Signosis
Innovative Plate Assay Solutions

Wnt/β-Catenin TF Activation Profiling Plate Array

Catalog Number FA-1007 **(For Research Use Only)**

Introduction

The Wnt signaling pathway is important to both embryonic development and tumorigenesis. β-Catenin, the central component of the pathway, functions as a cofactor for a number of transcription factors. These transcription factors then activate transcription of Wnt target genes involved in cell proliferation, survival, and migration. Characterization of the activation of TF involved in the Wnt/ β-Catenin pathway can lead to greater understanding of various disease states and the development of treatments.
Signosis has developed the Wnt/β-Catenin TF Activation Profiling Plate Array, which can be used to simultaneously monitor 16 Wnt/β-Catenin related TFs, including AP-1, CEBP, c-Myc,GBX2, GLI-1,Mitf, NFAT, NR5A2,Oct3/4, OLIG1, Pitx2, Prop1,Sox2,TCF/LEF, VAX2.

Principle of the assay

Signosis' 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 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)
- Six Isolation columns (RT)
- TF binding buffer mix (-20 °C)
- TF Probe mix (-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°C)
- Substrate B (4°C)

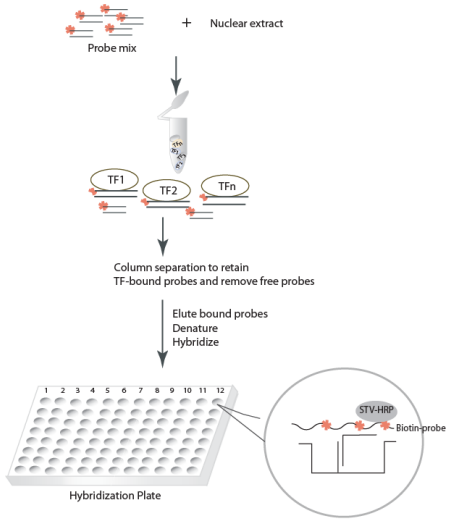

Diagram of TF Activation Profiling Plate Array

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 (DNAase 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 °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
   15ul TF binding buffer mix
   3ul TF Probe mix
   Xul nuclear extract (5µg-15µg)
   Xul ddH2O
   30ul
2. Incubation at room temperature (20-23^{o}C) for 30 minutes.

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 30ul reaction mix directly onto the filter in the center of the Isolation Column (avoiding bubbles).
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 50ul of Elution buffer onto the center of column, and incubate at room temperature for 5 minutes.
12. Put the column on a 1.5 ml microcentrifuge tube, and centrifuge at 10,000 rpm for 2 minutes at room temperature.
13. Chill 200ul 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 ^{o}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 hybridization or store -20 ^{o}C for the future use (the probe must be denatured again before use).

Hybridization of Eluted Probe with Hybridization Plate

16. Remove the sealing film from the plate.
17. Add 2ml warmed Hybridization buffer to a dispensing reservoir (DNase free) and then add 200ul denatured probes. Mix them together by gently shaking the reservoir.
18. Dispensing 100ul of the mixture into the corresponding wells with 8 multi-channel pipette **immediately**.
    Note: the 96 well hybridization plate is divided into 6 sections for 6 samples. Two columns for each sample.
    If a blank well is desired to perform, select one TF well you may not be interested in from the diagram below as a blank well and add 1x Hybridization buffer only without the eluted probe.
19. Seal the wells with foil film securely and hybridize at 42 ^{o}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

20. Remove the foil film from the experimental wells with a blade. Keep the unused well sealed.
21. 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 by **row** with a **12 multi-channel pipette**. At each wash, incubate the plate for 5 minutes with gently shaking at room temperature.
22. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
23. 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 gently shaking.
24. Invert the plate over an appropriate container to remove block buffer.
25. Add 20 µl of streptavidin-HRP conjugate in 10ml blocking buffer (1:500) dilution, enough for the whole plate (6 sections). Add 95 µl of diluted streptavidin-HRP conjugate to each well by **row** with a **12 multi-channel pipette** and incubate for 45 min at room temperature with gently shaking.
26. Wash the plate 3 times by adding 200ul 1X Detection wash buffer to each well by **row** with a **12 multi-channel pipette**. At each wash, incubate the plate for 5 minutes with gently shaking at room temperature.
27. 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.

28. Freshly prepare the substrate solution:
    For the whole plate:
    1ml Substrate A
    1ml Substrate B
    8ml Substrate dilution buffer
29. Add 95µl substrate solution to each well by **row** with a **12 multi-channel pipette** and incubate for 1 min.
30. 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.

Wnt/β-Catenin TF Activation Profiling Plate Array Diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	AP-1	Oct3/4	AP-1	Oct3/4	AP-1	Oct3/4	AP-1	Oct3/4	AP-1	Oct3/4	AP-1	Oct3/4
B	CEBP	OLIG1	CEBP	OLIG1	CEBP	OLIG1	CEBP	OLIG1	CEBP	OLIG1	CEBP	OLIG1
C	c-Myc	Pitx2	c-Myc	Pitx2	c-Myc	Pitx2	c-Myc	Pitx2	c-Myc	Pitx2	c-Myc	Pitx2
D	GBX2	Prop1	GBX2	Prop1	GBX2	Prop1	GBX2	Prop1	GBX2	Prop1	GBX2	Prop1
E	GLI-1	Sox2	GLI-1	Sox2	GLI-1	Sox2	GLI-1	Sox2	GLI-1	Sox2	GLI-1	Sox2
F	Mitf	TCF/LEF	Mitf	TCF/LEF	Mitf	TCF/LEF	Mitf	TCF/LEF	Mitf	TCF/LEF	Mitf	TCF/LEF
G	NFAT	VAX2	NFAT	VAX2	NFAT	VAX2	NFAT	VAX2	NFAT	VAX2	NFAT	VAX2
H	NR5A2	Blank	NR5A2	Blank	NR5A2	Blank	NR5A2	Blank	NR5A2	Blank	NR5A2	Blank