Signosis
BioSignal Capture

Stem Cell TF Activation Profiling Plate Array I

Catalog Number 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. Hence, 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. Analyzing the activities of these TFs can provide valuable clues in the study and manipulation of stem cells. Signosis has developed Stem Cell Transcription Factor Activation Plate Array to analyze activities of 16 stem cell-specific TFs simultaneously in mammalian samples. The assay can be used with the whole cell lysate from 1000-10000 cells.

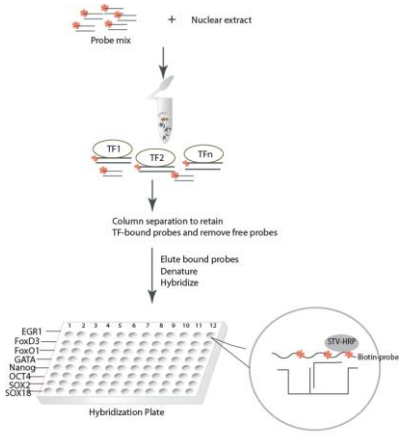

Diagram of Stem Cell TF Activation Profiling Plate Array

Principle of the assay

Signosis' stem cell TF activation profiling plate array is used for monitoring the activation of multiple TFs simultaneously. In the technology, a series of biotin-labeled probes are made based on the consensus sequences of TF DNA-binding sites. When the probe mix incubates nuclear extracts, individual probes will find its corresponding TF and form TF/probe complexes, which can be easily separated from free probes through a simple 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. Luminescence is reported as relative light units (RLUs) on a microplate luminometer.

Materials provided with the kit

- One 96-well Hybridization Plate (RT)
- 6 Isolation columns (RT)
- TF binding buffer mix (-20 ˚C)
- TF stem cell probe mix I (-20 ˚C)
- Filter binding buffer (4 ˚C)
- Filter wash buffer (4 ˚C)
- Elution buffer (RT)
- Streptavidin-HRP conjugate (4˚C)
- Plate hybridization buffer (RT)
- 5x Plate hybridization wash buffer (RT)
- Blocking buffer (RT)
- 5x Detection wash buffer (RT)
- Substrate A (4^{o}C)
- Substrate B (4^{o}C)
- Substrate dilution buffer (RT)
- Foil film

Material required but not provided

- Nuclear Extraction Kit from Signosis (SK-0001)
- PCR machine
- Microcentrifuge working at 4 °C
- Hybridization incubator
- Shaker
- Plate reader for luminescent detection
- ddH2O (RNAase free)

Reagent preparation before starting experiment

- Keep Filter binding buffer and Filter wash buffer on ice
- Warm up Plate Hybridization Buffer and Hybridization Wash buffer at 42 ^{o}C before use.
- Dilute 30ml of 5x Plate Hybridization wash buffer with 120 ml of dH_2O before use.
- Dilute 40ml of 5x Detection wash buffer with 160 ml of dH_2O before use.
- Dilute streptavidin-HRP 500 times with blocking buffer before use.

Assay Procedure
Read the procedure carefully before you start

TF DNA Complex Formation

1. Mix the following components for each reaction in a tube or one well of a PCR plate
   15ul TF binding buffer mix
   2ul TF stem cell probe mix I
   Xul nuclear extract (5µg-15µg)
   Xul ddH2O
   30ul
2. Incubation at 16˚C for 30 minutes in a PCR machine

Separation of TF DNA Complex from Free Probes

3. Equilibrate the Isolation Column by adding 200ul cold Filter binding buffer, and centrifuge at 6000 rpm for 1 min in microcentrifuge at room temperature.
4. Transfer the entire mix onto the filter in the center of the Isolation Column.
5. Incubate on ice for 30 minutes.
   Don't incubate longer than 30 minutes, which results in high background.
6. Add 500ul cold Filter wash buffer to the column, and incubate for 2-3 minutes on ice.
7. Centrifuge at 6000 rpm for 1 min in microcentrifuge at 4^{o}C, and discard the flow through.
8. Wash the column by adding 500ul cold Filter wash buffer to the column on ice.
9. Centrifuge for 1 min at 6000 rpm in microcentrifuge at 4^{o}C, and discard the flow through.
10. Repeat the step 8-9 for additional 3 time washes.

