Signosis, Inc.

Innovative Plate Assay Solutions

Single Cell RT-PCR Assay Kit

Catalog Number CL-0002 **(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 micro sections. Signosis has developed a sensitive single cell RT-PCR directly in cell lysates without RNA preparation. Therefore, a small number of cells can be used for RT-PCR analysis. Signosis now offers the optimized buffers for subsequent RT-PCR followed cell lysis with Direct cDNA lysis buffer. All of reagents for cDNA synthesis and PCR 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
- PCR buffer mix
- DNA polymerase

Material that may be required but not provided

- Gene specific PCR primers

1. Sample preparation procedure

(1) Estimate the number of cells. Wash the cells with 200 µl ice cold 1XPBS. If the range of cell number is from 1000 to 10,000, add 50 µl ice-cold Cell lysis buffer and then subject to snap-frozen at -80 ºC. If the range of cell number is from 50-1000, add 20 µl Cell lysis buffer. The cell number is 1- 50, add 5 µl Cell Lysis buffer. **** Note:** Keep the cells on ice during the procedure to prevent cells from degrading. ******

(2) Incubate in Cell Lysis buffer for 10 minutes. Remove contaminated DNA by spinning the sample at 12,000rpm for 5 minutes.
Optional: Add 0.25-1 µl DNase I, and incubate at 37 ºC for 10 minutes and inactivate at 75 ºC for 10 minutes.

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

2. cDNA synthesis using PCR machine

(1) Sample preparation
1.0 - 4.0 ul total RNA (0.1-1 µg) or cell lysate
1.0 µl oligo dT
1.0 µl random primer or dT+ random primer
X µl ddH2O

11µl

(2) Incubate for 5 minutes at 65 ºC, and chill on ice.
(3) Add 8 µl Reverse transcription buffer mix and 1 µl RT to each tube, and incubate for 1 hour at 45 ºC.
(4) Heat the reaction to 98 ºC for 5 minutes, and chill on ice.

3. PCR amplification

(1) Prepare PCR reaction
Mix the following component for one reaction:
18.0 µl PCR Buffer Mix (including DNA Polymerase)
0.5 µl cDNA
2 µl control primer (5-10 µM) or gene specific primer
**** Note**: Make a master mix by multiplying the volume by the number of your reactions. ******

(2) Heat the PCR reaction at 98 ºC for 1 minutes. Proceed PCR for 30-35 cycles as follows:

98 ºC	20-30 seconds
55 ºC	30 seconds
72 ºC	30 seconds/kb

(3) Run PCR products on 1.2% agarose gel electrophoresis. The size is around 260bp.

Data example

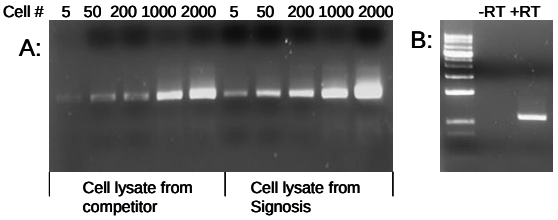

Figure 1: Single Cell RT-PCR Assay Kit. A: The indicated cells were lysed with cell lysis buffer from Signosis and competitor respectively, and subjected to RT-PCR for β-actin with 30 cycles. B: Testing no genomic DNA contamination. Lane1. β-actin was amplified with 36 PCR cycles directly from cell lysate without reverse transcription (RT). Lane2. β-actin was amplified with 36 PCR cycles directly from cDNA transcribed from cell lysate.