



TF Reporter Plate Array I

Catalog Number AP-2101

(For Research Use Only)

Introduction

Transactivation of a transcription factor (TF) is often measured by a reporter construct which contains a cis-element (DNA binding sequence) of the TF and a reporter gene such as a luciferase gene. When the reporter is delivered into cells, the activated TF binds to the cis-element and mediates the induction of the reporter gene. The induction can be measured via biochemical analysis of the reporter gene product. However, it is a single assay, one TF at a time. As any cellular change could result in the activation of multiple TFs, monitoring these TFs need multiple transfections and biochemical measurements. In addition, normalization is needed in order to normalize the transfection efficiencies. Signosis has developed a proprietary technology for monitoring the activation of multiple TFs simultaneously. In TF reporter plate array I, seven TF reporter vectors are mixed for monitoring the activation of seven TFs simultaneously, including NFkB, HIF-1, AP1, ISRE, Stat1, Stat3, and p53, along with a control TA vector without cis-element.

Principle of the assay

Signosis' proprietary TF reporter plate array I is a plate-based array for quantitatively profiling the activities of seven TFs simultaneously. In the technology, a series of reporter constructs are made, each of which contains a cis-element and a reporter tag sequence to a specific TF. When the constructs are delivered as a library into cells, the activation of TFs will bind to its corresponding constructs and mediate the expression of the tag sequences. The expressed tag sequences are analyzed via a cDNA plate array, with which each specific tag sequence is analyzed by a corresponding complementary tag capture oligo immobilized onto an assigned well. The captured cDNAs are further detected with streptavidin-HRP. Luminescence is reported as relative light units (RLUs) on a microplate luminometer. The TF activity of TFs is directly proportional to the luminescent intensity.

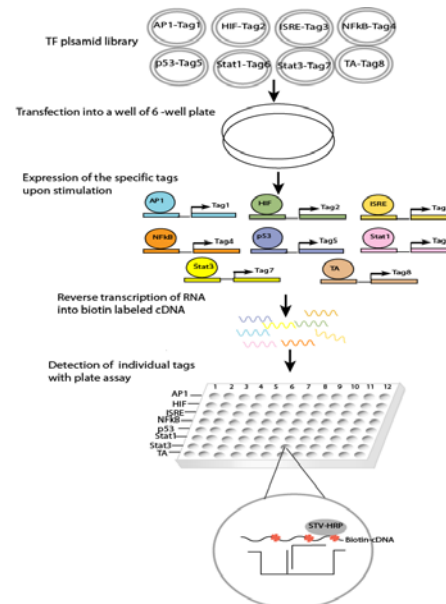


Diagram of TF reporter plate array

Materials provided with the kit

- A 96-well plate coated with 8 different capture oligos (4°C)
- TF reporter plasmid mix (-20 °C)
- TF reporter primer (-20 °C)
- Reverse transcription buffer mix (-20 °C)
- Reverse transcriptase RT (-20 °C)
- Streptavidin-HRP conjugate (4°C)
- Plate hybridization buffer (RT)
- 5x Plate hybridization wash buffer (RT)
- Block buffer (RT)
- 5xDetection wash buffer (RT)
- Substrate A (4°C)
- Substrate B (4°C)
- Substrate dilution buffer (RT)

Material required but not provided

PCR machine
Incubator
0.2ml PCR tube
Plate reader for chemiluminescence detection
ddH₂O (RNAase free)

Reagent preparation before starting experiment

- Dilute 30ml of 5x Plate hybridization wash buffer with 120 ml of dH₂O before use.
- Dilute 40ml of 5x Detection Wash Buffer with 160 ml of dH₂O before use.
- Warm up Plate hybridization buffer and Hybridization Wash buffer at 45 °C incubator.
- Dilute 1000 times of streptavidin-HRP with block buffer before use at Step 3(3).

Assay procedure

1. Transfection of TF Plasma Mix into the cells

We recommend using FuGene 6 Roche to transfect Plasmid Mix into the adherent cells seeded on a six-well tissue culture plate.

- (1) Seed the cells with the proper medium containing serum at 80% confluency at the time of transfection.
- (2) For each transfection, dilute 6ul FuGene 6 with 100ul serum-free medium (without antibiotics); and add 6ul of plasmid mix to diluted FuGene 6.
- (3) Tap the tube to mix the contents, and incubate for 20-40 minutes.
- (4) Add complex to the cells in a drop-wise manner. Swirl the wells to ensure the distribution over the entire surface. And incubate for overnight.
- (5) If the starvation is required for treatment, the serum medium can be replaced with serum-free medium.
- (6) The treatment commonly takes 3-6 hours for induction.
- (7) RNA can be prepared with Tri reagent or column purification kit.

