



## Mouse Cytokine cDNA Plate Array

Catalog Number AP-1141

(For Research Use Only)

### Introduction

Cytokines are essential molecules play crucial roles in many biological functions, including viral infection, inflammation, immunity, and hematopoiesis. Cytokines are produced by a variety of cell types in response to different stimuli. In addition, the expression of cytokine genes appears to be regulated by complex mechanism. Expression of one cytokine gene could be regulated by other cytokines. Dysregulation of cytokine gene expression may be caused by chromosomal alterations or by infection of viruses that induce activation or inactivation of the expression machinery. Therefore, gene expression profiling of cytokine genes could provides a useful tool to uncover the mechanism underlying the regulation. Signosis developed a plate-based array for profiling 20+ cytokine 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. Luminescence is reported as relative light units (RLUs) on a microplate luminometer.

The expression level of genes is directly proportional to the luminescent intensity.

### Materials provided with the kit

- A 96-well plate coated with 24 different capture oligos ( RT)
- Mouse Cytokine 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)
- Blocking buffer (RT)
- 5xDetection wash buffer (RT)
- Substrate A (4°C)
- Substrate B (4°C)
- Substrate dilution buffer (RT)

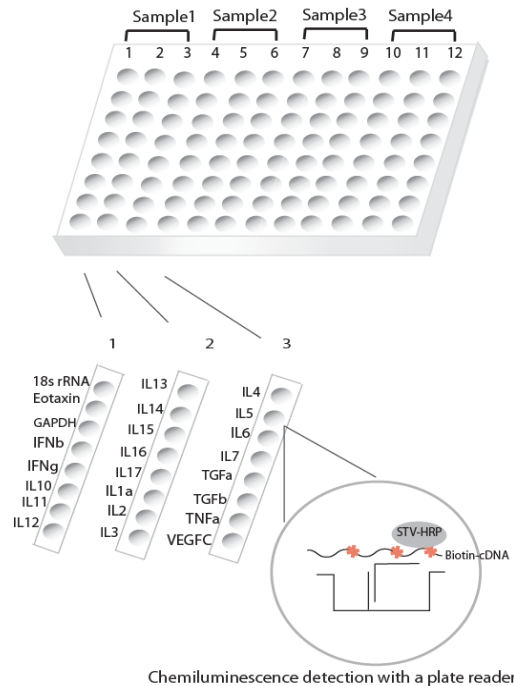


Diagram of Mouse Cytokine cDNA Plate Array

### 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. Stir the solution with a 10ml or 5ml pipette to facilitate the dissolving process.
- Dilute 500 times of streptavidin-HRP with blocking buffer before use at Step 3(4).

## Assay procedure

### 1. cDNA synthesis

**Note: Briefly spin tubes before opening**

(1) Sample preparation

- X µl 1-10µg total RNA
- 2 µl Mouse Cytokine Primer Mix
- X µl ddH<sub>2</sub>O

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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.

### 2. Plate hybridization

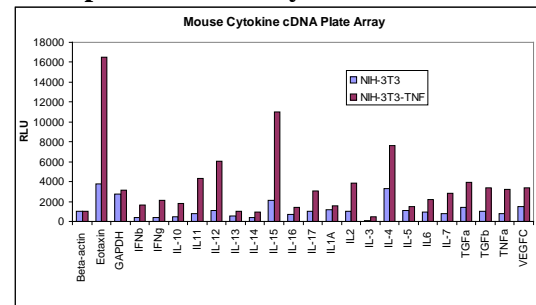
- (1) Remove the sealing film
- (2) Arrange the appropriate number of the wells of the plate based on your experiment. The whole plate is divided into 4 repeat sections, Column 1-3, 4-6, 7-9, 10-12 for 4 different samples.
- (3) Mix 20ul cDNA with 2.8ml pre-warmed Plate hybridization buffer, and dispense 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.
- (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. *Put an open container with water in the incubator to keep humidity and prevent evaporation from experimental wells.*

### 3. 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 1 minutes.
- (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 Data Analysis



**Figure1: Mouse cytokine cDNA plate assay for analysis of 23 genes.**

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

### Diagram of Mouse Cytokine cDNA Plate Array

	1	2	3	4	5	6	7	8	9	10	11	12
A	18S rRNA	IL-13	IL-4	18S rRNA	IL-13	IL-4	18S rRNA	IL-13	IL-4	18S rRNA	IL-13	IL-4
B	Eotaxin	IL-14	IL-5	Eotaxin	IL-14	IL-5	Eotaxin	IL-14	IL-5	Eotaxin	IL-14	IL-5
C	GAPDH	IL-15	IL-6	GAPDH	IL-15	IL-6	GAPDH	IL-15	IL-6	GAPDH	IL-15	IL-6
D	IFNβ	IL-16	IL-7	IFNβ	IL-16	IL-7	IFNβ	IL-16	IL-7	IFNβ	IL-16	IL-7
E	IFNγ	IL-17	TGFα	IFNγ	IL-17	TGFα	IFNγ	IL-17	TGFα	IFNγ	IL-17	TGFα
F	IL-10	IL1A	TGFβ	IL-10	IL1A	TGFβ	IL-10	IL1A	TGFβ	IL-10	IL1A	TGFβ
G	IL11	IL2	TNFα	IL11	IL2	TNFα	IL11	IL2	TNFα	IL11	IL2	TNFα
H	IL-12	IL-3	VEGFC	IL-12	IL-3	VEGFC	IL-12	IL-3	VEGFC	IL-12	IL-3	VEGFC