EZ-press Cell to cDNA Kit PLUS

Catalog No.: B0003

Description

The *EZ*Bioscience® EZ-press Cell to cDNA Kit PLUS 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. It also has improved genome DNA degrading efficiency than the previous EZ-press Cell to cDNA Kit (Catalog No. B0001). 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 25 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 even more effectively by the gDNA Remover contained in this kit than the previous version (Catalog No. B0001). Moreover, the reagents used in this kit are safe and non-toxic, which is pleasant compared with the smelly and hazardous TRIzol reagent. And Compared with EZ-press Cell to cDNA Kit, the template RNA in cell lysate can be precipitated by ethanol precipitation, and quantified after dissolvement. So the same amount of RNA can be employed as template in RT reactions for each sample. Furthermore, the EZ-press Cell to cDNA Kit PLUS has higher RT efficiency. The reverse transcription reactions carried out by this kit could be finished in only 15 minutes.

Components

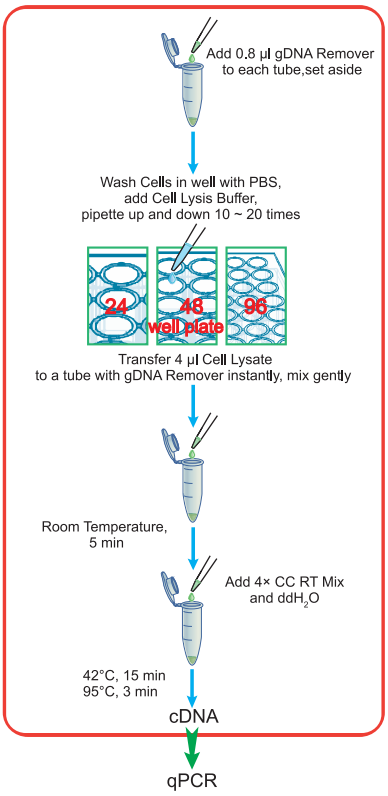
Components	B0003-S (10 Rxns)	B0003 (100 Rxns)
Cell Lysis Buffer	2.2 ml	22 ml
gDNA Remover	10 µl	100 µl
4× CC RT Mix	55 µl	550 µl
RNase free ddH_2O	1 ml	2 ml

Storage

Store at -20°C.

Protocol

Experimental Procedure Overview:



Preparation of cells

Cells are recommended to be passaged in 24, 48, or 96 well cell culture plates, and should have a confluence of more than 50% before use.

Thaw the ***Cell Lysis buffer***, invert or flick the tube several times to **mix thoroughly** (**do not use vortex**), and place the tube at room temperature before use.

Cell Lysis

1. **First, add 0.8 µl gDNA Remover to each tube before starting cell lysis.**

2A. For adherent cells:

a) Aspirate the culture medium from the wells, Wash the wells with PBS (~200 µl/well).

b) Lysis cells in turn:

c) Remove PBS in one well as completely as possible without disturbing the cells. Add Cell Lysis Buffer to this sample with the proportion of 50 µl Cell Lysis Buffer/1×10^5 cells, pipette up and down for 10 times to lyse the cells (be avoid of producing too much bubbles). [Note: Cells should be lysed by pipetting up and down, until no cells residues could be seen at the bottom of the wells]. Transfer 4 µl of the cell lysate to a tube with 0.8 µl gDNA Remover immediately and mix gently, then placed on ice.

d) Treat the remaining sample with the above procedure, one cell sample at a time.

2B. For suspension cells:

a) The cell suspension is collected into a RNase-free centrifuge tube, centrifuged at 500 g for 3 minutes to remove the medium, and then the cells are suspended in PBS and counted.

b) For cells with similar size to B and T cells, aspirate PBS suspension containing 1×10^5 to 3×10^5 into a new 1.5 ml centrifuge tube for each sample, centrifuge cells at 500 g for 3 minutes, then aspirate the PBS carefully without disturbing the cell pellets; then add 10 ~ 30 µl Cell Lysis Buffer to one sample, pipetting up and down for 10 times to lyse the cells.

For cells with similar size to macrophages and CHO cells, aspirate PBS suspension containing 1×10^5 to 3×10^5 into a new 1.5 ml centrifuge tube for each sample, centrifuge cells at 500 g for 3 minutes, aspirate the PBS carefully without disturbing the cell pellets; then add 50 ~ 150 µl Cell Lysis Buffer to one sample, pipetting up and down for 10 times to lyse the cells

c) After cells are lysed thoroughly, transfer 4 µl of the cell lysate to a tube with 0.8 µl gDNA Remover immediately and mix gently, placed on ice.

3. **After all samples completely treated, Incubate at room temperature (19 ~ 27°C) for 5 minutes.**

4. **Put the cell lysates on ice; carry out the RT reactions as soon as possible (no more than 30 min).**

Reverse Transcription

5. **Add 4× CC RT Mix and ddH_2O to the cell lysates:**

Components	Volume (20 µl)
Cell lysate	4.8 µl
4× CC RT Mix	5 µl
RNase free ddH_2O	10.2 µl

6. **Once assembled, mix reactions gently, then centrifuge briefly to collect the contents at the bottom of the reaction tube. Perform the reverse transcription reaction at 42°C for 15 minutes, then with 95°C for 3 minutes.**

Note:

1. Read the manual carefully before use to ensure the experiments are done right.
2. The lysis capacity of 50 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 well cell culture plates with the cell number of not more than 4×10^5 can be used for lysis directly after PBS washing. When doing with cells more than 4×10^5/well (or dish), follow the procedure 2B.
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.

Real-time PCR

The reverse transcription products can be used in PCR reactions immediately without need to dilute, or diluted based on the abundance of gene expression; if not used immediately, it can be stored in aliquots at -80°C for use within 3 month. Avoid repeated freeze-thaw cycles.

Set up the reactions as the following table with using undiluted reverse transcription products:

Components	Volume (20 µl)
2X SYBR Green qPCR Master Mix	10 µl
Template	0.4 µl
Forward primer (10 µM)	0.4 µl
Reverse primer (10 µM)	0.4 µl
ROX Reference Dye*	0.4 µl
ddH_2O	8.4 µl

*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

Cell Name	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 PLUS and TRIzol-reverse transcription method were evaluated by real-time qPCR with 5 pairs of primers (in HEK-293T cells). Highly consistent results between EZ-press Cell to cDNA Kit PLUS and TRIzol -RT method can be observed in the following figure, which indicate that **EZ-press Cell to cDNA Kit PLUS can completely replace TRIzol-RT method in preparing cDNAs from cells.**

Troubleshooting

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 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 10 times. The cells must be completely covered by the Cell Lysis Buffer;
4) Cells should be lysed at room temperature(19 ~ 27°C) for 5 minutes after the addition of gDNA Remover;
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 1×10^5 per 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;
c. If the Ct value is just slightly larger than your expectation, try to transfer 20 ~ 100 µl cell lysate to a new tube, add ethanol to the cell lysate (final ethanol concentration 70%), and centrifuge at 12000 rpm for 2 minutes to precipitate the RNA. Discard the supernatant and air dry for 5 minutes. Dissolve in 4 µl Cell Lysis Buffer and 0.8 µl gDNA Remover, and incubate at room temperature(19 ~ 27°C) for 5 minutes. Add 10.2 µl ddH_2O and 5 µl 4× CC RT Mix, then perform reverse transcription.

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 4 ul cell lysate;
c. Decrease the volume of cDNA used in qPCR, e.g. add 0.4 µl of cDNA to the 20 µl qPCR reactions instead of 0.8 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.