



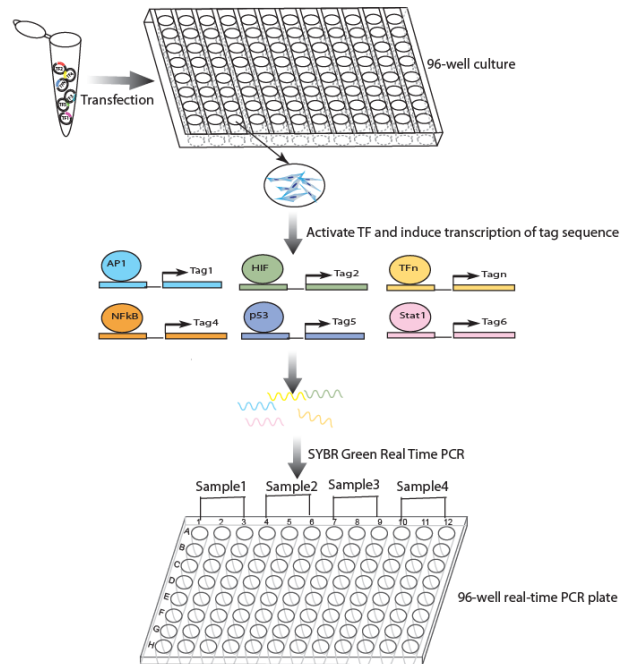
Multiplex TF Reporter Real-Time PCR Assay I

Catalog Number CL-2001

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Introduction

Transactivation of a transcription factor (TF) is often measured by a reporter construct which contains a cis-element (DNA binding sequence) of the TF and a reporter gene such as a luciferase gene. When the reporter is delivered into cells, the activated TF binds to the cis-element and mediates the induction of the reporter gene. The induction can be measured via biochemical analysis of the reporter gene product. However, it is a single assay, one TF at a time. As any cellular change could result in the activation of multiple TFs, monitoring these TFs need multiple transfections and biochemical measurement. In addition, normalization is needed in order to normalize the transfection efficiencies. Signosis has developed a proprietary technology for monitoring the activation of multiple TFs simultaneously. These selected 24 TFs in array I represent the most important signal transduction pathways. In TF reporter real-time PCR assay I, 24 TF reporter vector mix is transfected into the cells in one transfection. The activation of 24 TFs is monitored simultaneously with SYBR green based real-time PCR directly in cell lysates without RNA preparation with CL™ cell lysis buffer, which is included in the kit.



Principle of the assay

Signosis' proprietary multiplex TF reporter real-time PCR I is a real-time based assay for quantitatively profiling the activities of 24 TFs simultaneously. In the technology, a series of reporter constructs are made, each of which contains a cis-element and a reporter tag sequence to a specific TF. When the constructs are delivered as a library into cells in a well in 96-well plate, the activation of TFs will bind to its corresponding constructs and mediate the expression of the tag sequences. The cell lysates are prepared, transcribed into cDNA and subsequently subjected to real-time PCR. The difference in TFs can be identified through comparison of two samples.

Materials provided

- Cell lysis buffer
- TF Plasmid mix
- TF Reverse transcription buffer mix
- Reverse transcriptase
- TF SYBR Green PCR Master Mix
- DNA polymerase
- 24 TF specific PCR primers

Diagram of Multiplex TF Reporter Real-Time PCR

Material may required but not provided

- RNase free ddH₂O
- Fugene 6 (Roche, cat#11 815 091 001)
- Real-time PCR instrument
- PCR plate and film for real-time PCR

Assay procedure

1. Transfection of TF Plasma Mix into the cells

We recommend using FuGene 6 Roche to transfect Plasmid Mix into the adherent cells seeded on a 96-well plate.

- (1) Seed the cells with 100ul proper medium containing serum without antibiotics at 80% confluency at the time of transfection.
- (2) For each transfection well, dilute 3ul FuGene 6 with 20ul serum-free medium (without antibiotics); and dilute 4ul of plasmid mix to 20ul serum-free medium (without antibiotics). Add the diluted DNA to dilute FuGene 6.
- (3) Tap the tube to mix the contents, and incubate for 20-30 minutes.
- (4) Add complex to the cells in a drop-wise manner. Swirl the wells to ensure the distribution over the entire surface. And incubate for overnight.
- (5) If the starvation is required for treatment, the serum medium can be replaced with serum-free medium.
- (6) The treatment commonly takes 2-4 hours for induction.

2. Sample preparation procedure

- (1) Wash the cells with 200ul ice cold 1XPBS. Freeze the cell at -80°C for at least 10 minutes or longer for future usage.
- (2) Thaw the cells, and incubate 100ul Cell lysis buffer for 10 minutes on ice, and mix the cell lysate by pipette up and down and transfer to a PCR tube
- (3) Add 1ul DNase and incubate for 30 minute at 37°C ,and add EDTA to 5mM and heat at 75°C for 10 minutes to inactivate DNase.
- (4) 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
2 ul Cell lysate
8 ul Reverse transcription buffer mix
9 ul ddH₂O
1ul RT

20 µl

3. PCR amplification

- (1) Prepare PCR reaction
Mix the following component for one reaction:
20ul PCR Buffer Mix
0.2 ul DNA polymerase
0.5 ul cDNA
1ul gene specific primer
Note: make a master mix for 24 TF reporters by multiplying the volume by 25, and dispense 20ul PCR mix to each well of one column of PCR plate. And transfer 1ul of TF specific primer from the provided 24--well primer strip to each well.
- (2) Proceed PCR cycles:
Heating the reactions at 82°C for 60 seconds.
Proceed PCR 35 cycles as follows:
94 °C 15 seconds
58 °C 45 seconds
72 °C 50 seconds
- (3) Conduct real-time PCR analysis

Example of Analysis Data

