



## 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)
- 5xDetection wash buffer (RT)
- Substrate A (4 °C)
- Substrate B (4 °C)
- Substrate dilution buffer (RT)

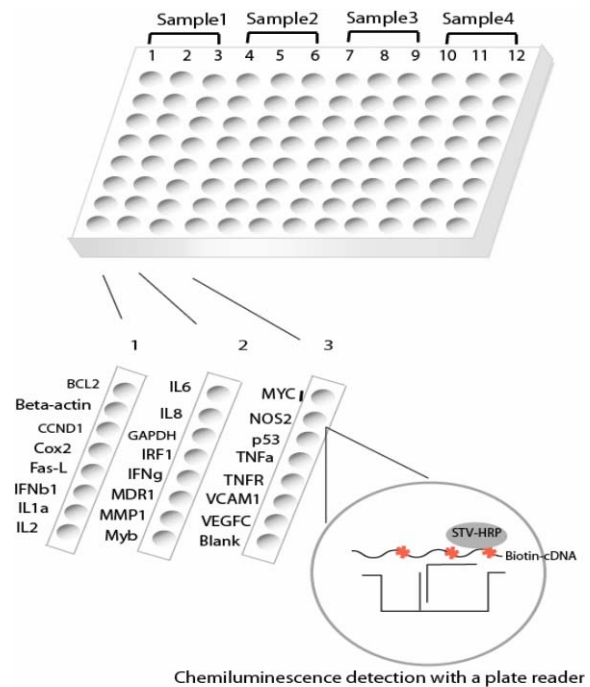


Diagram of human NFκB cDNA plate assay

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

