Signosis, Inc.
Innovative Plate Assay Solutions

Mitochondrial UPR TF Activation Profiling Plate Array

Catalog # FA-1010 **(For Research Use Only)**

Introduction

Protein folding within the mitochondria faces challenges from organelle structure, ROS production from mitochondria's role in bioenergetics metabolism, and assembly of complex macromolecules in stoichiometric manner. To maintain protein homeostasis, a stress response known as Mitochondrial Unfolded Protein Response (UPRmt) upregulates chaperones' gene expression and increases mitochondrial folding capacity during stress. This prevents deleterious protein aggregation and maintains proper protein folding. UPRmt activates a large number of transcription factors (TFs) including AP1, ATF4, C/EBP, CHOP, E2F1, FOXO3, HIF, HSF, MEF2, NFkB, NRF1, NRF2/ARE, p53, SATB, TFEB and XBP. **Signosis, Inc.** has developed the *Mitochondrial UPR TF Activation Profiling Plate Array*, which can be used to monitor 16 mitochondrial UPR 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 tube
- 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 UPRmt 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

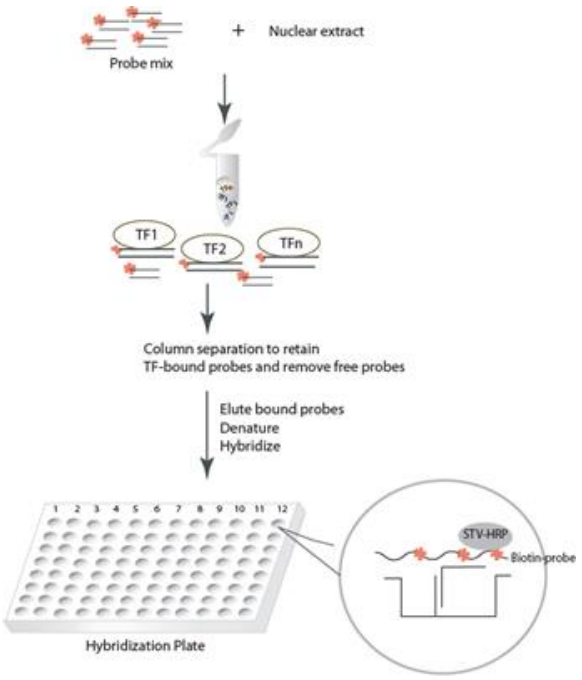
1. Mix the following components for each reaction in a tube
   15 µl *TF Binding Buffer Mix*
   3 µl *TF Probe mix*
   X µl Nuclear Extract (5µg-15µg recommended)
   X µl ddH2O (add up to final volume)
   30 µl Reaction Mix
2. Incubate the **Reaction Mix** at room temperature (20-23ºC) for **30 minutes**.

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**.

UPRmt TF Activation Profiling Array Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	AP1	MEF2	AP1	MEF2	AP1	MEF2	AP1	MEF2	AP1	MEF2	AP1	MEF2
B	ATF4	NFkB	ATF4	NFkB	ATF4	NFkB	ATF4	NFkB	ATF4	NFkB	ATF4	NFkB
C	C/EBP	NRF1	C/EBP	NRF1	C/EBP	NRF1	C/EBP	NRF1	C/EBP	NRF1	C/EBP	NRF1
D	CHOP	NRF2/ARE	CHOP	NRF2/ARE	CHOP	NRF2/ARE	CHOP	NRF2/ARE	CHOP	NRF2/ARE	CHOP	NRF2/ARE
E	E2F1	P53	E2F1	P53	E2F1	P53	E2F1	P53	E2F1	P53	E2F1	P53
F	FOXO3	SATB	FOXO3	SATB	FOXO3	SATB	FOXO3	SATB	FOXO3	SATB	FOXO3	SATB
G	HIF	TFEB	HIF	TFEB	HIF	TFEB	HIF	TFEB	HIF	TFEB	HIF	TFEB
H	HSF	XBP	HSF	XBP	HSF	XBP	HSF	XBP	HSF	XBP	HSF	XBP

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-1006	ER (UPR) Stress TF Activation Profiling Plate Array