Signosis, Inc.
Innovative Plate Assay Solutions

Lysosomal Stress TF Activation Profiling Plate Array

Catalog Number: FA-1012 **(For Research Use Only)**

Introduction

The lysosome plays a key role in cellular homeostasis by controlling both cellular clearance and energy production to respond to environmental cues. It is well known that transcription factor EB (TFEB) and mTOR complex 1 (mTORC1) are master regulators of lysosomal biogenesis. Under starvation, cells initiate the process of autophagy and increase the capacity of the lysosome by the lysosome stress response. mTORC1 dissociates from the lysosome and becomes inactivated, leading to dephosphorylation of TFEB and autophagy. Dephosphorylated TFEB enters the nucleus, binds to the enhancer element CLEAR, and activates transcription of genes related to the lysosome as well as autophagy. The lysosome stress response is closely related to various diseases such as Huntington's disease, liver disease and lysosomal storage diseases including Pompe disease. Recently, more TFs have been reported to be associated with lysosome stress, such as p53, FOXO1, PPAR, Stat3, NFkB, HIF, HSF and MITF. **Signosis, Inc.'s** *Lysosomal Stress TF Activation Profiling Plate Array* is used for monitoring 8 different 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
- 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	12	RT
Elution Buffer	1.2mL	RT
TF Plate Hybridization Buffer	25mL	RT
5X Plate Hybridization Wash Buffer	30mL	RT
5X Detection Wash Buffer	40mL	RT
Blocking Buffer	30mL	RT
Filter Wash Buffer	25mL	4°C
Filter Binding Buffer	2.4mL	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	180µL	-20°C
TF Lysosomal Stress Probe Mix	36µL	-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* **42˚C** before use.
3. Aliquot **500µL** of ddH_2O 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 **30mL** of *5X Plate Hybridization Wash Buffer* with **120mL** of ddH2O before use.
5. Dilute **40mL** of *5X Detection Wash Buffer* with **160mL** of ddH2O 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

1. Mix the following components for each reaction in a tube
   15µL *TF Binding Buffer Mix*
   3µL *Lysosomal Stress Probe MixI*
   XµL Nuclear Extract (5µg-15µg recommended)
   YµL ddH2O (add up to final volume)
   30µL Reaction Mix [final volume]
2. Incubate the **Reaction Mix** at room temperature (20-23°C) for **30 minutes**.

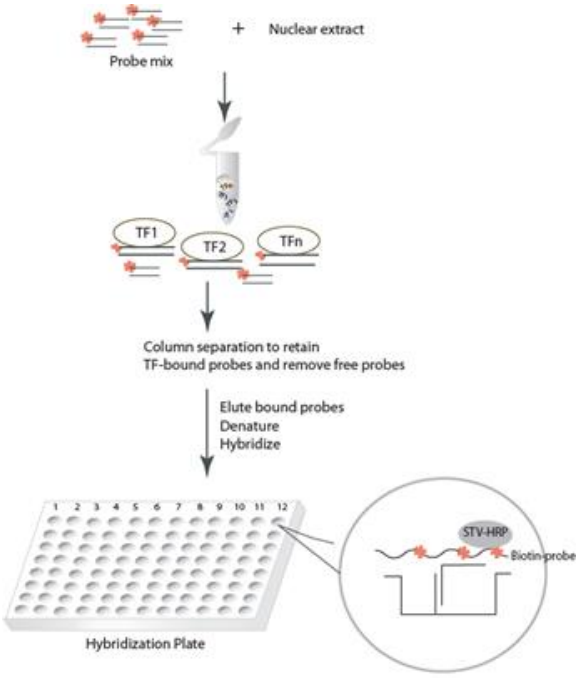
Separation of TF DNA Complex from Free Probes

3. Equilibrate an *Isolation Column* by adding **200µL** 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 **30µL 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 **500µL** 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 **4°C**. Discard the flow through from the collection tube.
8. Wash the column by adding **500µL** 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 **4°C.**
10. Repeat steps 8-9 for an additional **3 times** for a total a 4 washes.

Elution of Bound Probe

11. Place the *Isolation Column* on a new 1.5mL microcentrifuge tub. Add **100µL** of *Elution Buffer* onto the center of *Isolation Column*, and incubate at room temperature for **5 minutes**.
12. Centrifuge the column in the new 1.5mL tube at **10,000 rpm** for **2 minutes** at room temperature.
13. If you have yet to do so, chill **500µL** ddH2O (DNAase free) in a 1.5mL 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 **-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**.

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 **10mL** pre-warmed *TF Plate Hybridization Buffer* to a dispensing reservoir (DNase free) and then add **600µL** denatured probes. Mix them together by gently shaking the reservoir.
19. Using a 8 multi-channel pipette **100µL** of the mixture from step 18. into the corresponding wells with 8 multi-channel pipette **immediately**.
    Note: the *96-Well Plate* is divided into 2 sections of six columns each 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 **100µL** *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 *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 **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** *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:
    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**.

