**Signosis, Inc.**
Innovative Plate Assay Solutions

# TF Activation Profiling Plate Array II

**Catalog Number: FA-1002** **(For Research Use Only)**

## Introduction

Transcription factors (TFs) are a group of cellular proteins that play essential roles in regulating gene expression. They act as sensors to monitor cellular changes and convert signals into gene expression. Often, a specific cellular signal pathway can activate multiple TFs. The expression of a specific gene can also be under the control of multiple TFs. Thus, monitoring the activation of multiple TFs simultaneously is critical to understanding the molecular mechanism of cellular regulation underlying cell signaling and gene expression. **Signosis, Inc.'s** *TF Activation Profiling Plate Array II* is used for monitoring 96 different TFs simultaneously from one sample.

## Principle of the assay

**Signosis, Inc.'s** *TF Activation Profiling Plate Array II* is used for monitoring the activation of multiple TFs simultaneously. With 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 the *96-Well 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 Plates (with aluminum adhesive seal)** | 2 | RT |
| **Isolation Columns** | 2 | RT |
| **Elution Buffer** | 400 µL | RT |
| **TF Plate Hybridization Buffer** | 20 mL | RT |
| **5X Plate Hybridization Wash Buffer** | 60 mL | RT |
| **5X Detection Wash Buffer** | 60 mL | RT |
| **Blocking Buffer** | 60 mL | RT |
| **Filter Wash Buffer** | 5 mL | 4°C |
| **Filter Binding Buffer** | 1 mL | 4°C |
| **Substrate A** | 2 mL | 4°C |
| **Substrate B** | 2 mL | 4°C |
| **Streptavidin-HRP Conjugate** | 40 µL | 4°C |
| **Substrate Dilution Buffer** | 16 mL | 4°C |
| **TF Binding Buffer Mix** | 60 µL | -20°C |
| **TF Probe Mix II** | 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* and *Hybridization Wash Buffer* to **42˚C** before use.
3. Aliquot **500 µL** 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 **60 mL** of *5X Plate Hybridization Wash Buffer* with **240 mL** of ddH2O before use.
5. Dilute **60 mL** of *5X Detection Wash Buffer* with **240 mL** of ddH2O before use.
6. Dilute **40 µL** *Streptavidin-HRP* in **20 mL** Blocking Buffer (1:500 dilution).

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

# Assay Procedure

**TF/ DNA Complex Formation**

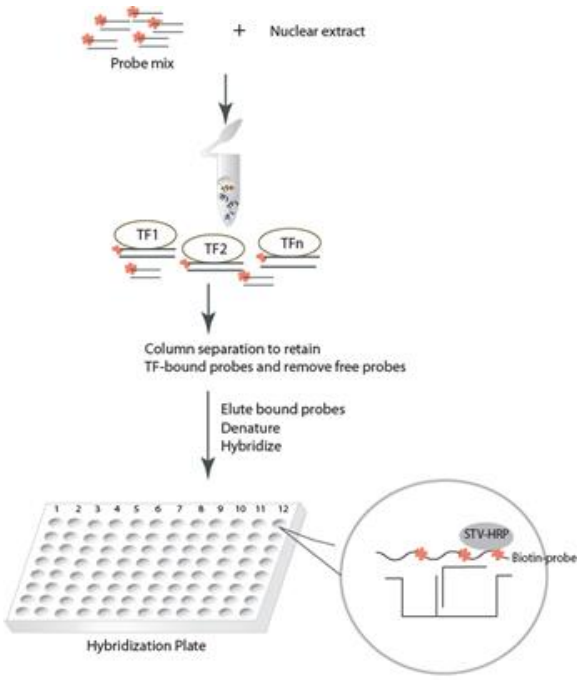
1. Mix the following components for each reaction in a tube
   **15 µL** *TF Binding Buffer Mix*
   **5 µL** *TF Probe Mix II*
   **X µL** Nuclear Extract (5µg-15µg recommended)
   **X µL** ddH2O (add up to final volume)
   **30 µL Reaction Mix [**final volume]
2. Incubate the **Reaction Mix** at room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

**Separation of TF DNA Complex from Free Probes**

3. Equilibrate an *Isolation Column* by adding **200 µL** pre-chilled *Filter Binding Buffer*. Centrifuge the column with the collection tube at **6,000 rpm** 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 **6,000 rpm** in a microcentrifuge at **4ºC.** Then discard the flow through.
10. Repeat steps 8-9 for an additional **3 times** for a total of 4 washes.

