Cat. # 3734

For Research Use

TaKaRa

CellAmp™ Whole Transcriptome Amplification Kit (Real Time) Ver.2

Product Manual

v202201Da

Table of Contents

I. Description

The CellAmp Whole Transcriptome Amplification Kit (Real Time) Ver.2 allows direct amplification of cDNA from a small number of cells. The amplified cDNA can be used as a template for real-time PCR. When using conventional techniques for working with trace amounts of nucleic acids, loss of nucleic acid during purification steps is a frequent problem. However, this kit provides good yield because loss-inducing purification steps for RNA and cDNA are not required.

The procedure begins with cell lysis, followed by a reverse transcription reaction with the dT adapter primer (RT dT Primer 2) to synthesize cDNA from mRNA. Next, Terminal Deoxynucleotidyl Transferase (TdT) is used to add a dA tail to the synthesized cDNA. The resulting cDNA products with 3' dA tails are used as template for PCR, and require fewer cycles for amplification.
In addition to directly amplifying cDNA from cells, this product can be used to amplify cDNA from trace amounts of RNA.

Ver.2 uses an additional Exonuclease I treatment step, which can suppress nonspecific primer-derived amplification and can increase the amplification yield for genes with low expression levels.

II. Components (100 reactions, 5 μl reaction volume)

1.	Lysis Buffer (4X)	150 μl
2.	Recombinant RNase Inhibitor (40 U/μl)	30 μl
3.	RT dT Primer 2	12 μl
4.	dNTP Mixture (2.5 mM each)	12 μl
5.	$MgCl_2$ (22.5 mM)	36 μl
6.	RT Enzyme Mix 2*[1]	36 μl
7.	Exonuclease I (5 U/μl)	72 μl
8.	TdT Buffer (5X)	144 μl
9.	dATP (90 mM)	24 μl
10.	TdT Enzyme Mix*[2]	54 μl
11.	PCR Primer Mix 2	300 μl
12.	RNase Free dH_2O	1 ml

＊1 Contains PrimeScript™ Reverse Transcriptase and RNase Inhibitor.
＊2 Contains Terminal Deoxynucleotidyl Transferase and RNase H.

III. Storage

-20℃

IV. Materials Required but not Provided

- DNA Polymerase
  TaKaRa Ex Taq® Hot Start Version (Cat. #RR006A/B)
- 1.5 ml microtube
- 0.2 ml microtube
- Micropipette and tips
- Phosphate-buffered saline (PBS)
- Thermal cycler
  TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600)*
  TaKaRa PCR Thermal Cycler Dice *Touch* (Cat. #TP350)* etc.

  * Not available in all geographic locations. Check for availability in your area.

V. Precautions Before Use

1. Prepare master mix for the number of reactions plus a few extra, then aliquot into individual PCR tubes.
   When running only a small number of reactions, prepare enough master mix for at least 5 reactions.
2. When each step of the reaction is completed, put the reaction tubes on ice and gently centrifuge before proceeding to the next step.
3. Use 0.2 ml microtubes and a thermal cycler for the reaction.

VI. Protocol

A. Amplifying cDNA directly from cells

1. Prepare the cell suspension, starting with no more than 1,000 cells. Remove the medium from cultured cells and wash with phosphate buffered saline (PBS). Then, resuspend the cells in PBS.

2. Prepare the master mix as shown below (including all components except the cell suspension) for the number of reactions plus a few extra, then aliquot into 0.2 ml PCR tubes. Next, add 0.5 μl of cell suspension to each reaction.

<per reaction>

Reagent	Volume
Lysis Buffer	1.25 μl
Recombinant RNase Inhibitor	0.25 μl
RT dT Primer 2	0.1 μl
dNTP Mixture	0.1 μl
Cell suspension*1	0.5 μl
RNase Free dH_2O	2.8 μl
Total	5 μl

＊1 Do not add more than 0.5 μl of cell suspension, equivalent of up to 1,000 cells.

3. Lyse the cells by incubating at 70℃ for 90 sec

4. Prepare a second master mix containing $MgCl_2$ and RT Enzyme Mix 2, as described below. To each tube from step 3, add 0.6 μl of the second master mix to result in a total volume of 5.6 μl per reaction.

<per reaction>

Reagent	Volume
Reaction from step 3	5 μl
$MgCl_2$	0.3 μl
RT Enzyme Mix 2	0.3 μl
Total	5.6 μl

5. Perform the cDNA synthesis reaction under the following conditions:

42℃ 5 min
85℃ 5 sec

6. Add 0.6 μl of Exonuclease I to result in a total volume of 6.2 μl. Perform the Exonuclease I treatment under the following conditions

   37℃ 15 min
   80℃ 15 min

7. Prepare a third master mix containing TdT Buffer, dATP, TdT Enzyme Mix, and RNase Free dH_2O as described below. Add 6 μl of this master mix to each of the tubes from step 6 to result in a total reaction volume of 12.2 μl.

   <per reaction>

Reagent	Volume
Reaction from step 6	6.2 μl
TdT Buffer	1.2 μl
dATP	0.2 μl
TdT Enzyme Mix	0.45 μl
RNase Free dH_2O	4.15 μl
Total	12.2 μl

8. Perform the poly dA tail addition under the following conditions:

   37℃ 15 min
   70℃ 10 min

9. Prepare a fourth master mix containing 10X *Ex Taq* Buffer, dNTP Mixture, PCR Primer Mix 2, *TaKaRa Ex Taq* HS, and RNase Free dH_2O. Aliquot 22.5 μl of this master mix into a new 0.2 μl tube for each reaction. Add 2.5 μl of the reaction solution from step 8 to result in a total reaction volume of 25 μl.

