



Human NFκB-Regulated cDNA Plate Array

Catalog Number AP-0101

(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+ 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. 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 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)
- Blocking buffer (RT)
- 5x Detection wash buffer (RT)
- Substrate A (4 °C)
- Substrate B (4 °C)
- Substrate dilution buffer (4 °C)

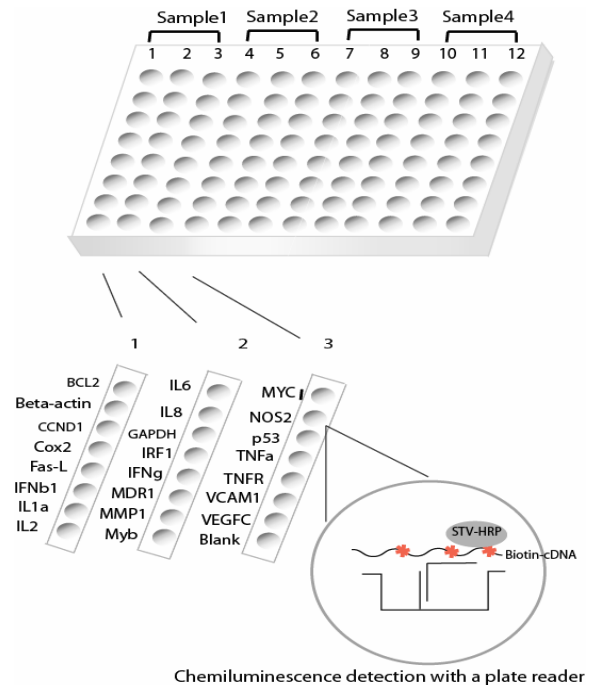


Diagram of human NFκB cDNA plate assay

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

Assay procedure

1. cDNA synthesis using PCR machine

Note: Briefly spin tubes before opening

- (1) Sample preparation
 - X µl 1-10µg total RNA
 - 2 µl Human NFκB Reg. Primer Mix
 - X µl ddH₂O
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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. Add 100ul Plate hybridization buffer without cDNA to the 'blank' well.
- (4) Seal the wells with foil film securely and hybridize 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) Remove the foil film from the experimental wells with a blade. Keep the unused well sealed.
- (2) Invert the Hybridization Plate over an appropriate container and expel the contents forcibly, and wash the plate 3 times by adding 200ul of pre-warmed 1x Plate hybridization wash buffer to each well. At each wash, incubate the plate for 5 minutes with gently shaking at room temperature.

- (3) Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels.
- (4) Add 200µl of Blocking buffer to each well and incubate for 15 minutes at room temperature with gently shaking.
- (5) Invert the plate over an appropriate container to remove block buffer.
- (6) Add 20 µl of streptavidin-HRP conjugate in 10ml blocking buffer (1:500) dilution, enough for one plate. Add 95 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gently shaking.
- (7) Wash the plate 3 times by adding 200ul 1X Detection wash buffer to each well, At each wash, incubate the plate for 10 minutes with gently shaking at room temperature.
- (8) Complete removal of liquid at each wash by firmly tapping the plate against clean paper towels. At the last wash, invert plate on clean paper towels for 1-2 min to remove excessive liquid.
- (9) Freshly prepare the substrate solution:
 - For the whole plate:
 - 1ml Substrate A
 - 1ml Substrate B
 - 8ml Substrate dilution buffer
- (10) Add 95µl substrate solution to each well and incubate for 1 min.
- (11) Place the plate in the luminometer. Allow plate to sit inside machine for 5 min before reading. 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

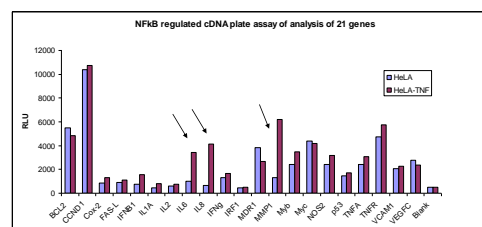


Figure1: NFκB-regulated cDNA plate assay for analysis of 21 genes.

HeLa cells were treated with and without 20ng/ul TNF for 30 minutes respectively. Total RNAs were prepared. RNAs were subjected to NFκB regulated cDNA plate assay, and detected with chemiluminescence by a plate reader. The results showed that TNF induced the expression level of IL6, IL8 and MMP1 by 5 fold (indicated by arrow)

Diagram of 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	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