Table of Contents

I. Description

This kit is designed to perform cDNA amplification directly from small amounts of cell. The amplified cDNA can be applied for real-time PCR as template. In general, there is a problem in loss of nucleic acids during purification process when dealing with small amounts of nucleic acid. However, by using this kit, cDNA can be amplified efficiently without purifying RNA or cDNA.

In this kit, perform cell lysis at first, followed by synthesize cDNA from mRNA by reverse transcription using dT adapter primer (RT dT Primer). Next, add dA tail by TdT to synthesized cDNA, which will be used as template for low cycle PCR and amplify the cDNA.

This kit is useful to directly amplify cDNA from cells, and also to amplify cDNA from small amount of RNA.

II. Kit Components (for 50 reactions)

1.	Lysis Buffer	350 μl
2.	RNase Inhibitor (40 U / μl)	25 μl
3.	RT dT Primer	20 μl
4.	dNTP Mixture (2.5mM each)	20 μl
5.	RT Enzyme Mix ＊1	50 μl
6.	TdT Buffer	300 μl
7.	dATP (100 mM)	30 μl
8.	TdT Enzyme Mix ＊2	25 μl
9.	PCR Primer Mix	600 μl × 2
10.	RNase Free dH_2O	1 ml × 2

＊1：Contains PrimeScript™ Reverse Transcriptase and RNase Inhibitor

＊2：Contains Terminal Deoxynucleotidyl Transferase and RNase H

III. Reagents and instruments not supplied in this kit

- PCR reagent
  TaKaRa Ex Taq™ Hot Start Version (Cat.# RR006A/B)
- 1.5 ml microtube
- 0.2 ml microtube
- Micropipette and tips
- Thermal Cycler
  TaKaRa PCR Thermal Cycler Dice™ (Cat.#TP600/TP650) etc.

IV. Storage

－20℃

V. Notes

1. To accurately prepare the reaction solution, it is recommended to, first, prepare master mixture for numbers of reaction and several extra, and then dispense this to each reaction tubes.
2. When reaction in each steps are completes, cool down the reaction tubes on ice, spin down briefly, and then move to next step.
3. It is recommended to use 0.2ml tube and Thermal Cycler for reaction.

VI. Protocols

Protocol A. Amplification of cDNA directly from cell

1. Prepare reaction mixture shown in below. Prepare necessary quantities of components other than cell suspension. It is recommended to disperse 12 μl of the master mixture to each 0.2 ml microtube and to add 4 μl of cell suspension.

	Amount
Lysis Buffer	5.6 μl
RNase Inhibitor	0.5 μl
RT dT Primer	0.4 μl
dNTP Mixture	0.4 μl
Cell suspension ＊1	4.0 μl
RNase Free dH_2O	5.1 μl
Total	16 μl

＊1：Amounts of cell suspension to be added should be limited to 4 μl and cell number should be limited to 200 cells.
Cell suspension is cells dispersed in PBS etc., after removed culture medium.

2. Incubate at 75 ℃ for 1 minute and dissolve the cells.

3. Add the following reagents to the tube from step 2. Total amount should be 20 μl.
(Prepare master mixture by using components other than the reaction mixture from step 2 and add each 4 μl to reaction tube.)

	Amount
Reaction mixture of step 2	16 μl
Lysis Buffer	1.4 μl
RT Enzyme Mix	1.0 μl
RNase Free dH_2O	1.6 μl
Total	20 μl

4. Perform cDNA synthesis reaction
42 ℃ for 5 minutes
85 ℃ for 5 seconds

5. Add the following reagents to the tube from step 4. Total amount should be 40 μl.
(Prepare master mixture by using components other than the reaction mixture from step 4 and add 20 μl to the reaction tube)

	Amount
Reaction mixture from step 4	20 μl
TdT Buffer	6.0 μl
dATP	0.6 μl
TdT Enzyme Mix	0.5 μl
RNase Free dH_2O	12.9 μl
Total	40 μl

6. Perform Poly(A) addition reaction.
   37 ℃ for 15 minutes
   70 ℃ for 10 minutes

7. Prepare master mixture by using components other than reaction mixture from step 6 and add 23 μl to new each 0.2 ml microtube. Add 2 μl of the reaction mixture from step 6 to prepare the reaction mixture shown below.

	Amount
Reaction mixture from step 6	2.0 μl
10X *Ex Taq* Buffer ＊2	2.5 μl
dNTP Mixture (2.5mM each) ＊2	2.0 μl
PCR Primer Mix	3.0 μl
TaKaRa Ex Taq™ HS (5 U/ μl) ＊2	0.125 μl
RNase Free dH_2O	15.375 μl
Total	25 μl

＊2：Use *TaKaRa Ex Taq*™ Hot Start Version (Cat.# RR006A).

