**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 | 4°C |
| **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*, *Blocking 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**

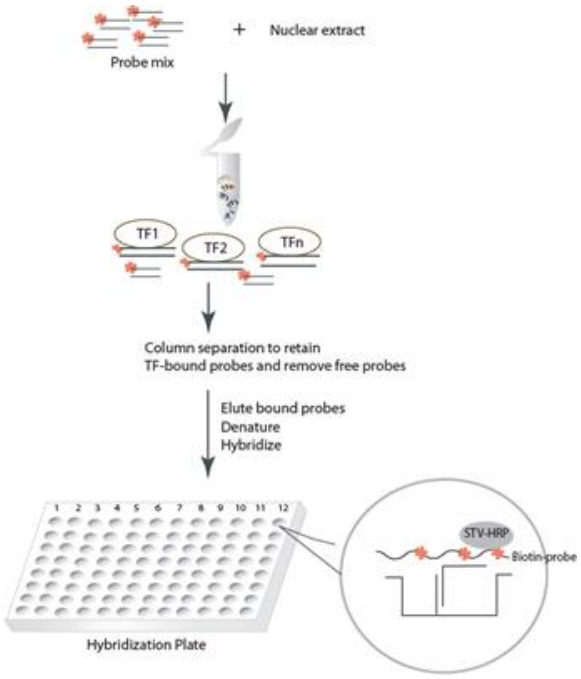
1. Mix the following components for each reaction in a tube
   **15µL** *TF Binding Buffer Mix*
   **3µL** *Lysosomal Stress Probe MixI*
   **XµL** Nuclear Extract (5µg-15µg recommended)
   **YµL** ddH2O (add up to final volume)
   **30µL Reaction Mix [**final volume]
2. Incubate the **Reaction Mix** at room temperature (20-23ºC) 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 **4ºC**. 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 **4ºC.**
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 **-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 **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**

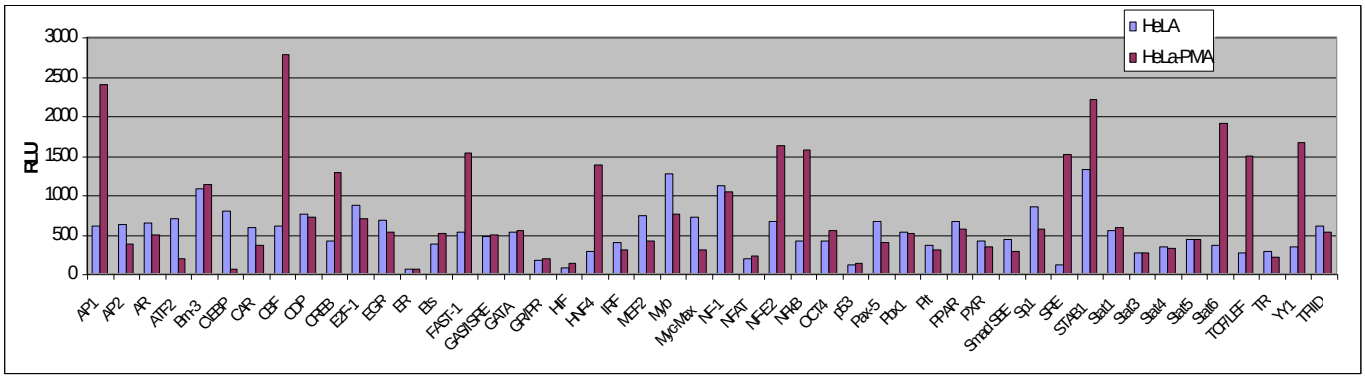



**Figure**: TF Activation Profiling Plate Array Assay acquired RLUs. HeLA cells were treated with and without PMA. Nuclear Extracts prepared and subjected to the TF Profiling Assay I (Cat# FA-1001).

**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 |