Signosis
Innovative Plate Assay Solutions

Real-Time PCR Assay for Monitoring NFκB-Regulated Genes

Catalog Number CL-1001 **(For Research Use Only)**

Introduction

NFkB 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. When it is activated, NFkB mediates the expression of genes involved in immune and inflammatory responses, cell growth control, and apoptosis. To facilitate the monitoring of gene expression representing these biological functions with a limited number of cells, Signosis developed a real-time PCR assay to analyze 8 genes in total RNA or directly in cell lysates.

Principle of the assay

Signosis' Real-Time PCR assay is to monitor the expression of 8 genes through reverse transcription of mRNA into cDNA and real-time PCR. The purified total RNA or cell lysate is first reverse transcribed into cDNA. Targeted genes are then analyzed with real-time PCR.

Materials provided

- Cell lysis buffer
- Oligo dT (18mer)
- Random primer
- Reverse transcription buffer mix
- Reverse transcripase
- 2X SYBR Green PCR Master Mix
- 8 Gene specific PCR primers

Material may required but not provided

- RNase free ddH2O
- Real-time PCR instrument
- PCR plate and film for real-time PCR

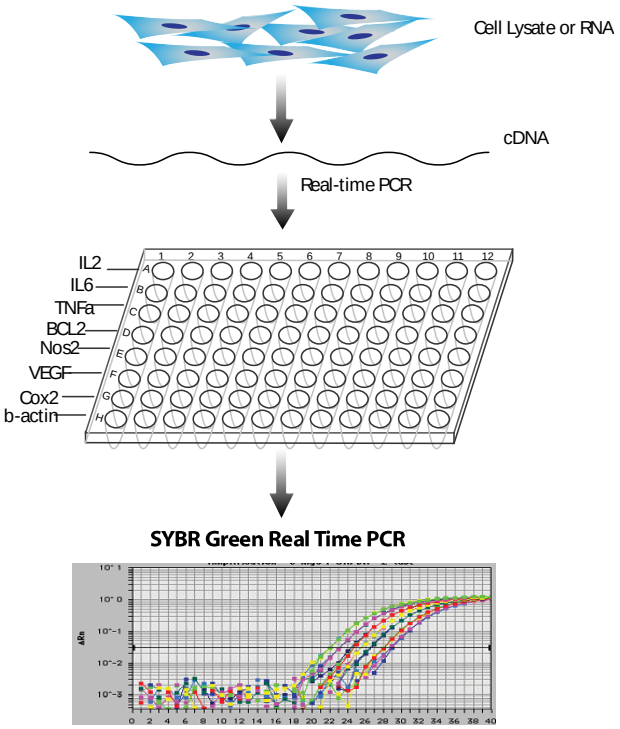

Diagram of Real-time PCR Assay for Monitorning NFkB Regulated Genes

1. Sample preparation procedure

(1) Estimate the number of cells. Wash the cells with 200ul ice cold 1XPBS. If the range of cell number is from 1000 to 10,000, and add 50ul ice-cold Cell lysis buffer and then subject to snap-frozen at -80°C. If the range of cell number is from 50-1000, add 20 ul Cell lysis buffer. The cell number is 1- 50, add 5 ul Cell lysis buffer.

(2) Incubate in Cell lysis buffer for 10 minutes. Remove contaminated DNA by spinning the sample at 12,000rpm for 5 minutes.

(3) Transfer the supernatant to a fresh tube. Heat at 75°C for 10 minutes, and put on ice. The cell lysate is ready for use or can be stored at -80 °C for the future usage.

2. cDNA synthesis using PCR machine

(1) Sample preparation
1-4 ul total RNA (0.1-1ug) or cell lysate
2 µl oligo dT, random primer or dT+ random primer
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 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.

3. PCR amplification

(Note: make small aliquot of 2X PCR Buffer into several small tubes to avoid free-thaw cycle)

(1) Prepare PCR reaction
Mix the following component for one reaction:
10ul 2x SYBR Green PCR Master Mix
8.3ul ddH_2O
0.5 ul cDNA
1 ul gene specific primer
Note: make a master mix for 8 genes by multiplying the volume by 8.5, and dispense 20ul PCR mix to each well of one column of PCR plate. And transfer 1ul of gene specific primer from the provided 8-well primer strip to each well.

(2) Proceed PCR cycles:
Heating the reactions at 95°C for 30 sec.
Proceed PCR 35 cycles as follows:
95 °C 15 seconds
58 °C 30 seconds
72 °C 30 seconds

(3) Conduct real-time PCR analysis

Example of Analysis Data

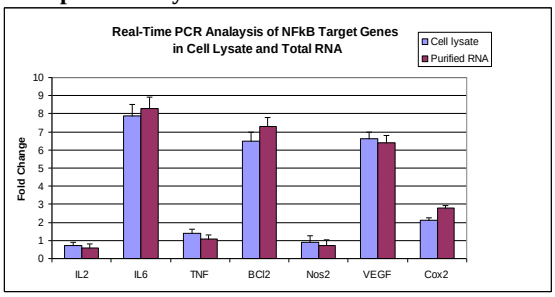

Figure 1: Real-Time PCR Analysis of NFkB Target Genes in Cell Lysate and Total RNA. The HeLa cells were treated with TNFa for 6 hours. The cell lysate from 1000 cells and 0.1 ug of total RNA were subjected to the assay. The real-time PCR was conducted on ABI7700.