8. Perform cDNA amplification.

95 ℃	3 minutes	1 cycle
50 ℃	2 minutes	
72 ℃	3 minutes	
95 ℃	30 seconds	20 cycles
67 ℃	1 minute	
72 ℃	3 minutes	
72 ℃	10 minutes	

Dilute cDNA amplification products 1/10 to 1/100 and use them as real-time PCR template. As appropriate amounts of template would differ by expression amount of targeted gene, adjust as needed. If cDNA amplification product is not used immediately, store at − 20 ℃.

【Note】 It is recommended to design primers for real-time PCR within about 1 kb from 3′ end of mRNA, because a reaction using this kit is adjusted to synthesize moderate length of 1st strand cDNA, resulting in equivalent PCR amplification of cDNA products. Therefore, note that PCR amplification in sites distant from 3′ end of mRNA may not be performed efficiently, when using the cDNA products amplified by this kit as PCR template.

Protocol B. Amplification of cDNA from total RNA

1. Prepare reaction mixture shown in below. Prepare necessary amounts of the components other than total RNA. It is recommended to dispense 16 μl to each 0.2 ml microtube and add 4 μl of total RNA.

	Amount
Lysis Buffer	7.0 μl
RT Enzyme Mix	1.0 μl
RT dT Primer	0.4 μl
dNTP Mixture	0.4 μl
total RNA ＊3	4.0 μl
RNase Free dH_2O	7.2 μl
Total	20 μl

＊3：The amount of total RNA should be less than 20 ng.

2. Perform cDNA synthesis reaction.

42 ℃ 5 minutes
85 ℃ 5 seconds

Complete the operation by following the step 5 of the protocol A.

VII. Appendix: Experimental example

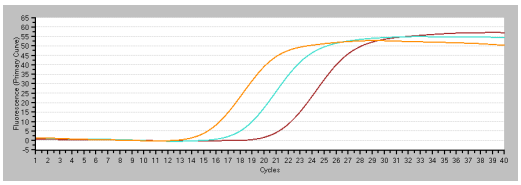
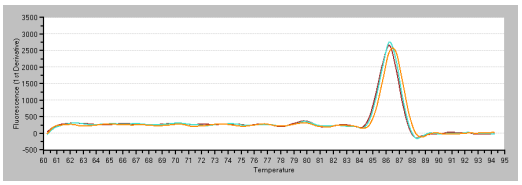
【Procedure】

Following the protocol of this kit, amplification of cDNA from mouse cell (2, 20 or 200 cells) and mouse total RNA (20 pg, 200 pg or 2 ng) were performed. The obtained cDNA amplified products were diluted to 1/10, and 2 μl of those was used as PCR template.

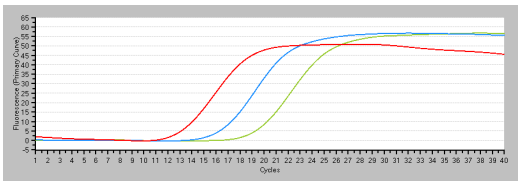
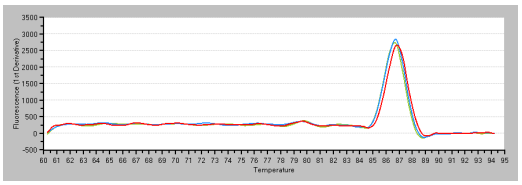
【Result】

The figures (next page) show the result of Mouse Rplp1 which was obtained by performing real-time PCR. The template used for real-time PCR corresponds to mouse cell (8×10^{-4}, 8×10^{-3}, 8×10^{-2} cells) and mouse total RNA (8×10^{-3} pg, 8×10^{-2} pg, 8×10^{-1} pg) when re-calculated to initial template amount. This result shows that efficient amplification of cDNA was performed.

Mouse cell

Total RNA from mouse liver

Instrument：**Thermal Cycler Dice™ Real Time System**
Reagent：**SYBR® *Premix Ex Taq*™ II (Perfect Real Time)**
Target：**Mouse Rplp1**

VIII. Reference

Brady G. and Iscove NN. (1993) Construction of cDNA libraries from single cells. *Methods Enzymol*., **225**, 611-623.

IX. Related Products

SYBR® *Premix Ex Taq*™ II (Perfect Real Time) (Cat.#RR081A/B)
Premix Ex Taq™ (Perfect Real Time) (Cat.#RR039A/B)
TaKaRa Ex Taq™ Hot Start Version (Cat.#RR006A/B)
TaKaRa PCR Thermal Cycler Dice™ (Cat.#TP600/TP650)

Phone: +81-77-543-7247 Fax: +81-77-543-9254