TF Activation Profiling Plate Array II
Catalog Number: FA-1002

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

<table>
<thead>
<tr>
<th>Component</th>
<th>Qty</th>
<th>Store at</th>
</tr>
</thead>
<tbody>
<tr>
<td>96-Well Plates (with aluminum adhesive seal)</td>
<td>2</td>
<td>RT</td>
</tr>
<tr>
<td>Isolation Columns</td>
<td>2</td>
<td>RT</td>
</tr>
<tr>
<td>Elution Buffer</td>
<td>400 µL</td>
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<tr>
<td>TF Plate Hybridization Buffer</td>
<td>20 mL</td>
<td>RT</td>
</tr>
<tr>
<td>5X Plate Hybridization Wash Buffer</td>
<td>60 mL</td>
<td>RT</td>
</tr>
<tr>
<td>5X Detection Wash Buffer</td>
<td>60 mL</td>
<td>RT</td>
</tr>
<tr>
<td>Blocking Buffer</td>
<td>60 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Filter Wash Buffer</td>
<td>5 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Filter Binding Buffer</td>
<td>1 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Substrate A</td>
<td>2 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Substrate B</td>
<td>2 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Streptavidin-HRP Conjugate</td>
<td>40 µL</td>
<td>4°C</td>
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<tr>
<td>Substrate Dilution Buffer</td>
<td>16 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>TF Binding Buffer Mix</td>
<td>60 µL</td>
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</tr>
<tr>
<td>TF Probe Mix II</td>
<td>20 µL</td>
<td>-20°C</td>
</tr>
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</table>

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 to 42°C before use.
3. Aliquot 500 µL of ddH2O 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 (20-23°C) 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 4°C. 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 4°C. 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 -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 **10 µL** 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 IX 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 wells.
27. If you if you have yet to do so: add 40 µL of Streptavidin-HRP Conjugate in 20 µL Blocking Buffer (1:500 dilution), enough for the whole plate (6 sections). This is the diluted Streptavidin-HRP Conjugate.
28. Wash the 96-Well Plate by adding 200 µL IX 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.
30. 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

TF Activation Profiling Array II Diagram

<table>
<thead>
<tr>
<th>A</th>
<th>AP1</th>
<th>CDP</th>
<th>GATA</th>
<th>NF-1</th>
<th>Pit</th>
<th>Stat3</th>
<th>XBP</th>
<th>FOXG1</th>
<th>HoxA-5</th>
<th>NRP2(ARE)</th>
<th>Prox1</th>
<th>SOX2</th>
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<tbody>
<tr>
<td>B</td>
<td>AP2</td>
<td>CREB</td>
<td>GR/PR</td>
<td>NFAT</td>
<td>PPAR</td>
<td>Stat4</td>
<td>AP3</td>
<td>FOXO1(FKBP)</td>
<td>HIF</td>
<td>Oct-1</td>
<td>RB</td>
<td>SOX9</td>
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<tr>
<td>C</td>
<td>AR</td>
<td>E2F-1</td>
<td>HIF</td>
<td>NF-E2</td>
<td>PXR</td>
<td>Stat5</td>
<td>AP4</td>
<td>FREAC2 (FOXF2)</td>
<td>KLF4</td>
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<td>RUNX</td>
<td>SOX18</td>
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<tr>
<td>D</td>
<td>ATF2</td>
<td>EGR</td>
<td>HIF4</td>
<td>NFkB</td>
<td>SMAD</td>
<td>Stat6</td>
<td>COUP-TF</td>
<td>Gcr-1</td>
<td>MyoD</td>
<td>Pbx3</td>
<td>ROR/RZR</td>
<td>SRY</td>
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<tr>
<td>E</td>
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<td>ER</td>
<td>IRF</td>
<td>OCT4</td>
<td>Sp1</td>
<td>TCF/LEF</td>
<td>ELK</td>
<td>Gfr-1</td>
<td>MZF</td>
<td>Pbx8</td>
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<tr>
<td>F</td>
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<td>p53</td>
<td>SRF</td>
<td>YY1</td>
<td>FOXA1</td>
<td>HEN (NSCL-1)</td>
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<td>PT1</td>
<td>SFT</td>
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<td>G</td>
<td>CAR</td>
<td>FAST-1</td>
<td>Myb</td>
<td>Pbx-5</td>
<td>SATB1</td>
<td>TR</td>
<td>FoxC1</td>
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<td>Nkx3-2</td>
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<td>VDR</td>
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<tr>
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<td>Pbx1</td>
<td>Stat1</td>
<td>TFIIID</td>
<td>FOXD3</td>
<td>HOX4C</td>
<td>NRF1</td>
<td>MEF1</td>
<td>Snail</td>
<td>WT1</td>
</tr>
</tbody>
</table>

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.

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**Related Products**

<table>
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<tr>
<th>Catalog #</th>
<th>Product Description</th>
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<td>FA-1001</td>
<td>TF Activation Profiling Plate Array I</td>
</tr>
<tr>
<td>FA-1003</td>
<td>Stem Cell TF Activation Profiling Plate Array</td>
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<tr>
<td>FA-1004</td>
<td>Cancer Stem Cell TF Activation Profiling Plate Array</td>
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<tr>
<td>FA-1005</td>
<td>Oxidative Stress TF Activation Profiling Plate Array</td>
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<tr>
<td>FA-1006</td>
<td>ER (UPR) Stress TF Activation Profiling Plate Array</td>
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</table>
### 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, subclass A
Brm-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 homeobox
GATA | GATA transcription factor | HoxA-5 | homeobox A5
GR/PR | Glucocorticoid receptor/Progestosterone 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 myelocytomatisis 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