**Elution of Bound Probe**

11. Place the *Isolation Column* in a new 1.5mL microcentrifuge tube. Add **100 µL** of *Elution Buffer* onto the center of *Isolation Column* and incubate at room temperature for **5 minutes**.
12. Centrifuge the microcentrifuge tube with the *Isolation Column* at **10,000 rpm** for **2 minutes** at room temperature.
13. If you have yet to do so, chill **500 µL** 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 **10 mL** pre-warmed *TF Plate Hybridization Buffer* to a dispensing reservoir (DNase free) and then add **600 µL** denatured probes. Mix them together by gently shaking the reservoir.
19. Using a 12 multi-channel pipette **100 µL** of the mixture from step 18. into the corresponding wells with 8 multi-channel pipette **immediately**.

    **Note**: **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.
25. If you have yet to do so: add **40 µL** of *Streptavidin-HRP Conjugate* in **20 mL** *Blocking Buffer* (1:500 dilution), enough for the whole plate (6 sections). This is the *diluted Streptavidin-HRP Conjugate*
26. 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.
27. 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.
28. 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.
29. Repeat step 28. two more times. At the third and final wash, invert plate on clean paper towels for **1 minute** to remove excessive liquid.
28. 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*
29. 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.
30. 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**.

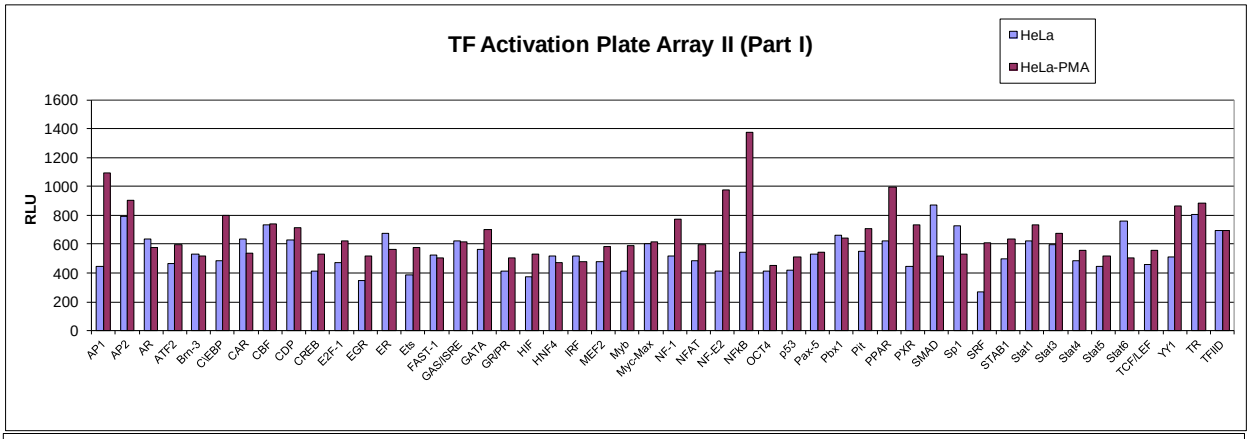
**TF Activation Profiling Array II Diagram**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | AP1 | CDP | GATA | NF-1 | Pit | Stat3 | XBP | FOXG1 | HoxA-5 | NRF2(ARE) | Prox1 | SOX2 |
| B | AP2 | CREB | GR/PR | NFAT | PPAR | Stat4 | AP3 | FOXO1(FKHR) | HSF | Oct-1 | RB | SOX9 |
| C | AR | E2F-1 | HIF | NF-E2 | PXR | Stat5 | AP4 | FREAC2 (FOXF2) | KLF4 | Pax2 | RUNX | SOX18 |
| D | ATF2 | EGR | HNF4 | NFkB | SMAD | Stat6 | COUP-TF | Gli-1 | MyoD | Pax3 | ROR(RZR) | SRY |
| E | Brn-3 | ER | IRF | OCT4 | Sp1 | TCF/LEF | ELK | Gfi-1 | MZF | Pax8 | RXR | TFE3 |
| F | C\EBP | Ets | MEF2 | p53 | SRF | YY1 | FOXA1 | HEN (NSCL-1) | Nkx2-5 | PIT1 | SF-1 | USF-1 |
| G | CAR | FAST-1 | Myb | Pax-5 | SATB1 | TR | FoxC1 | HNF-1 | Nkx3-2 | PLAG1 | SMUC | VDR |
| H | CBF | GAS/ISRE | Myc-Max | Pbx1 | Stat1 | TFIID | FOXD3 | HOX4C | NRF1 | MEF1 | Snail | WT1 |

