EZ-press SINGLE Cell to cDNA Kit

Catalog No.: B0011

Description

The *EZ*Bioscience® EZ-press SINGLE Cell to cDNA Kit allows researchers to analyze the gene expression of single or very low cell numbers (1 ~ 1,000 cells) in a reliable, sensitive and easy way. Cells are first lysed by the unique Cell Lysis Buffer. Then cDNA is produced and pre-amplified using gene specific primers from the cell lysate in the same tube. Thanks to the pre-amplification following reverse transcription, extremely low concentration of RNA from a very small amount of cells can be detected by the subsequent qPCR.

Components

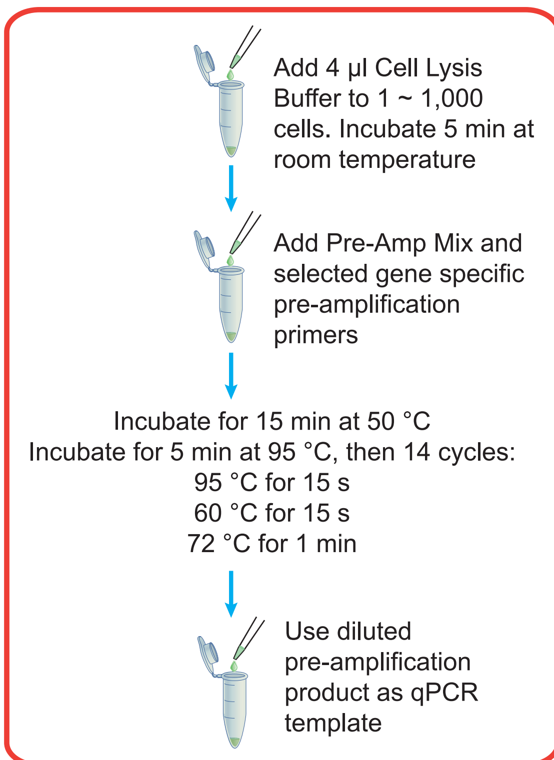
Component	B0011 (100 Rxns)
Cell Lysis Buffer	5.5 ml
2× Pre-Amp Mix	1.1 ml
ddH_2O	1 ml

Storage

Store at -20 °C.

Protocol

Experimental Procedure Overview:



Before starting the experiment:

1. Determine the genes to be analyzed.
2. Design primers of genes to be analyzed for real-time PCR. To get better results, the primers should be near the 3' end of mRNA. It is recommended to include at least one intron in the region to be amplified to avoid the amplification of genome DNA.
3. Design gene specific pre-amplification primers for the genes of interest according to the amplicon of qPCR, which means that the pre-amplification products should contain the qPCR amplicon. The length of pre-amplification products should be between 300~700 bp.

   β-Actin or GAPDH is recommended as the reference gene, while 18S rRNA is not recommended.

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

Single Cell Lysis

Add 4 µl Cell Lysis Buffer to 1 ~ 1,000 cells, incubate at room temperature for 5 minutes to lyse the cells. For cells more than 1000, add 4 µl Cell Lysis Buffer/ 500 cells. (For T / B Cells or other cells with extremely small cell volume, add 4 µl Cell Lysis Buffer to each 1~2000 cells, then incubate at room temperature for 5 minutes to lyse the cells.)

Reverse Transcription and Pre-Amplification

Set up the following mixture in RNase free centrifuge tube:

Component	Volume (20 µl)
Cell Lysate (containing 1 ~ 1,000 cells)	4 µl
2× Pre-Amp Mix	10 µl
Gene specific pre-amplification primers (10 µM)	0.2 µl each (0.1 µM each)
RNase free ddH_2O	to 20 µl

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	Cycles
50 °C	15 min	1
95 °C	5 min	1
95 °C	15 s	14
60 °C	15 s	
72 °C	1 min	

Real-time PCR

Dilute the pre-amplification reaction by 10 times using ddH_2O. The diluted pre-amplification products can be used in qPCR reactions immediately, or stored in aliquots at -80°C. Avoid repeated freeze-thaw cycles.

