Signosis, Inc.

Innovative Plate Assay Solutions

Direct cDNA Cell Lysis Buffer

Catalog Number CL-0001 **(For Research Use Only)**

Introduction

The study of gene expression often needs RNA preparation followed by cDNA synthesis and PCR. However, difficulty in obtaining a large amount of cells for RNA preparation is the bottleneck in some of the studies, such as laser dissected samples, FACS sorted cells, the cultured cells in 96-wells, and liquid biopsy. Cell number is limited (<2,000 cells), which restrict RNA preparation. Signosis' Direct cDNA cell lysis buffer allows preparation of cell lysate, which can be used for direct reverse transcription without RNA preparation. Cell lysate made from a few cells is good enough for reverse transcription of RNA to cDNA and following traditional PCR and real time PCR analysis or cDNA plate array assays.

Materials provided

- 10ml Direct cDNA cell lysis buffer (-20ºC)

Material may require but not provided

- RT-PCR kit (Cat# CL-0002 from Signosis, Inc.)
- SYBR Green PCR Buffer Master Mix (Cat# CL-0003 from Signosis, Inc.)

I. 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.

Suggested Procedures

II. cDNA synthesis

(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.

III. PCR amplification

1. Add 1µl cDNA and 1.25µl each of forward and reverse primer to DNA polymerase reaction mix without polymerase
2. Heat the reaction at 98ºC for 1 minute, add 1µl DNA polymerase.
3. Proceed PCR cycles:
   98ºC 30 seconds
   55ºC 30 seconds
   72ºC 30 seconds
4. PCR products on 1.2% agarose gel electrophoresis.

*** Please note that the parameters will have to be optimized if different DNA polymerase is used. ***

IV. Real-time PCR

Mix the following component for one reaction:
20ul SYBR Green PCR Master Mix
1ul primer (5-10uM)
1ul cDNA

Proceed with the following PCR cycles:

1. Heating the reaction at 82ºC for 60 seconds.
2. Proceed two-step PCR 35 cycles:
   95ºC 40 seconds
   60ºC 60 seconds

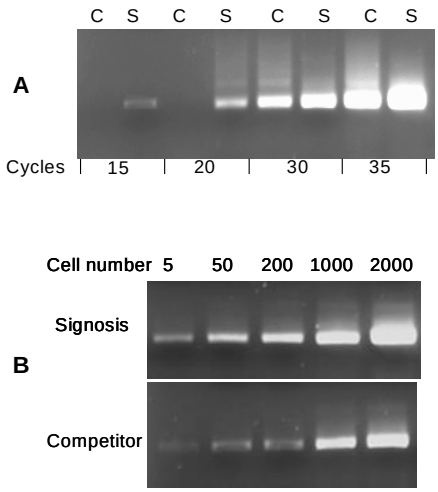

Figure 1. Comparison of cell lysate amplification kit from Signosis with the related kit from competitor.
A: 2000 HeLa cells were lysed with cell lysis buffer from a competitor (C) and from Signosis cell lysis buffer (S). cDNAs were synthesized with RT-PCR kit and the endogenous gene (actin) amplified with different cycles using Signosis PCR amplification system.
B: The cell lysate from different cell numbers were used for actin amplification with 30 cycles.

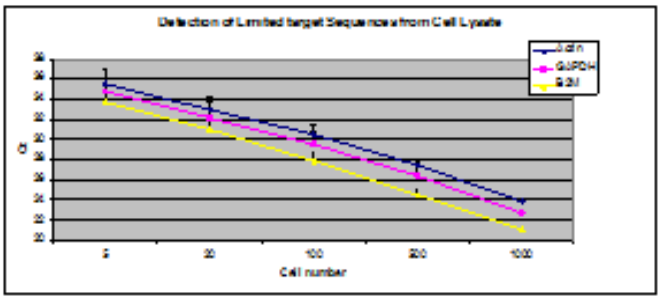

Figure 2. Detection of Limited Target Sequences from Cell Lysate.
Increasing amounts of HeLa cells were lysed with cell lysis buffer. All samples were reverse transcribed by the RT reagents provided in the kit. Real-time PCR was conducted for beta-actin, GAPDH and B2M genes on all samples in triplicate reactions on a 7700 system.