash Buffer	15mL	4°C
Filter Binding Buffer	1.5mL	4°C
Substrate A	1mL	4°C
Substrate B	1mL	4°C
Streptavidin-HRP Conjugate	20µL	4°C
Substrate Dilution Buffer	8mL	4°C
TF Binding Buffer Mix	90µL	-20°C
TF ER Stress Probe Mix	20µL	-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 **200µL** of ddH<sub>2</sub>O in a 1.5mL microcentrifuge tube (per sample; 3 samples would be 600µL ddH<sub>2</sub>O) on ice so that it is chilled for the assay (for at least **10 minutes**).
4. Dilute **30mL** of *5X Plate Hybridization Wash Buffer* with **120mL** of ddH<sub>2</sub>O before use.
5. Dilute **40mL** of *5X Detection Wash Buffer* with **160mL** of ddH<sub>2</sub>O before use.
6. Dilute **20µL** *Streptavidin-HRP* in **10mL** *Blocking Buffer* (1:500 dilution).



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

# Assay Procedure

## TF/ DNA Complex Formation

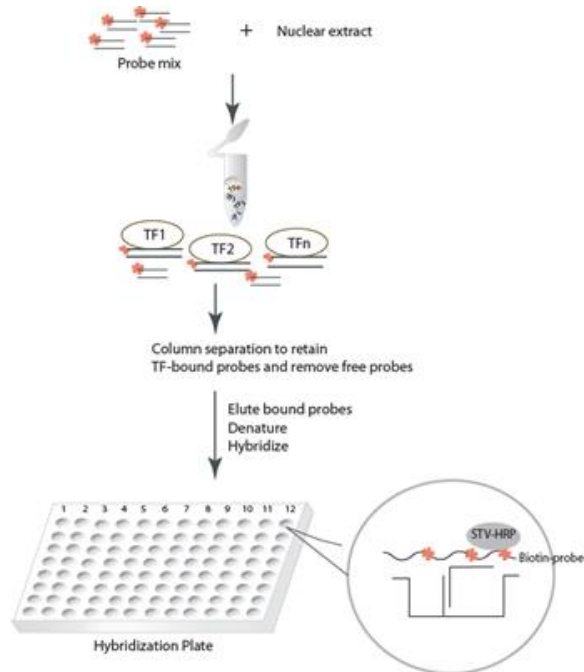
- Mix the following components for each reaction in a tube  
**15 $\mu$ L** *TF Binding Buffer Mix*  
**3 $\mu$ L** *TF Probe mix*  
**X $\mu$ L** Nuclear Extract (5 $\mu$ g-15 $\mu$ g recommended)  
**Y $\mu$ L** ddH<sub>2</sub>O (add up to final volume)  
**30 $\mu$ L** *Reaction Mix*
- Incubate the **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 1.5mL 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 (DNase 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 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 **2mL** 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 an 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 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 $\mu$ 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 **200µL** 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 **200µL** 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 **20µL** of *Streptavidin-HRP Conjugate* in **10mL Blocking Buffer** (1:500 dilution), enough for the whole plate (6 sections). This is the *diluted Streptavidin-HRP Conjugate*

28. Add **95µL** 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 **200µL 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:  
**1mL Substrate A**  
**1mL Substrate B**  
**8mL Substrate Dilution Buffer**  
**10mL Substrate Solution**
33. Add **95µL 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**.

#### Insulin Resistant TF Activation Profiling Plate Array Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	AP2	NEUROD	AP2	NEUROD	AP2	NEUROD	AP2	NEUROD	AP2	NEUROD	AP2	NEUROD
B	CREB	NKX2.2	CREB	NKX2.2	CREB	NKX2.2	CREB	NKX2.2	CREB	NKX2.2	CREB	NKX2.2
C	EGR-1	NKX6.1	EGR-1	NKX6.1	EGR-1	NKX6.1	EGR-1	NKX6.1	EGR-1	NKX6.1	EGR-1	NKX6.1
D	FOXO1	PAX4	FOXO1	PAX4	FOXO1	PAX4	FOXO1	PAX4	FOXO1	PAX4	FOXO1	PAX4
E	HNF-1	PAX6	HNF-1	PAX6	HNF-1	PAX6	HNF-1	PAX6	HNF-1	PAX6	HNF-1	PAX6
F	HSF-1	PDX1	HSF-1	PDX	HSF-1	PDX	HSF-1	PDX	HSF-1	PDX	HSF-1	PDX
G	LXR	PPAR	LXR	PPAR	LXR	PPAR	LXR	PPAR	LXR	PPAR	LXR	PPAR
H	MAFA	SREBP1	MAFA	SREBP1	MAFA	SREBP1	MAFA	SREBP1	MAFA	SREBP1	MAFA	SREBP1

#### 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-1005</b>	Oxidative Stress TF Activation Profiling Plate Array

