EZ-press Cell to cDNA Kit

Catalog No.: B0001

Description

The *EZ*Bioscience® EZ-press Cell to cDNA Kit offers a fast and easy method to produce cDNA from cells in culture without RNA isolation. It is a good substitution for the TRIzol method in quantitative analysis of gene expression. This kit offers several significant advantages compared with TRIzol method: 1. Easy to use. Starting from cells in culture, high quality cDNA can be synthesized in just two steps (cell lysis and reverse transcription), no RNA isolation is needed. 2. Fast. The whole experiment (from cells to cDNA) can be completed in less than 35 minutes. 3. Stable and highly reproducible. The total RNA is perfectly reserved throughout the experiment, enabling stable and highly reproducible results. 4. High sensitivity. The lower detection limit for each sample is only 100 cells. 5. Genome DNA is removed effectively by the DNase contained in the reagents. Moreover, the reagents used in this kit are safe and non-toxic, which is pleasant compared with the smelly and hazardous TRIzol reagent.

Components

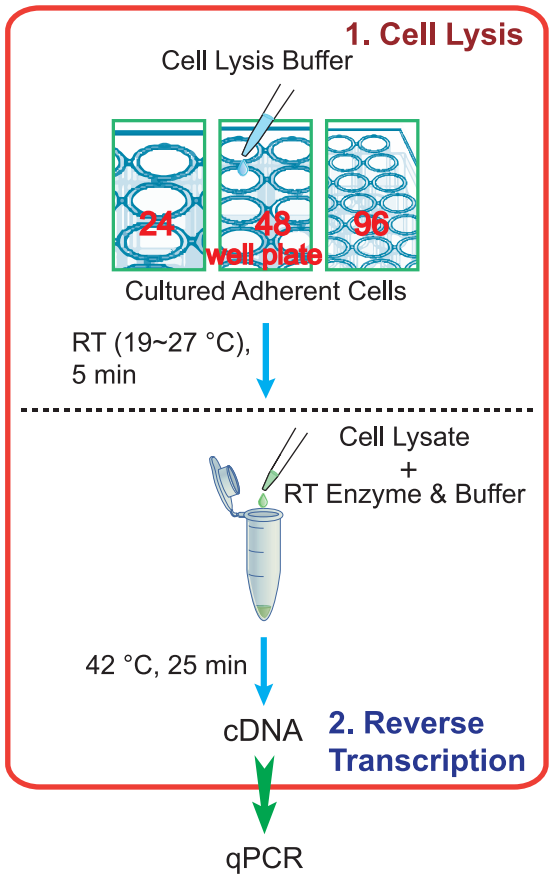
Component	B0001-S (10 Rxns)	B0001 (100 Rxns)
Cell Lysis Buffer	2.2 ml	22 ml
RT Enzyme Mix	22 µl	220 µl
2× RT Buffer	110 µl	1.1 ml
ddH_2O	1 ml	2 ml

Storage

Store at -20 °C.

Protocol

Experimental Procedure Overview:



Thaw the ***Cell Lysis buffer*** and ***2× RT Buffer***, invert or flick the tubes several times to **mix thoroughly** (**do not use vortex**), and place the tubes on ice.

Cell Lysis

1A. For adherent cells with a cell number of less than 3×10^5/sample:

a) Aspirate the culture medium from the wells.

b) Wash the wells with PBS (200 µl/well). Remove PBS as completely as possible without disturbing the cells.

c) Add Cell Lysis Buffer to each sample with the proportion of 80 µl Cell Lysis Buffer/1×10^5 cells, gently pipette up and down for 5 times to lyse the cells.

d) Incubate the cell lysates for 5 minutes at room temperature (19~27 °C).

1B. For adherent cells with a cell number of more than 3×10^5/sample or suspension cells:

a) (*For adherent cells only, for suspension cells, start at step b*) Detach cells using the subculturing method routinely employed in your laboratory.

b) Count then gently pellet the cells, discard the growth medium.

c) Wash cells with PBS by resuspending them in ~200 µl PBS per 10^5 cells (or ~100 µl PBS for <1×10^5 cells).

d) Transfer a certain amount of cells (~1×10^5) to a new 1.5ml centrifuge tube for each sample, centrifuge cells in low speed, then aspirate the PBS carefully without disturbing the cell pellets. Place the cells on ice.

e) Add 80 µl Cell Lysis Buffer to each sample, gently pipette up and down for 5 times to lyse the cells.

f) Incubate the cell lysates for 5 minutes at room temperature (19~27 °C).