**Data analysis notes:**

1. TF readings within ±10% of a 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.

**Data Example**



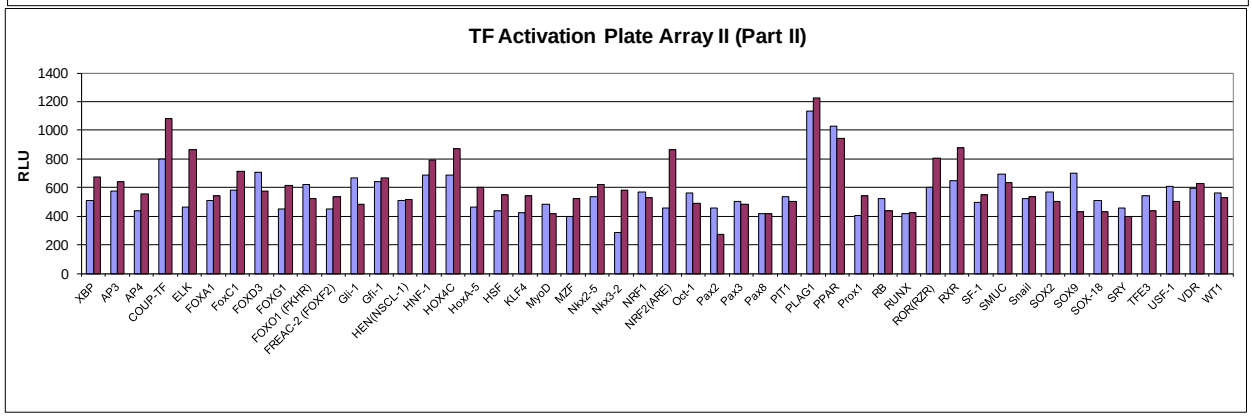




**Figure**: TF Activation Profiling Plate Array Assay acquired RLUs. HeLA cells were treated with and without PMA. Nuclear Extracts prepared and subjected to the TF Profiling Assay I.

| Related Products | |
|---|---|
| ***Catalog #*** | ***Product Description*** |
| **FA-1001** | TF Activation Profiling Plate Array I |
| **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 |
| **FA-1006** | ER (UPR) Stress TF Activation Profiling Plate Array |

