



## 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 a TF and a reporter gene such as a luciferase gene. When a 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 TF assay, therefore, only one TF is measured at a time. As any cellular changes 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.

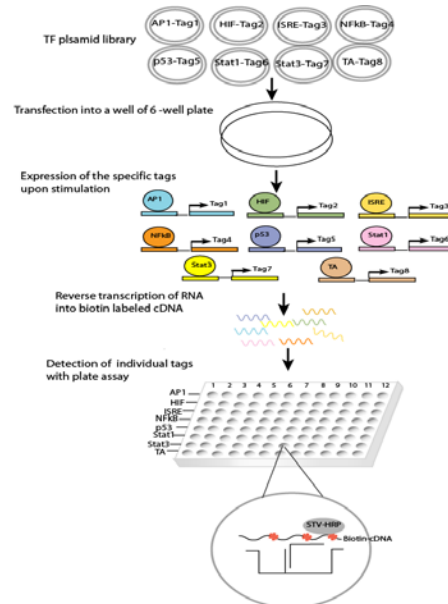


Diagram of TF reporter plate array

### Materials provided with the kit

- A 96-well plate coated with 8 different capture oligos (RT)
- 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)
- Blocking buffer (RT)
- 5xDetection wash buffer (RT)
- Substrate A (4°C)
- Substrate B (4°C)
- Substrate dilution buffer (RT)
- Foil sealing film

### Material required but not provided

PCR machine  
Incubator  
0.2ml PCR tube  
Luminometer plate reader  
ddH<sub>2</sub>O (RNAase free)

## Reagent preparation before starting experiment

- Dilute 30ml of 5x Plate hybridization wash buffer with 120 ml of dH<sub>2</sub>O before use.
- Dilute 40ml of 5x Detection Wash Buffer with 160 ml of dH<sub>2</sub>O before use.
- Warm up Plate hybridization buffer and Hybridization Wash buffer at 45 °C until no visible precipitate before use. **The buffer may need to be stirred with a 5ml or 10ml pipette to dissolve completely during 45 °C warm-up.**
- Dilute 1000 times of streptavidin-HRP with blocking buffer before use at Step 3(4).

## Assay procedure

### 1. Transfection of TF Plasmid 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 6µl 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 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 Trizol reagent or column purification kit.

### 2 cDNA synthesis

**Note: Briefly spin tubes before opening**

- (1) Sample preparation  
X µl 1-10µg total RNA  
2 µl TF Report Primer Mix  
X µl ddH<sub>2</sub>O  
-----  
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 98 °C for 5 minutes, and chill on ice.
- (5) The 20ul cDNA is synthesized and labeled with biotin and ready for hybridization on the plate.

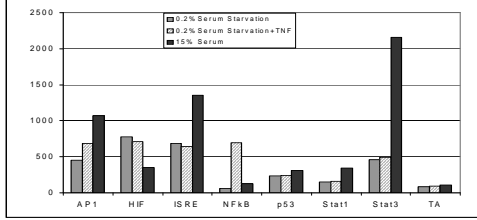
### 3. Plate hybridization

- (1) Remove the sealing film
- (2) Calculate how many columns are used. Seal the unused columns tightly with a foil sealing film (provide).
- (3) Mix 20ul cDNA with 2.0ml pre-warmed Plate hybridization buffer, and dispense 95ul mixture to each well in two columns **immediately** for duplication.
- (4) Seal the whole plate with foil film (provided) securely and incubate the plate at 45 °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.

### 4. Plate detection

- (1) Removing the top foil sealing film with a blade to expose the experimental wells. Keep the unused well sealed for the future usage.
- (2) Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate by adding 300µl of warmed 1x Plate hybridization wash buffer. Repeat the washing process two times for a total of three washes. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
- (3) Add 200µl of Blocking buffer incubate for 15 minutes at room temperature with gentle shaking.
- (4) Invert the plate over an appropriate container to remove blocking buffer. And add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
- (5) Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate with 200ul 1X Detection wash buffer for 5 min at room temperature with gently shaking. Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
- (6) Repeat (5) for additional 2 time washes.
- (7) Freshly prepare the substrate solution  
For the whole plate:  
1ml Substrate A  
1ml Substrate B  
8ml Substrate dilution buffer
- (8) Add 95µl substrate solution to each well and incubate for 1minutes.
- (9) Place the plate in the luminometer, and read. Set integration time to 1 second with no filter position. For the best results, read the plate within 5-20 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 (shade 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 reporter 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