2. Put the lysates on ice; carry out the RT reactions in 30 minutes.

Note:

1. Read the manual carefully before use to ensure the experiments are done right.

2. The lysis capacity of 80 µl Cell Lysis Buffer is about 1×10^5 cells, varies according to the cell type. To achieve the best results, it is strongly recommended to do a pilot experiment to determine the best number of cells.

3. Cells cultured in 96, 48, 24 and 12 well cell culture plates with the cell number of not more than 3×10^5can be used for lysis directly after PBS washing. When doing with cells more than 3×10^5/well (or dish), follow the procedure 1B: cells must be detached first (step a).And then, add cell culture medium to the trypsin detached cells, count, low speed centrifuge and discard the supernatants (step b), resuspend with proper volume of PBS (step c), and then take ~1×10^5 cells/sample for lysis with 80 µl Cell Lysis Buffer (step d).

4. The cell lysate is incompatible with any other RT reagents commonly used (no or few cDNA can be produced using other RT reagents). So the cell lysates **MUST** be reverse transcribed using the specially optimized RT reagents supplied in this kit.

Reverse Transcription

Set up the following mixture in RNase free centrifuge tube:

Component	Volume (20µl)
Cell Lysate	4 µl
RT Enzyme Mix	2µl
2× RT Buffer	10 µl
RNase free ddH_2O	4µl

Once assembled, mix reactions gently, then centrifuge briefly to collect the contents at the bottom of the reaction tube.
Perform the reverse transcription reaction following the procedure below:

Temperature	Time
42°C	25 min

Real-time PCR

Dilute the 20 µl reverse transcription reaction by 80µl ddH_2O. The diluted reverse transcription products can be used in PCR

reactions immediately (reverse transcription product should be not more than 1/5 of the PCR reaction volume), or stored in aliquots at -80°C for use within 1 month. Avoid repeated freeze-thaw cycles.

Set up the reactions as the following table:

Component	Volume (20μl)
2× SYBR Green qPCR Master Mix	10μl
Template (diluted RT product)	4μl
Forward primer (10 μM)	0.5μl
Reverse primer (10 μM)	0.5μl
ROX Reference Dye*	0.4μl
ddH_2O	4.6μl

*The usage of ROX depends on the real-time PCR instrument used.

Then set the real-time PCR amplification program according to the product manual of the qPCR Kit, and run the reaction.

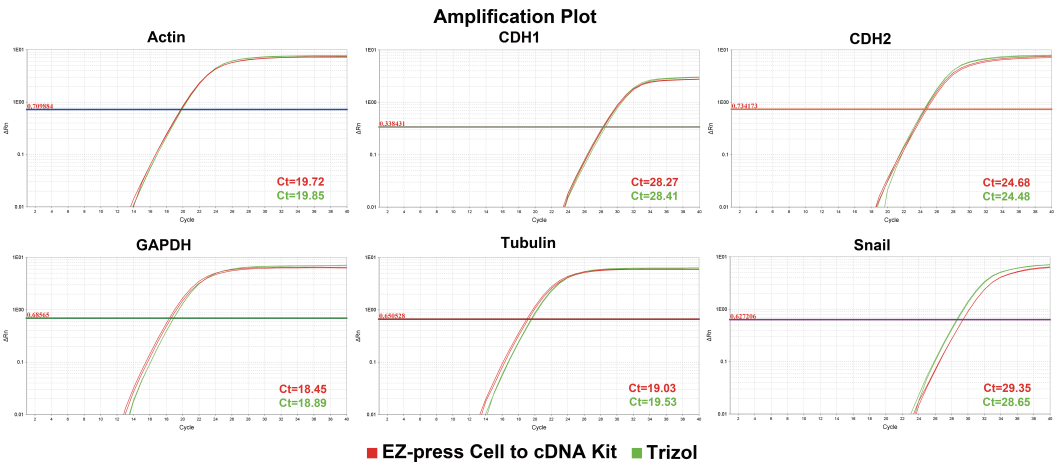
Cell types tested by EZ-press Cell to cDNA Kit

