



## Mouse NFκB-Regulated cDNA Plate Array

Catalog Number AP-1101

(For Research Use Only)

### Introduction

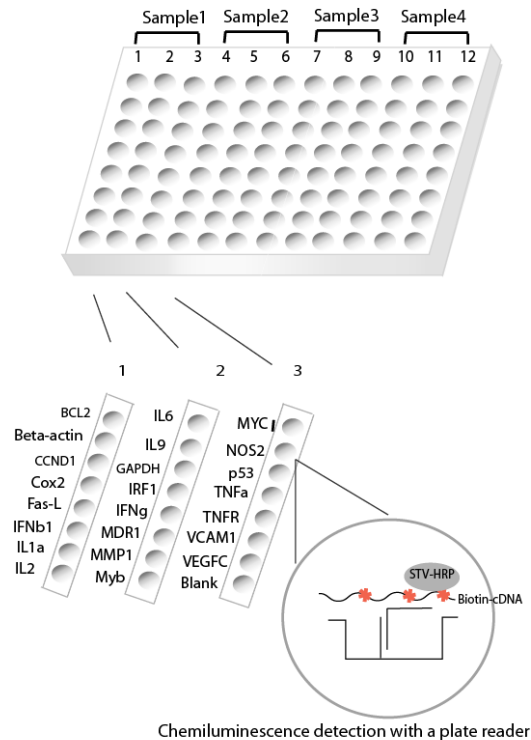
NFκB is a ubiquitous transcription factor that plays a key role in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized LDL, and bacterial or viral antigens. Incorrect regulation of NFκB has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development. When NFκB is activated, it is dissociated from its inhibitor IκB and moves from the cytoplasm to the nucleus, where it binds to target DNA elements and positively regulates the transcription of genes involved in immune and inflammatory responses, cell growth control, and apoptosis. Signosis developed a plate-based array for profiling 20+ mouse NFκB-regulated genes.

### Principle of the assay

Signosis' proprietary cDNA plate array is a plate-based hybridization profiling analysis for monitoring the expression of dozens of genes through reverse transcription of mRNA into cDNA. Like array analyses, total RNA is first reverse transcribed into cDNA in the presence of biotin-dUTP in the assay. Targeted genes are then specifically captured onto individual wells on a plate, instead of membranes, through a pre-coated gene-specific oligonucleotide. The captured cDNAs are further detected with streptavidin-HRP. The chemiluminescent signals are read with a plate reader. The expression level of genes is directly proportional to the chemiluminescent intensity.

### Materials provided with the kit

- A 96-well plate coated with 23 different capture oligos (RT)
- Mouse NFκB Reg. Primer Mix (-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)
- 5x Detection wash buffer (RT)
- Substrate A (4 °C)
- Substrate B (4 °C)
- Substrate dilution buffer (RT)



Chemiluminescence detection with a plate reader

Diagram of Mouse NFκB-Regulated cDNA Plate Array

### Material required but not provided

- PCR machine
- Incubator
- 0.2ml PCR tube
- Plate reader for chemiluminescence detection
- 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
- Dilute 40ml of 5x Detection wash buffer with 160 ml of dH<sub>2</sub>O
- Warm up Plate hybridization buffer and Hybridization Wash buffer at 45 °C incubator for 2 hours to overnight until no visible precipitate before use
- Dilute 1000 times of streptavidin-HRP with block buffer before use at Step 3(3).

## Assay procedure

### 1. cDNA synthesis

**Note: Briefly spin tubes before opening**

- (1) Sample preparation
    - X µl 1-10µg total RNA
    - 2 µl Mouse NFκB. Reg 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, ready for hybridization on the plate.

### 2. Plate hybridization

- (1) Arrange the appropriate number of the wells of the plate based on your experiment by removing the top foil sealing film with a blade. Keep the unused well sealed. The whole plate is divided into 4 repeat sections, Column 1-3, 4-6, 7-9, 10-12 for 4 different samples.

**Note: keep the removed sealing film for resealing during hybridization**

- (2) Mix 20ul cDNA with 2.8ml pre-warmed Plate hybridization buffer, and dispense 95ul mixture to each well in a section **immediately**. A reagent reservoir can be used for dispensing cDNA mixture into the wells with a 8 multichannel pipette. Add 100ul Plate hybridization buffer without cDNA to the 'blank' well.
- (3) Seal the wells with foil film 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.

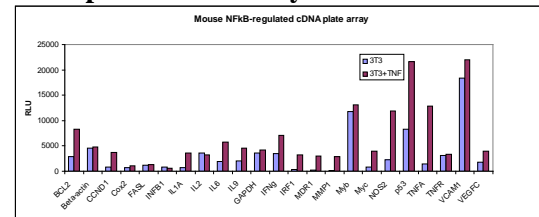
### 3. Plate detection

- (1) Invert the plate over an appropriate container and expel the contents forcibly, and wash the plate by adding 300µl of 1X warmed 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 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. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
- (4) 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.
- (5) Repeat Step (4) for additional 2 time washes.
- (6) Freshly prepare the substrate solution  
For the whole plate:  
1ml Substrate A  
1ml Substrate B  
8ml Substrate dilution buffer
- (7) Add 95µl substrate solution to each well and incubate for 1minutes.
- (8) Determine the chemiluminescence of each well with a microplate reader within 5 minutes.

## Example of Data Analysis



**Figure1: Mouse NFκB-regulated cDNA plate assay for analysis of 21 genes.**

NIH3T3 cells were treated with and without 20ng/ul TNF for 90 minutes respectively. Total RNAs were prepared. RNAs were subjected to NFκB regulated cDNA plate assay, and detected with chemiluminescence by a plate reader.

## Diagram of Mouse NFκB cDNA plate assay

1	2	3	4	5	6	7	8	9	10	11	12
BCL2	IL6	Myc	BCL2	IL6	Myc	BCL2	IL6	Myc	BCL2	IL6	Myc
Beta-actin	IL9	NOS2	Beta-actin	IL9	NOS2	Beta-actin	IL9	NOS2	Beta-actin	IL9	NOS2
CCND1	GAPDH	p53	CCND1	GAPDH	p53	CCND1	GAPDH	p53	CCND1	GAPDH	p53
Cox2	IFNg	TNFA	Cox2	IFNg	TNFA	Cox2	IFNg	TNFA	Cox2	IFNg	TNFA
FASL	IRF1	TNFR	FASL	IRF1	TNFR	FASL	IRF1	TNFR	FASL	IRF1	TNFR
INFB1	MDR1(Abc)	VCAM1	INFB1	MDR1(Abc)	VCAM1	INFB1	MDR1(Abc)	VCAM1	INFB1	MDR1(Abc)	VCAM1
IL1A	MMP1	VEGFC	IL1A	MMP1	VEGFC	IL1A	MMP1	VEGFC	IL1A	MMP1	VEGFC
IL2	Myb	blank	IL2	Myb	blank	IL2	Myb	blank	IL2	Myb	blank