Signosis, Inc.
Innovative Plate Assay Solutions

Stem Cell TF Activation Profiling Plate Array

Catalog # FA-1003 **(For Research Use Only)**

Introduction

Stem cells are important cells characterized by their ability to self-renew or differentiate into many cell types which is governed by intracellular signaling pathway and transcription regulation. Transcription regulation plays a determining role in conferring cellular identity and function. The step-wise maturation of stem cells into terminally differentiated cell types requires the timely activation of a cascade of transcription programs governed by lineage-specifying transcription factors. Multiple transcription factors and target genes have been widely reported to associate with stem cell self-renewal and pluripotency including EGR1, OCT4, FOXD3, FOXO, Nanog, SOX2, SOX18, ETS, GLI, KLF4, MEF2, Myc, RNUX1, Pax6, TCF/LEF and GATA. However, stem cells are previous and limited. **Signosis, Inc.** has developed *Stem Cell Transcription Factor Activation Plate Array* to analyze activities of 16 stem cell-specific TFs simultaneously in mammalian samples. This assay can be used with Whole Cell Lysis Buffer for limited cell numbers ranging from 1000 – 10,000 cells.

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

- 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	6	RT
Elution Buffer	600µL	RT
TF Plate Hybridization Buffer	20mL	RT
5X Plate Hybridization Wash Buffer	30mL	RT
5X Detection Wash Buffer	40mL	RT
Blocking Buffer	30mL	RT
Filter Wash Buffer	15mL	4°C
Filter Binding Buffer	1.5mL	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	90µL	-20°C
TF Stem Cell Probe Mix	20µL	-20°C
1X Whole Cell Lysis Buffer	600µL	-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 **200µL** of ddH_2O in a 1.5mL microcentrifuge tube (per sample; 3 samples would be 600µL ddH_2O) 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

Preparation of Cell Lysate from pellet

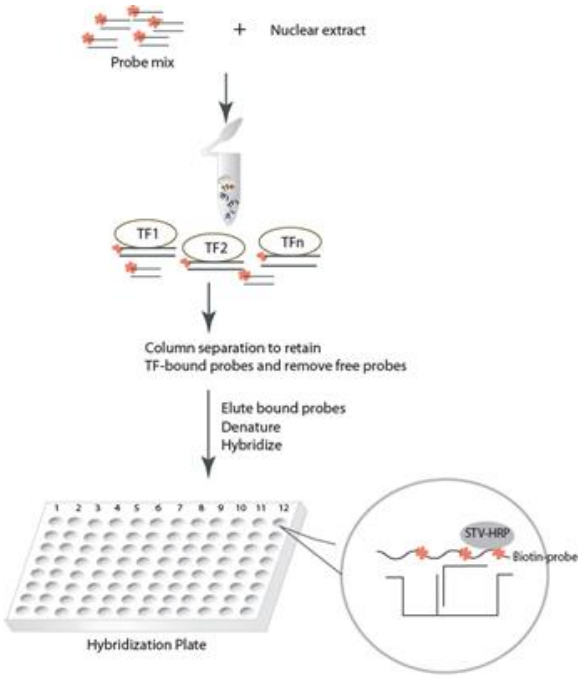
1. In your tube containing your stem cell pellet in ~10µl of your buffer, add 1X whole cell lysis buffer.
   - Use 50µl of the 1X whole cell lysis buffer for a tube containing between 1,000-10,000 cells.
   - For a tube containing at least 1×10^4, add up to 100µl of 1X whole cell lysis buffer.
   - If your pellet contains more than 1×10^6, we recommend using our Nuclear Extraction kit (Cat# SK-0001) instead of preparing cell lysate.
2. Pipet up and down to make sure the cells float in the solution.
3. Incubate for 30 minutes on ice with gentle shaking.
4. Centrifuge at 5,000 RPM for 2 minutes.
5. Use the supernatant as your whole cell lysate.

TF/ DNA Complex Formation

6. Mix the following components for each reaction in a tube
   15µL *TF Binding Buffer Mix*
   3µL *TF Stem Cell Probe mix*
   XµL Cell lysate or nuclear extract (5µg-15µg recommended)
   YµL ddH2O (add up to final volume)
   30µL Reaction Mix
7. Incubate the **Reaction Mix** at room temperature (20-23ºC) for **30 minutes**.

Separation of TF DNA Complex from Free Probes

8. 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.
9. Transfer the **30µL Reaction Mix** directly onto the filter in the center of the *Isolation Column* (avoiding bubbles).
10. Incubate on ice for **30 minutes**. DO NOT incubate longer than 30 minutes; this will result in high background**.**
11. Add **500µL** pre-chilled *Filter Wash Buffer* to the *Isolation Column* and incubate for **3 minutes** on ice.
12. 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.
13. Wash the column by adding **500µL** pre-chilled *Filter Wash Buffer* to the *Isolation Column* on ice.
14. Centrifuge the *Isolation Column* with the collection tube for **1 minute** at **6,000rpm** in a microcentrifuge at **4ºC.** Then discard the flow through.
15. Repeat steps 8-9 for an additional **3 times** for a total a 4 washes.



Elution of Bound Probe

11. Add **50µL** 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 1.5mL microcentrifuge tube and centrifuge at **10,000 rpm** for **2 minutes** at room temperature.
13. If you have yet to do so, chill **200µL** 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 **2mL** pre-warmed *TF Plate Hybridization Buffer* to a dispensing reservoir (DNase free) and then add **200µL** 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 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 **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**.

Stem Cell TF Activation Profiling Array Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	EGR1	Ets	EGR1	Ets	EGR1	Ets	EGR1	Ets	EGR1	Ets	EGR1	Ets
B	FoxD3	Gli	FoxD3	Gli	FoxD3	Gli	FoxD3	Gli	FoxD3	Gli	FoxD3	Gli
C	FoxO1	KLF4	FoxO1	KLF4	FoxO1	KLF4	FoxO1	KLF4	FoxO1	KLF4	FoxO1	KLF4
D	GATA	MEF2	GATA	MEF2	GATA	MEF2	GATA	MEF2	GATA	MEF2	GATA	MEF2
E	Nanog	Myc	Nanog	Myc	Nanog	Myc	Nanog	Myc	Nanog	Myc	Nanog	Myc
F	OCT4	RNUX1	OCT4	RNUX1	OCT4	RNUX1	OCT4	RNUX1	OCT4	RNUX1	OCT4	RNUX1
G	SOX2	Pax6	SOX2	Pax6	SOX2	Pax6	SOX2	Pax6	SOX2	Pax6	SOX2	Pax6
H	SOX18	TCF/LEF	SOX18	TCF/LEF	SOX18	TCF/LEF	SOX18	TCF/LEF	SOX18	TCF/LEF	SOX18	TCF/LEF