Signosis, Inc.

Innovative Plate Assay Solutions

Single Cell RT-PCR Assay Kit (Modified for Single Cell)

Catalog Number CL-0002 **(For Research Use Only)**

Introduction

Gene expression is regulated at the level of individual cells. It is desirable 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 and mouse
- PCR buffer mix
- DNA polymerase

Material that may be required but not provided

- Gene specific PCR primers

1. Sample preparation procedure

1. For single cell or 5-100 cells, add 1-5 µl of Cell Lysis Buffer in PCR tube.
   **** Note:** Keep the cells on ice during the procedure to prevent cells from degrading. ******
2. Add 20 µl of 1X Cell Lysis Buffer and incubate on ice for 10 minutes.
3. Conduct freeze and thaw for three times
4. Heat at 75 ºC for 10 minutes in thermal cycler and put on ice when completed.
5. 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 µl 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

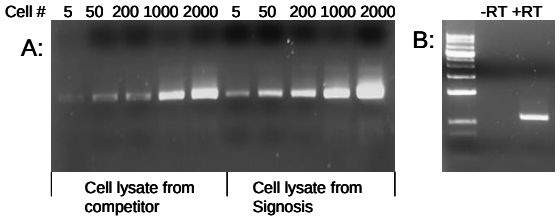

Figure 1: Single Cell RT-PCR Assay Kit. A: The indicated cells were lysed with cell lysis buffer from Signosis and competitor respectively, and subjected to RT-PCR for β-actin with 30 cycles. B: Testing no genomic DNA contamination. Lane1. β-actin was amplified with 36 PCR cycles directly from cell lysate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