



Human Stat3-Regulated cDNA Plate Array

Catalog Number AP-0151

(For Research Use Only)

Introduction

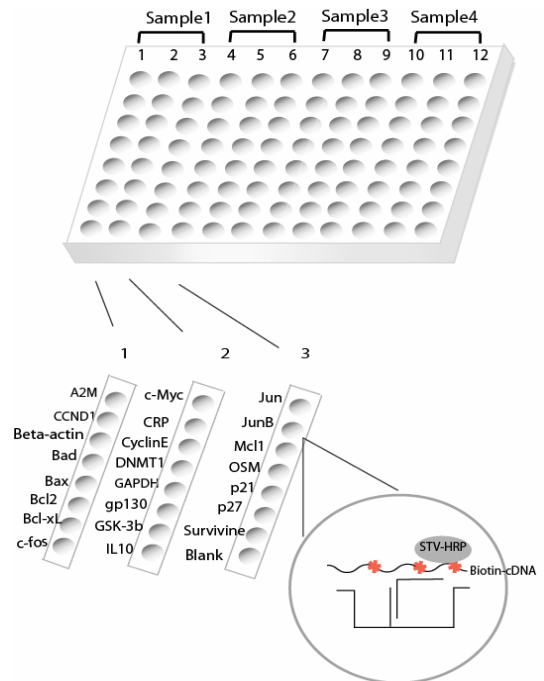
Signal Transducer and Activator of Transcription 3 (Stat3) is a transcription factor that mediates the nuclear action of many different cytokines and growth factors. It plays roles in cell growth and apoptosis. Stat3 also participates in cellular transformation and tumorigenesis. The aberrant activation of Stat3 in tumor cells has been considered as a drug target. Stat3 is activated by tyrosine phosphorylation in response to a wide range of cytokines, growth factors, and hormones. Tyrosine phosphorylation triggers dimerization and nuclear translocation of the protein. Stat3 mediates the expression of its target genes that are involved in a diverse array of biological processes such as oncogenesis, cell growth, and differentiation. Signosis' Stat3-regulated cDNA plate array allows profiling 20+ Stat3-target 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 23 different capture oligos (RT)
- Human Stat3 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)
- Blocking buffer (RT)
- 5xDetection wash buffer (RT)
- Substrate A (4 °C)
- Substrate B (4 °C)
- Substrate dilution buffer (RT)



Chemiluminescence detection with a plate reader

Diagram of human Stat3-regulated cDNA plate array

Material required but not provided

PCR machine
Incubator
0.2ml PCR tube
luminometer plate reader
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 until no visible precipitate before use.
- Dilute 1000 times of streptavidin-HRP with blocking buffer before use at Step 3(4).

Assay procedure

1. cDNA synthesis using PCR machine

Note: Briefly spin tubes before opening

- (1) Sample preparation
 - X μ l 1-10 μ g total RNA
 - 2 μ l Human Stat3 Reg. Primer Mix
 - X μ l ddH₂O

11 μ l

- (2) Incubate for 5 minutes at 65 °C, and chill on ice.
- (3) Add 8 μ l Reverse transcription buffer mix and 1 μ l 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 20 μ l 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 20 μ l cDNA with 2.8ml pre-warmed Plate hybridization buffer, and dispense 95 μ l 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 100 μ l Plate hybridization buffer without cDNA to the 'blank' well.
- (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.

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 300 μ l 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 200 μ l 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 μ l 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 200 μ l 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 95 μ l 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

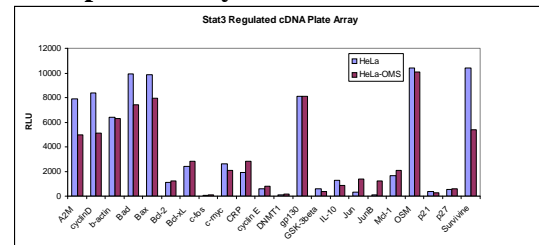


Figure 1. Stat3 Regulated cDNA Plate Assay for analysis of oncostatin M induced Stat3 target genes. HeLa cells were starved for 17 hours in 0.2% serum medium, and then treated with or with out 100ng/ml oncostatin M for 1 hour. RNAs were subjected to Sata3 cDNA plate array assay.

Stat3 Regulated cDNA plate Array diagram

	1	2	3	4	5	6	7	8	9	10	11	12
A	A2M	c-myc	Jun	A2M	c-myc	Jun	A2M	c-myc	Jun	A2M	c-myc	Jun
B	CCND1	CRP	JunB	CCND1	CRP	JunB	CCND1	CRP	JunB	CCND1	CRP	JunB
C	b-actin	cyclin E	Mcl-1	b-actin	cyclin E	Mcl-1	b-actin	cyclin E	Mcl-1	b-actin	cyclin E	Mcl-1
D	Bad	DNMT1	OSM	Bad	DNMT1	OSM	Bad	DNMT1	OSM	Bad	DNMT1	OSM
E	Bax	GAPDH	p21	Bax	GAPDH	p21	Bax	GAPDH	p21	Bax	GAPDH	p21
F	Bcl-2	gp130	p27	Bcl-2	gp130	p27	Bcl-2	gp130	p27	Bcl-2	gp130	p27
G	Bcl-xL	GSK-3beta	Survivine	Bcl-xL	GSK-3beta	Survivine	Bcl-xL	GSK-3beta	Survivine	Bcl-xL	GSK-3beta	Survivine
H	c-fos	IL-10	Blank	c-fos	IL-10	Blank	c-fos	IL-10	Blank	c-fos	IL-10	Blank