Elution of Bound Probe

11. Add 100ul of Elution buffer onto the center of column, and incubate at room temperature for 5 minutes.
12. Put the column on a clean 1.5 ml microcentrifuge tube, and centrifuge at 10,000 rpm for 2 minutes at room temperature.

Hybridization of Eluted Probe with Hybridization Plate

13. Remove the sealing film from the plate.
14. Add 2ml warmed Hybridization buffer to a dispensing reservoir (DNase free) and then add 100ul denatured probes. Mix them together by gently shaking the reservoir.
16. Dispensing 95ul of the mixture into the corresponding wells with 8 channel pipette **immediately**.
    Note: 96 well hybridization plate is divided into two sections. Section one (Column 1-6) for one sample and Section two (Column 7-12) for another sample.
17. Seal the wells with foil film securely and hybridize at 42 ˚C for overnight. Ensure the numbers and letters on the plate are clearly visible from under foil seal by pressing the foil down on every single experimental well.

Detection of Bound Probe

18. Remove the foil film from the experimental wells with a blade. Keep the unused well sealed.
19. Invert the Hybridization Plate over an appropriate container and expel the contents forcibly, and wash the plate 3 times by adding 200ul of pre-warmed 1x Plate hybridization wash buffer to each well. At each wash, incubate the plate for 5 minutes with gently shaking at room temperature.
20. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
21. Add 200µl of Blocking buffer to each well and incubate for 15 minutes at room temperature with gently shaking.
22. Invert the plate over an appropriate container to remove block buffer.
23. Add 20 µl of streptavidin-HRP conjugate in 10ml blocking buffer (1:500) dilution, enough for one plate. Add 95 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gently shaking.
24. Wash the plate 3 times by adding 200ul 1X Detection wash buffer to each well, At each wash, incubate the plate for 10 minutes with gently shaking at room temperature.
25. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels. At the last wash, invert plate on clean paper towels for 1-2 min to remove excessive liquid.
26. Freshly prepare the substrate solution:
    For the whole plate:
    1ml Substrate A
    1ml Substrate B
    8ml Substrate dilution buffer
27. Add 95µl substrate solution to each well and incubate for 1 min.
29. Place the plate in the luminometer. Allow plate to sit inside machine for 5 min before reading. Set integration time to 1 second with no filter position. For the best results, read the plate within 5-20 minutes.

Data Example

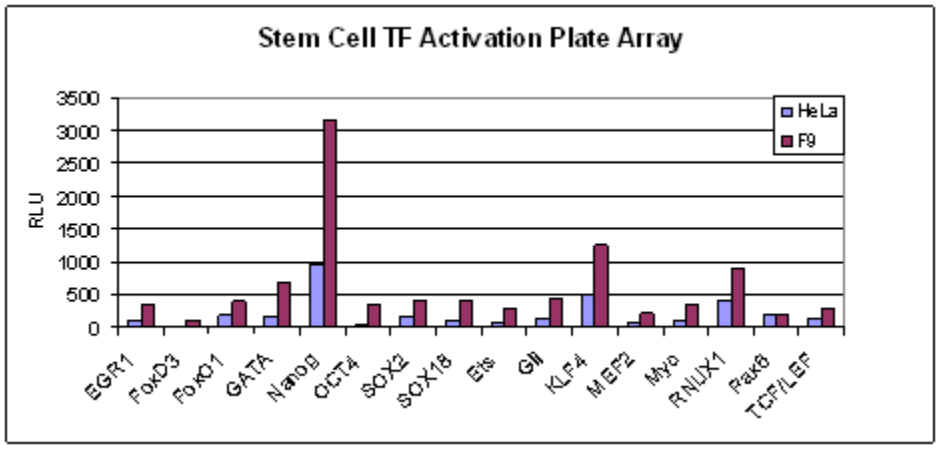

Figure: Stem cell TF activation profiling plate array analysis of TF activities in HeLa and Stem cell F9. Nuclear extracts were prepared and subjected to TF profiling assay.

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