Signosis, Inc.
Innovative Plate Assay Solutions

Cholesterol Metabolism TF Activation Profiling Plate Array

Catalog Number FA-1008 **(For Research Use Only)**

Introduction

Cholesterol is an essential component of cell membranes to ensure cell viability and cell growth. Changes in cholesterol level could lead to abnormal biological processes and high levels are associated with several diseases, including cardiovascular disease, heart attack, stroke and Huntington's disease. It has been widely reported that cholesterol homeostasis and metabolism are regulated by a group of transcription factors (TF). Profiling the regulation and activation patterns of these associated transcription factors is crucial to further understanding and unraveling the intricate cholesterol metabolism pathway. **Signosis, Inc.** has developed and produced this array to help you detect specific activation of the many TFs involved in cholesterol metabolism including SREBP, LXR, FXR, USF-1, CREB, FOXO, HNF4, C/EBP, PPAR, COUP-TF, NUR77, EGR, GATA, NFkB and CHOP.

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
- ddH2O (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 Cholesterol Metabolism 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_2O in a 1.5 ml microcentrifuge tube (per sample; 3 samples would be 600 µl ddH_2O) 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 ddH2O before use.
5. Dilute **40 ml** of *5X Detection Wash Buffer* with **160 ml** of ddH2O 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

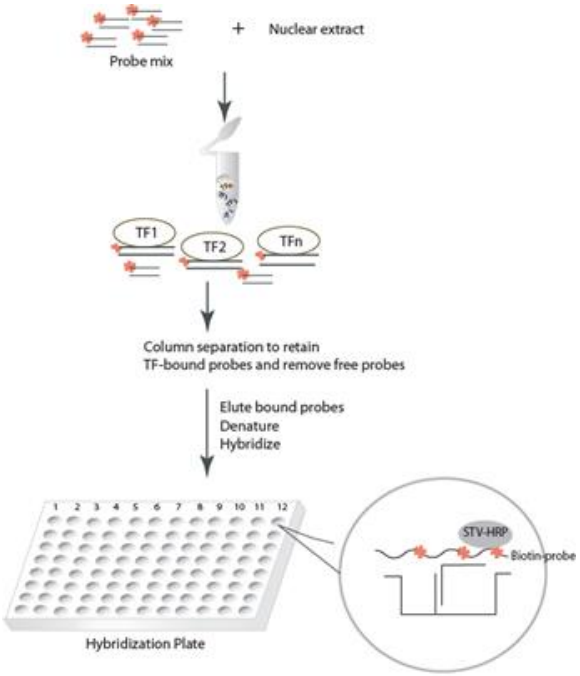
1. Mix the following components for each reaction in a tube
   15 µl *TF Binding Buffer Mix*
   3 µl *TF Probe mix*
   X µl Nuclear Extract (5µg-15µg recommended)
   Y µl ddH2O (add up to final volume)
   30 µl Reaction Mix
2. Incubate the **Reaction Mix** at room temperature (20-23ºC) for **30 minutes**.

Separation of TF DNA Complex from Free Probes

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

Elution of Bound Probe

11. Add **50 µl** of *Elution Buffer* onto the center of *Isolation Column*, and incubate at room temperature for **5 minutes**.
12. Place the *Isolation Column* on a new 1.5 ml microcentrifuge tube and centrifuge at **10,000 rpm** for **2 minutes** at room temperature.
13. If you have yet to do so, chill **200 µl** ddH2O (DNAase free) in a 1.5 ml microcentrifuge tube on ice for at least **10 minutes**, and **keep on ice**.
14. Transfer the eluted probe to a PCR tube and denature the eluted probes at **98°C** for **5 minutes**.
15. **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 **-20ºC** 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**.

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

Hybridization of Eluted Probe with Hybridization Plate

17. Remove the clear adhesive film sealing from the provided *96-Well Plate*.
18. Aliquot **2 ml** pre-warmed *TF Plate Hybridization Buffer* to a dispensing reservoir (DNase free) and then add **200 µl** denatured probes. Mix them together by gently shaking the reservoir.
19. Using a 8 multi-channel pipette **100 µl** 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 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 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 *1X 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** *1X 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**.

Cholesterol Metabolism TF Activation Profiling Array Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	SREBP	PPAR	SREBP	PPAR	SREBP	PPAR	SREBP	PPAR	SREBP	PPAR	SREBP	PPAR
B	LXR	Coup-TF	LXR	Coup-TF	LXR	Coup-TF	LXR	Coup-TF	LXR	Coup-TF	LXR	Coup-TF
C	FXR	Nur77	FXR	Nur77	FXR	Nur77	FXR	Nur77	FXR	Nur77	FXR	Nur77
D	USF-1	EGR	USF-1	EGR	USF-1	EGR	USF-1	EGR	USF-1	EGR	USF-1	EGR
E	CREB	GATA	CREB	GATA	CREB	GATA	CREB	GATA	CREB	GATA	CREB	GATA
F	FOXO	NFkB	FOXO	NFkB	FOXO	NFkB	FOXO	NFkB	FOXO	NFkB	FOXO	NFkB
G	HNF4	CHOP	HNF4	CHOP	HNF4	CHOP	HNF4	CHOP	HNF4	CHOP	HNF4	CHOP
H	C/EBP	RXR	C/EBP	RXR	C/EBP	RXR	C/EBP	RXR	C/EBP	RXR	C/EBP	RXR

Related Products

Catalog #	*Product Description*
FA-1001	TF Activation Profiling Plate Array I
FA-1002	TF Activation Profiling Plate Array II
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