



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 microsections. 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 CL™ cell lysis buffer. 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
- PCR buffer mix
- DNA polymerase

Material may required but not provided

- 1 ml Cell lysis buffer (cat# CL-0001 from Signosis)
- 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 -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.
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.
- (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

11 μl
- (2) Incubate for 5 minutes at 65°C , and chill on ice.
- (3) Add 8 μl Reverse transcription buffer mix and 1 μl 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

- (1) Prepare PCR reaction
Mix the following component for one reaction:
20ul PCR Buffer Mix
0.2 ul DNA polymerase
0.5 ul cDNA
1 ul control primer (5-10uM) or gene specific primer
Note: make a master mix by multiplying the volume by the number of your reactions
- (2) Proceed PCR cycles:
Heating the reactions at 82°C for 60 seconds. Proceed PCR 35 cycles as follows:

95 $^{\circ}\text{C}$	40 seconds
55 $^{\circ}\text{C}$	50 seconds
72 $^{\circ}\text{C}$	60 seconds

Note: PCR cycle can be adjusted according to a specific primer designing.
- (3) Run PCR products on 1.2% agarose gel electrophoresis. The size is around 260bp.

Data example

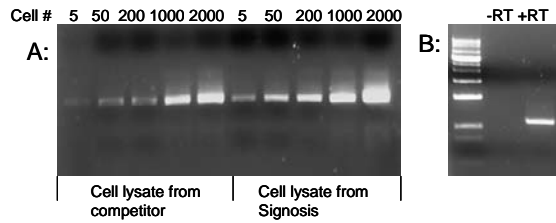


Figure: Single Cell RT-PCR Assay Kit. A: The indicated cells were lysed with cell lysis buffer from Signosis and competitor respectively, and subjected to RT-PCR for β -actin with 30 cycles.

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.