



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 and the cultured cells in 96-wells. Cell number is limited (<2,000 cells), which restrict RNA preparation. Signosis' CL™ 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 cell lysis buffer (-20°C)

Material may required but not provided

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

1. Sample preparation procedure

1. Estimate the number of cells. The number of cells should be between 10^4 - 10^5 cells. Wash the cells with 200ul ice cold 1XPBS and add 100ul ice-cold Cell lysis buffer and then subject to snap-frozen at -80°C. If the cell number is between 2000-10,000 cells, add 20ul Cell lysis buffer instead.

Notes: Keep the cells on ice at all time during the 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°C for 10 minutes and inactivate at 75°C for 10 minutes.
3. Heat the supernatant for 75°C for 15 minutes, and put on ice. The cell lysate is ready for use or can be stored at -80°C for the future usage.
4. 1-5ul cell lysate is used for cDNA synthesis.

2. cDNA synthesis

1. Sample preparation
4 ul cell lysate
2 µl oligo d(T) or random primer
X µl ddH2O

11µl

2. Add 8 µl Reverse transcription buffer mix and 1.5µl RT to each reaction tube, and incubate for 1 hour at 45 °C.
3. Heat the reaction to 98 °C for 5 minutes, and chill on ice.

3.PCR amplification

1. Add 2 µl cDNA and 1 µl control primer to 50 µl l full length PCR amplification buffer
2. Heat the reaction at 82°C for 1 minute, add 1 µl rtTH DNA polymerase.
3. Proceed PCR cycles:
94 °C 40 seconds
55 °C 40 seconds
72 °C 60 seconds
4. Run PCR products on 1.2% agarose gel electrophoresis.

Example

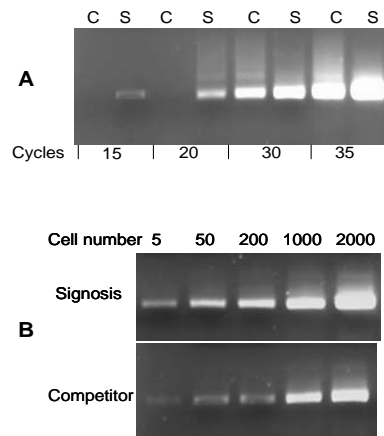


Figure 1: Comparison of cell lysate amplification kit from Signosis with the related kit from competitor. A: 2000 HeLa cells were lysed with cell lysis buffer from a competitor (C) and from Signosis cell lysis buffer (S). cDNAs were synthesized with RT-PCR kit and the endogenous gene (actin) amplified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

B: The cell lysate from different cell numbers were used for actin amplification with 30 cycles.

4. Real-time PCR

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

Proceed PCR cycles:

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

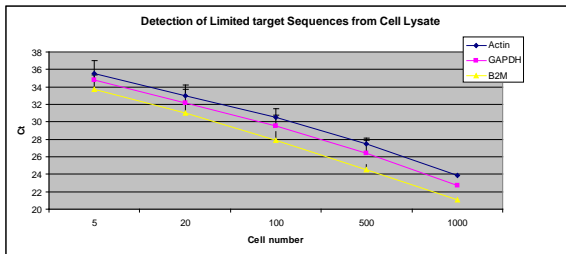


Figure 2. Detection of Limited Target Sequences from Cell Lysate. Increasing amounts of HeLa cells were lysed with cell lysis buffer. All samples were reverse transcribed by the RT reagents provided in the kit. Real-time PCR was conducted for beta-actin, GAPDH and B2M genes on all samples in triplicate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