Signosis
Innovative Plate Assay Solutions

Single Cell Real-Time RT-PCR Assay Kit

Catalog Number CL-0003 **(For Research Use Only)**

Introduction

Gene expression is regulated at the level of individual cells. It is desired to have methods capable of analyzing the gene expression pattern of individual cells such as stem cells, neurons, developmental tissues, and laser capture microsections. SYBR Green is the most widely used double-strand DNA-specific dye reported for real time PCR. Signosis has developed a single cell SYBR green based real-time RT-PCR directly in cell lysates without RNA preparation. This kit provides a simple, sensitive, quantitative and cost-effective method to monitor PCR reactions in real time. All of reagents for cDNA synthesis and PCR amplification in addition to CL™ cell lysis buffer are included in the kit.

Materials provided

- Cell lysis buffer
- Oligo dT (18mer)
- Random primer
- Reverse transcription buffer mix
- Reverse transcriptase
- β-actin control primer for human, mouse and rat
- SYBR Green PCR Master Mix

Material required but not provided

- Gene specific PCR primers

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 -80ºC. 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.
Notes: Keep the cells on ice at all time during procedure.

(2) Incubate for 10 minutes on ice, and centrifuge at 10,000g for 2 minutes. Transfer the supernatant to a fresh tube.
Optional: Add 0.25-1u DNase I, and incubate at 37 ºC for 30 minutes and inactivate at 75 ºC for 10 minutes.

(3) Heat the supernatant for 75 ºC for 15 minutes, and put on ice. The cell lysate is ready for use or can be stored at -80ºC for the future usage.

(4) 1-5ul cell lysate is used for cDNA synthesis.

2. cDNA synthesis using PCR machine

(1) Sample preparation
2-4 ul cell lysate
2 µl oligo dT, random primer or dT+ random primer
X µl ddH2O

11µl

(2) Add 8 µl Reverse transcription buffer mix and 1µl RT to each reaction tube, and incubate for 1 hour at 45 ºC.

(3) Heat the reaction to 98 ºC for 5 minutes, and chill on ice.

3. Real-time PCR

(1) Prepare PCR reaction
Mix the following component for one reaction:
X ul PCR-grade dH_2O
10ul 2X SYBR Green PCR Master Mix
1-2 ul cDNA
2 ul control primer (10uM) or gene specific primer
Total reaction mix: 20 ul
Note: make a master mix by multiplying the volume by the number of your reactions

(2) Proceed PCR cycles:
Heating the reactions at 98 ºC for 30 seconds. Proceed PCR 35 cycles as follows:

98 ºC	15 seconds
55 ºC	50 seconds
72 ºC	60 seconds

Note: PCR cycle can be adjusted according to a specific primer designing.

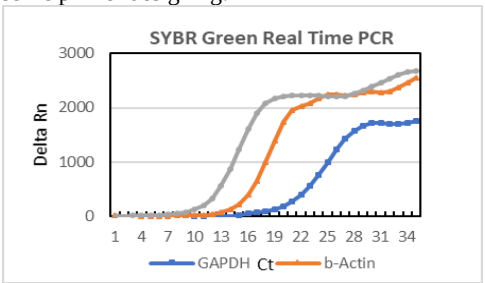

Figure 1. Detection of Limited Target Sequences from Cell Lysate. Increasing amounts of Hela cells were lysed with cell lysis buffer. All samples were reverse transcribed by the RT reagents provided in the kit. Real-time PCR was conducted for beta-actin, GAPDH and B2M genes on all samples in triplicate reactions on a 7700 system.