1. cDNA synthesis using PCR machine

Note: Briefly spin tubes before opening

- (1) Sample preparation
X µl 1-10µg total RNA
2 µl TF Reporter Primer
X µl ddH₂O

11µl

- (2) Incubate for 5 minutes at 65 °C, and chill on ice.
- (3) Add 8 µl Reverse transcription buffer mix and 1µl RT to each reaction tube, and incubate for 1 hour at 45 °C.
- (4) Heat the reaction to 92 °C for 5 minutes, and chill on ice.
- (5) The 20ul cDNA is synthesized and labeled with biotin, ready for hybridization on the plate.

2. Plate hybridization

- (1) Warm up the plate to room temperature, and arrange the appropriate number of the strips of the plate based on your experiment by removing the top foil sealing film with a blade. Keep the unused well sealed.
- (2) Mix 20ul cDNA with 2.2ml warmed Plate hybridization buffer, and dispense 95ul mixture to each well in two strips **immediately**. Don't use polystyrene reservoir for dispensation.

- (3) Seal the wells with foil film securely and incubate the plate at 45 °C incubator for overnight. Note: put an open container in the incubator, such as empty pipette tip box with **water** to keep the humidity

3. Plate detection

- (1) Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate by adding 300µl of warmed 1x cDNA Hybridization wash buffer. Repeat the washing process two times for a total of three washes. Complete removal of liquid at each wash by firmly taping the plate against clean paper towels.

Optional: To ensure remove all traces of the buffer in last washing step, invert the plate on a clean, dry paper towel and centrifuge at 240xg for 1 minute at room temperature.

- (2) Add 200µl of Block buffer incubate for 15 minutes at room temperature with gentle shaking.
- (3) Invert the plate over an appropriate container to remove block buffer. And add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 30 min at room temperature with gentle shaking.
- (4) Repeat the aspiration/wash as in 3(1) with 1x Detection Wash Buffer.
- (5) Freshly prepare the substrate solution
For the whole plate:
1ml Substrate A
1ml Substrate B
8ml Substrate dilution buffer
- (6) Add 95µl substrate solution to each well and incubate for 1minutes.
- (7) Determine the chemiluminescence of each well with a microplate reader within 5 minutes.

Example of Analysis Data

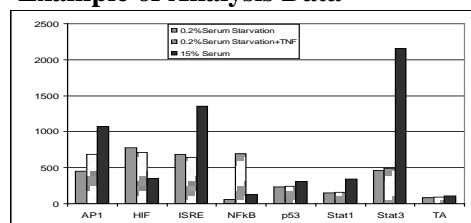


Figure1: TF reporter array analysis

HeLa cells were transfected with TF reporter plasmid mix without (grey bar) and with 20ng/ul TNF (shaded bar) or 15% serum treatment (black bar) for 4 hours. Total RNAs were prepared. RNAs were subjected to TF reporter plate array.

Diagram of TF Transactivation plate assay

	1	2	3	4	5	6	7	7	9	9	11	12
A	AP1	AP1	AP1	AP1	AP1	AP1	AP1	AP1	AP1	AP1	AP1	AP1
B	HIF	HIF	HIF	HIF	HIF	HIF	HIF	HIF	HIF	HIF	HIF	HIF
C	ISRE	ISRE	ISRE	ISRE	ISRE	ISRE	ISRE	ISRE	ISRE	ISRE	ISRE	ISRE
D	NFkB	NFkB	NFkB	NFkB	NFkB	NFkB	NFkB	NFkB	NFkB	NFkB	NFkB	NFkB
E	p53	p53	p53	p53	p53	p53	p53	p53	p53	p53	p53	p53
F	Stat1	Stat1	Stat1	Stat1	Stat1	Stat1	Stat1	Stat1	Stat1	Stat1	Stat1	Stat1
G	Stat3	Stat3	Stat3	Stat3	Stat3	Stat3	Stat3	Stat3	Stat3	Stat3	Stat3	Stat3
H	TA	TA	TA	TA	TA	TA	TA	TA	TA	TA	TA	TA