Set up the reactions as the following table:

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

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

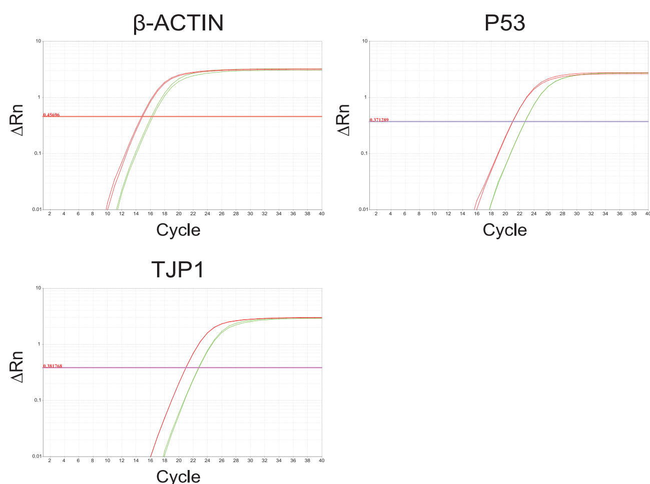
Then set the real-time PCR amplification program according to the product manual of the qPCR Kit, and run the reaction.

Representative experiment results

The gene expression of ten A549 cells was analyzed by *EZ*Bioscience® EZ-press SINGLE Cell to cDNA Kit. The results are compared with the gene expression profile got from purified RNA isolated using 300,000 cells. It can be seen that the results of both methods are highly consistent. So the gene

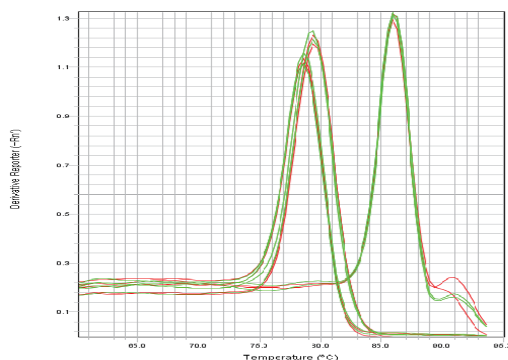
expression can be analyzed precisely using the **EZBioscience**® EZ-press SINGLE Cell to cDNA Kit at extremely high sensitivity.

Amplification Plot



Single Cell to cDNA Kit   TRIzol

Melt Curve



Troubleshooting

1. The qPCR results is not so good , or no amplification can be detected.

a) When harvesting the cells, after washing the cells by PBS and centrifuge, you should aspirate the supernatant very carefully, to avoid of losing the cells.

b) If too many cells per sample are used in the procedure, the RNase in the sample may not be totally inactivated and/or cellular components or debris could inhibit reverse transcription or PCR. Generally 1 ~ 1,000 cells can be used successfully using the Kit, **but if RT or PCR fails, try using fewer cells.**

c) If too much PBS remains in samples when the Cell Lysis Buffer is added, the Cell Lysis Buffer may be too dilute to fully inactivate cellular RNases. To avoid this, remove as much PBS as possible before adding Cell Lysis Buffer to the cells.

d) Do not allow cell lysates to sit longer than 10 min at room temperature once the Cell Lysis Buffer has been added. Then put the cell lysates on ice, and start the Pre-amplification reactions as soon as possible.

e) Check that the qPCR for your target works with your qPCR primers, reagents, and equipment by using cDNA generated from purified RNA from the same source (or a similar one) in qPCR. If the amplification does not give good results using cDNA from purified RNA, it will not work with this Kit.

f) It is important to exclude 18S assays from the preamplification pool, because the 18S rRNA is so highly expressed that its amplification would deplete the PCR reagents and other targets would not be amplified to any significant degree.

g) Make sure that preamplification is for only 14 cycles.

h) Preamplification reaction products must be diluted before using them in qPCR.