



Human NF κ B-Regulated cDNA Profiling in Cell Lysates

Catalog Number AP-3101

(For Research Use Only)

Introduction

NF κ B 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 NF κ B has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infection, and improper immune development. When NF κ B is activated, it is dissociated from its inhibitor I κ B 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. Through coupling its CLTM cDNA synthesis kit which allows reverse transcription directly in cell lysates without RNA preparation, Signosis has developed a plate-based hybridization array for profiling the expression of 20+ NF κ B-regulated genes directly in cell lysates.

Principle of the assay

Unlike conventional reverse transcription which needs total RNA, Signosis has developed a CLTM cDNA synthesis-based plate array. It can synthesize cDNA probes through reverse transcription directly in cell lysates without RNA preparation. The synthesized cDNA probes are then applied for a plate array hybridization. Targeted genes are specifically captured onto individual wells on a plate 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.

Material required but not provided

PCR machine
Incubator
0.2ml PCR tube
Luminometer plate reader
ddH₂O (RNAase free)

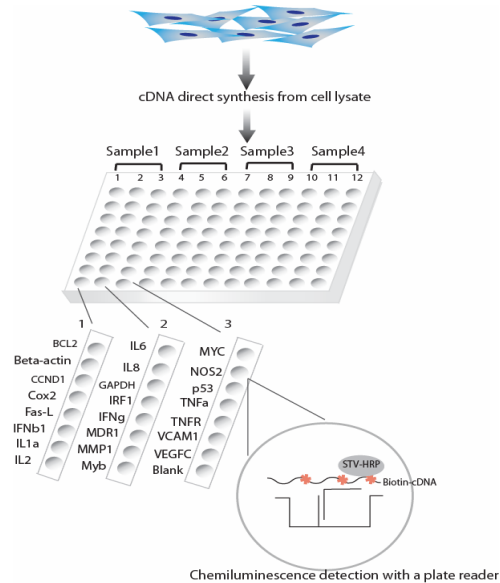


Diagram of human NF κ B cDNA profiling in cell lysates

Materials provided with the kit

- A 96-well plate coated with 23 different capture oligos (RT)
- Human NF κ B Reg. Primer Mix (-20 °C)
- Cell lysis Buffer (-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)

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

Assay procedure

1. Sample preparation

- (1) Estimate the number of cells. The number of cells should be between 10⁴-10⁵ cells. Wash the cells with 200ul ice cold 1XPBS and add 100ul ice-cold Cell lysis buffer and then subject to snap-frozen at -80°C. If the cell number is between 2000-10,000 cells, add 20 ul Cell lysis buffer instead.
Notes: Keep the cells on ice during the procedure.
- (2) Heat for 75 °C for 15 minutes, and put on ice. The cell lysates are ready for use or can be stored at -80 °C for the future usage.

2. cDNA synthesis using PCR machine

Note: Briefly spin tubes before opening

- (1) Sample preparation
4 ul cell lysate
2 µl Human NFκB Reg. Primer Mix
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 1.5µl 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) 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. **Add 100ul 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. *Put an open container with water in the incubator to keep humidity and prevent evaporation from experimental wells.*

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:
1 ml Substrate A
1 ml Substrate B
8 ml 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

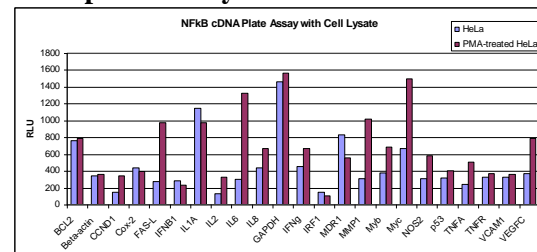


Figure1: NFκB-regulated cDNA plate assay for analysis of 21 genes from cell lysate

HeLa cells were cultured and treated with and without 10ng/ul PMA for 3 hours. Cell lysates were prepared and subjected to NFκB regulated cDNA plate assay, and detected with chemiluminescence by a plate reader.

Diagram of NFkB 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	IL8	NOS2	Beta-actin	IL8	NOS2	Beta-actin	IL8	NOS2	Beta-actin	IL8	NOS2
CCND1	GAPDH	p53	CCND1	GAPDH	p53	CCND1	GAPDH	p53	CCND1	GAPDH	p53
Cox-2	IFNg	TNFA	Cox-2	IFNg	TNFA	Cox-2	IFNg	TNFA	Cox-2	IFNg	TNFA
FAS-L	IRF1	TNFR	FAS-L	IRF1	TNFR	FAS-L	IRF1	TNFR	FAS-L	IRF1	TNFR
IFNB1	MDR1	VCAM1	IFNB1	MDR1	VCAM1	IFNB1	MDR1	VCAM1	IFNB1	MDR1	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