## Gene Description

| TF names | Gene Description | TF names | Gene Description |
|---|---|---|---|
| AP1 | Activator protein 1 (JUN/FOS) | XBP-1 | X-box binding protein 1 |
| AP2 | Activator protein 2 | AP3 | AP3 protein |
| AR | Androgen receptor | AP4 | AP4 protein |
| ATF2 | activating transcription factor 2 | COUP-TF | nuclear receptor subfamily 2, group F, |
| Brn-3 | POU domain, class 4, transcription factor 1 | ELK | ETS domain-containing protein Elk-1 |
| C/EBP | CCAAT/enhancer binding protein (C/EBP),alpha | FOXA1 | homeobox A1 |
| CAR | nuclear receptor subfamily 1, group I, member 3 | FoxC1 | homeobox C1 |
| CBF | CCAAT/enhancer binding protein (C/EBP), zeta | FOXD3 | forkhead box D3 |
| CDP | cut-like homeobox 1; CCAAT displacement protein | FOXG1 | FOXbox G1 |
| CREB | cAMP responsive element binding protein 1 | FOXO1 (FKHR) | FOXbox O1 |
| E2F-1 | E2F transcription factor 1 | FREAC-2 | Forkhead-related activator 2 |
| EGR | Early growth response | Gfi-1 | growth factor independent 1 transcription |
| ER | Estrogen receptor | Gli-1 | GLI zinc finger transcription factor |
| Ets | v-ets erythroblastosis virus E26 oncogene homolog 1 | HEN(NSCL-1) | helix-loop-helix protein |
| FAST-1(FOXH1) | Forkhead box H1 | HNF-1 | Hepatocyte Nuclear Factor 1 |
| GAS/ISRE | IFN-stimulated response element | HOX4C | HOX4c homobox |
| GATA | GATA transcription factor | HoxA-5 | homeobox A5 |
| GR/PR | Glucocorticoid receptor/Progesterone receptor | HSF | heat shock transcription factor 1 |
| HIF | Hypoxia inducible factor | KLF4 | Kruppel-like factor 4 |
| HNF4 | Hepatocyte nuclear factor 4 | MyoD | myogenic differentiation 1 protein |
| IRF | Interferon regulatory factor | MZF | zinc finger type transcription factor MZF |
| MEF2 | Myocyte enhancer factor 2 | Nkx2-5 | Homeobox protein Nkx-2.5 |
| Myb | v-myb myeloblastosis viral oncogene homolog | Nkx3-2 | Homeobox protein Nkx-3.2 |
| Myc-Max | v-myc myelocytomatosis viral oncogene homolog | NRF1 | nuclear respiratory factor 1 |
| NF-1 | Nuclear factor 1 | NRF2(ARE) | NRF2-related antioxidant responsive |
| NFAT | Nuclear factor of activated T-cells | Oct-1 | POU domain, class 2, transcription factor |
| NF-E2 | Nuclear factor (erythroid-derived 2) | Pax2 | Pair box-2 protein |
| NFkB | nuclear factor of kappa light polypeptide gene | Pax 3 | Pair box-3 protein |
| OCT4 | POU class 5 homeobox 1 | Pax8 | Pair box-8 protein |
| p53 | Tumor protein p53 | PIT1 | POU class 1 homeobox 1 |
| Pax-5 | Paired box 5 | PLAG1 | pleiomorphic adenoma gene 1 |
| Pbx1 | Pre-B cell leukemia transcription factor-1 | MEF1 | Myocyte enhancer factor 1 |
| Pit | Pituitary specific transcription factor 1 | Prox1 | Prospero homeobox protein 1 |
| PPAR | Peroxisome proliferator-activated receptor | RB | Retinoblastoma control element |
| PXR | Pregnane X Receptor | RUNX | Runt-related transcription factor 1 |
| SMAD (MADH) | SMAD family | ROR(RZR) | retinoic acid receptor-related orphan |
| Sp1 | SP1 transcription factor | RXR | retinoid X receptor |
| SRF | Serum response factor | SF-1 | Steroidogenic factor 1 |
| SATB1 | Special AT-rich sequence binding protein 1 | SMUC | snail-related transcription factor Smuc |
| Stat1 | Signal transducer and activator of transcription 1 | Snail | Snail 1 zinc finger protein |
| Stat3 | Signal transducer and activator of transcription 3 | SOX2 | SOX protein 2 |
| Stat4 | Signal transducer and activator of transcription 4 | SOX9 | SOX protein 9 |
| Stat5 | Signal transducer and activator of transcription 5 | SOX-18 | SOX protein 18 |
| Stat6 | Signal transducer and activator of transcription 6 | SRY | sex determining region Y |
| TCF/LEF | T cell factor / Lymphoid enhancer factor proteins | TFE3 | transcription factor binding to IGHM |
| YY1 | YY1 transcription factor | USF-1 | upstream transcription factor 1 |
| TR | Thyroid hormone receptor | VDR | vitamin D (1,25- dihydroxyvitamin D3) |
| TFIID | TATA box binding protein | WT1 | Wilms Tumor 1 suppresor protein1 |