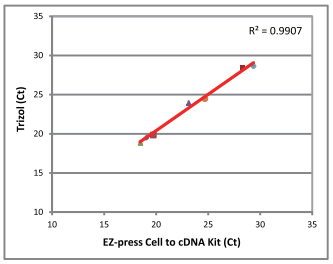
CellName	Growth	Species	Source Tissue
A431	Adherent	*H. sapiens*	Epidermoid Carcinoma
A549	Adherent	*H. sapiens*	Lung Carcinoma
CHO-K1	Adherent	*Hamster*	Ovary
COS-7	Adherent	*Monkey*	Kidney
HEK-293T	Adherent	*H. sapiens*	Kidney
HeLa	Adherent	*H. sapiens*	Cervical Adenocarcinoma
HepG2	Adherent	*H. sapiens*	Liver Carcinoma
Jurkat	Suspension	*H. sapiens*	Acute T-Cell Leukemia
NCI-H446	Adherent	*H. sapiens*	Small Cell Lung Cancer
NIH-3T3	Adherent	*M. musculus*	Embryonic Fibroblast

Cell types tested are listed in this table. When use other cells that are not included in this table, it is suggested optimizing the cell numbers to get better results.

Representative experimental results

cDNA synthesized by ***EZ*Bioscience**® EZ-press Cell to cDNA Kit and TRIzol-reverse transcription method were evaluated by real-time qPCR with 6 pairs of primers (in HEK-293T cells). Highly consistent results between EZ-press Cell to cDNA Kit and TRIzol -RT method can be observed in the following figure, which indicate that **EZ-press Cell to cDNA Kit can completely replace TRIzol-RT method in preparing cDNAs from cells.**





High correlation was observed between the results from the EZ-press Cell to cDNA Kit and Trizol purified RNA.

Trouble shooting

1. The Ct value of reference gene is too large or no amplification.

a. Make sure the reagents and primers are reliable. Fresh reagents are preferred. The solutions of primers should be stored in aliquots to avoid contamination;

b. Make sure the experimental process is correct, e.g.:

1) Mix the Cell Lysis Buffer and 2X RT Buffer before use;

2) Wash the cells with PBS before cell lysis and aspirate the PBS as clear as possible;

3) Add the Cell Lysis Buffer and gently pipette 5 times. The cells must be completely covered by the Cell Lysis Buffer;

4) Cells must be lysed at room temperature for 5 minutes. The temperature and time cannot be changed;

5) Mix all the reactions thoroughly before start;

6) Make sure the cell lysates are reverse transcribed using the RT reagents supplied in this kit.

c. In most conditions, be sure the cell number loaded is not more than 1×10^5per 80 μl Cell Lysis Buffer, if the result is still not so good, decrease the cell number by half, or double the volume of Cell Lysis Buffer used and try again;

d. Remove PBS as completely as possible before adding the Lysis Buffer to cells;

e. **Make sure there are really enough cells left after PBS washing**, especially when the cell number is less than 10,000. For small amount of cells (< 10,000), use 5 ~ 10 μl Cell Lysis Buffer.

2. The reference gene can be amplified normally, but genes of low expression level cannot.

a. Try to use more cells, use 50 μl Cell Lysis Buffer per 1×10^5 cells;

b. Make sure the primers are right, without contamination or degradation, and the target fragment can be amplified in the qPCR reaction properly.

3. Some genes' amplification plot or melt curve appears abnormal.

a. Decrease the cell number, or increase the volume of Cell Lysis Buffer to lyse the cells completely. For most conditions, the cell number is not more than 1×10^5 per 80 μl Cell Lysis Buffer;

b. Decrease the volume of cell lysates used in RT reactions, e.g. add 2 μl of cell lysate to each 20 μl RT reaction instead of 4ul cell lysate;

c. Decrease the volume of cDNA used in qPCR, e.g. add 2 μl of cDNA (diluted to 100 μl from 20 μl RT reaction) to the 20 μl qPCR reactions instead of 4 μl cDNA;

d. Make sure the reagents are reliable;

e. Make sure the primers are not contaminated or degraded. The volume of primers can be increased.