Lysosomal Stress TF Activation Profiling Array Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	FOXO1	FOXO1	FOXO1	FOXO1	FOXO1	FOXO1	FOXO1	FOXO1	FOXO1	FOXO1	FOXO1	FOXO1
B	HIF	HIF	HIF	HIF	HIF	HIF	HIF	HIF	HIF	HIF	HIF	HIF
C	HSF	HSF	HSF	HSF	HSF	HSF	HSF	HSF	HSF	HSF	HSF	HSF
D	MITF	MITF	MITF	MITF	MITF	MITF	MITF	MITF	MITF	MITF	MITF	MITF
E	NFkB	NFkB	NFkB	NFkB	NFkB	NFkB	NFkB	NFkB	NFkB	NFkB	NFkB	NFkB
F	p53	p53	p53	p53	p53	p53	p53	p53	p53	p53	p53	p53
G	PPAR	PPAR	PPAR	PPAR	PPAR	PPAR	PPAR	PPAR	PPAR	PPAR	PPAR	PPAR
H	STAT3	STAT3	STAT3	STAT3	STAT3	STAT3	STAT3	STAT3	STAT3	STAT3	STAT3	STAT3

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

Data Example

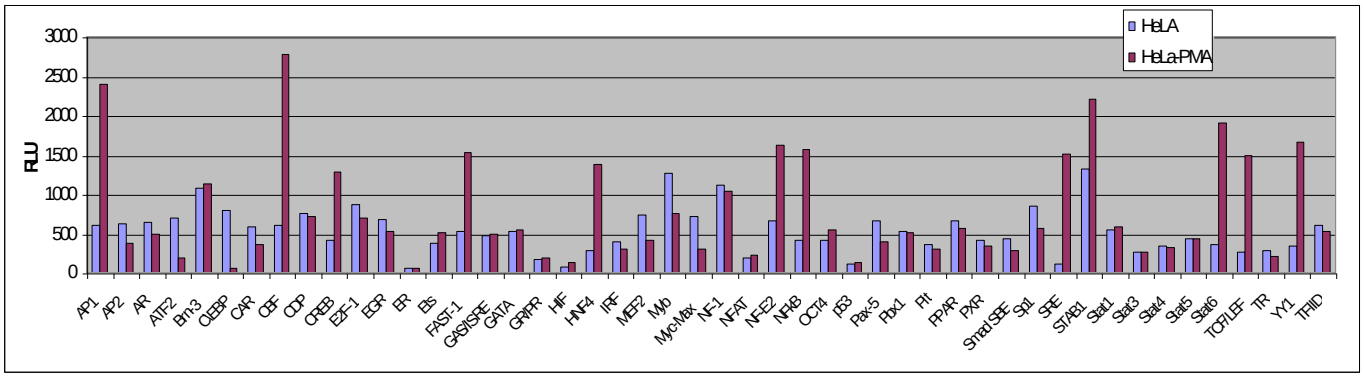


Figure: TF Activation Profiling Plate Array Assay acquired RLUs. HeLA cells were treated with and without PMA. Nuclear Extracts prepared and subjected to the TF Profiling Assay I (Cat# FA-1001).

Data analysis notes:

1. The TF readings within blank reading ±10%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.

Gene Description

TF	Description	TF	Description
AHR	aryl hydrocarbon receptor	MNF1	myocyte nuclear factor
ATF3	activating transcription factor 3	MTF	Myelin Trnascription factor
ATF6	activating transcription factor 6	Nanog	NANOG (Nanog Homeobox)
CHOP	DNA damage inducible transcript 3	Nhlh1 (HEN)	nescient helix loop helix 1
E12/E47	E2A immunoglobulin enhancer-binding factors E12/E47)	Notch	Notch homolog, translocation-associated (Drosophila)
EBF1	Early B-Cell Factor 1	NUR77	nerve growth factor IB (NGFIB)
EBP-80	Emopamil binding protein 80	OLIG1	oligodendrocyte transcription factor 1
EKLF	Erythroid Krüppel-like factor (EKLF)	PEA3/EVT4	ETS translocation variant 4 (ETV4)
Evl-1	myeloid transforming gene binds a consensus sequence	PITX2	Paired-Like Homeodomain 2
FXR	nuclear receptor subfamily 1 group H member 4 [Homo sapiens	PRDM14	PRDI-BF1 and RIZ homology domain containing
GBX2	gastrulation brain homeobox 2	PROP1	Paired-Like Homeobox 1
GKLF	gut-enriched Kruppel-like factor (GKLF)	pu.1	ETS-domain transcription factor, binding to a purine-rich
HES1	hes family bHLH transcription factor 1	REST	repressor element 1 silencing transcription factor/neuron-restrictive silencing factor (REST/NRSF)
HFH1	HNF-3/fkh homolog (HFH) genes	RREB	Ras Responsive Element Binding Protein 1
HLF	hepatic leukemia factor)	Sperm1	Sperm-related protein
HOXA4	homeobox A4	SREBP1	Sterol regulatory element-binding protein 1
INSM1	myeloid ecotropic viral integration site 1	TBET	T-cell-specific T-box transcription factor
ISGF	nterferon regulatory factor; ISGF	TBX3	T-box transcription factor
KRF1	keratinocyte-specific transcription factor, KRF1	Tead	Transcriptional enhancer factor TEF-1
LRH-1	liver receptor homolog-1	TFEB	T-Cell Transcription Factor EB
LXR	Liver X receptors (LXRs)	TGIF/Meis2	myeloid ecotropic viral integration site 1
MAF	v-maf avian musculoaponeurotic fibrosarcoma oncogene	Twist1	Twist Basic Helix-Loop-Helix Transcription Factor 1
MIBP	The c-myc intron binding protein 1	VAX2	Ventral Anterior Homeobox 2
MITF	Microphthalmia-Associated Transcription Factor	SIX	SIX homodomain protein