   <per reaction>

Reagent	Volume
Reaction from step 8	2.5 μl
10X *Ex Taq* Buffer*2	2.5 μl
dNTP Mixture (2.5 mM each)*2	2.5 μl
PCR Primer Mix 2	0.75 μl
TaKaRa Ex Taq HS (5 U/μl)*2	0.25 μl
RNase Free dH_2O	16.5 μl
Total	25 μl

   *2 Use *TaKaRa Ex Taq* Hot Start Version (Cat. #RR006A) (not supplied in this kit).

10. Perform the cDNA amplification under the following conditions:

95℃	1 min	1 cycle
50℃	1 min	
72℃	3 min	
↓		
95℃	30 sec	20 cycles
67℃	1 min	
72℃	3 min	
↓		
72℃	10 min	

Dilute the cDNA amplification product by 1/10 - 1/100, and use as template for real-time PCR. Since the optimal amount of template to use for real-time PCR varies depending on the expression level of the target gene, optimize the template quantity as necessary.
Store the cDNA amplification product at -20℃ if it will not be used immediately.

[Notes for performing real-time PCR using cDNA amplified with this product]
The synthesis of 1st strand cDNA by this kit is designed to facilitate uniform cDNA amplification by PCR when real-time PCR primers correspond to a position within 1 kb of the 3' end of mRNA transcripts. Primers for more distant regions are not recommended.

B. Amplifying cDNA from total RNA

1. Prepare a master mix including the reagents shown below except total RNA. Prepare enough volume for the required number of tubes plus a few extra. Aliquot 5.1 μl master mix into each 0.2 ml microtube, then add 0.5 μl total RNA to each tube for a total volume of 5.6 μl.

<per reaction>

Reagent	Volume
Lysis Buffer	1.25 μl
$MgCl_2$	0.3 μl
RT Enzyme Mix 2	0.3 μl
RT dT Primer 2	0.1 μl
dNTP Mixture	0.1 μl
total RNA*3	0.5 μl
RNase Free dH_2O	3.05 μl
Total	5.6 μl

＊3 Do not use more than 20 ng total RNA.

2. Perform the cDNA synthesis reaction under the following conditions.

42℃ 5 min
85℃ 5 sec

Complete the procedure by following Step 6 through Step 10 of VI. A.

VII. Experimental Examples

1. Amplification of cDNA from trace amounts of total RNA

[Method]

Using either this kit (Cat. #3734, Ver.2 kit) or the CellAmp Whole Transcriptome Amplification Kit (Real Time) (Cat.#3730: discontinued), cDNA was amplified from the total RNA derived from HeLa cells (20 pg, 200 pg, 2 ng, and 20 ng) according to the standard protocol for each kit. 5 μl of each of the cDNA amplification products was subjected to electrophoresis (Figure 1).

In addition, the cDNA amplification products were diluted 10-fold or 40-fold, and 2 μl aliquots of the dilutions were used as a template for real-time PCR (Figure 2). All of the real-time PCR experiments utilized TB Green® *Premix Ex Taq*™ II (Perfect Real Time). The volume of each real-time PCR reaction was 25 μl.

[Result]

(1) Figure 1 shows the results of the electrophoresis of the cDNA amplification products. Smeared bands were observed with CellAmp Whole Transcriptome Amplification Kit (Real Time), even for the no template control; these are believed to represent nonspecific amplification products derived from the primers. On the other hand, the band density with the Ver.2 kit correlated with the the amount of template present, and indicates the presence of target cDNA amplification products.

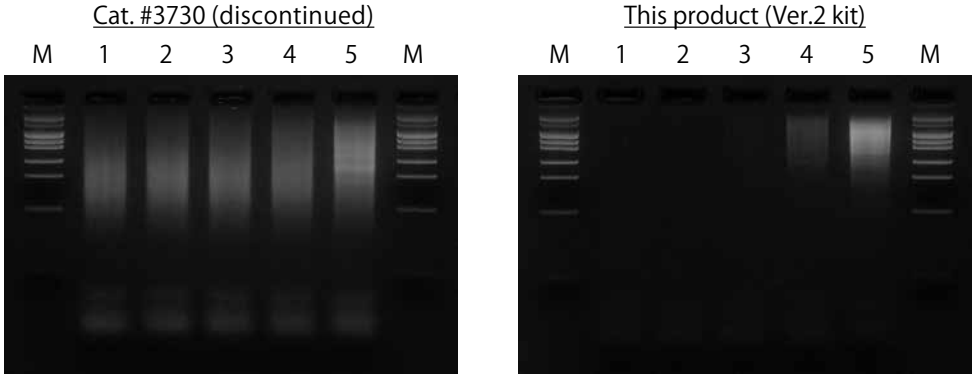
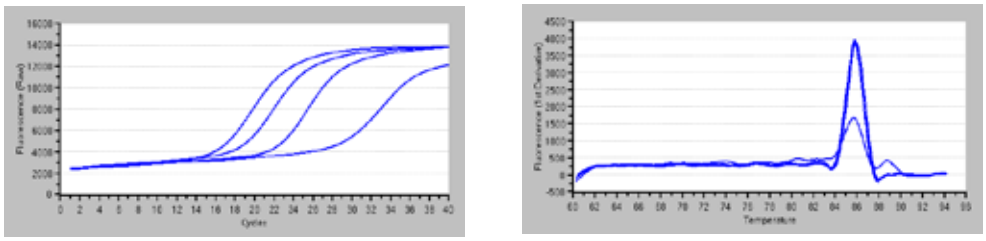


Figure 1. Comparison of cDNA amplification with varying amounts of template. 5 μl of the cDNA amplification product was run on a 3% Agarose gel.

Lane 1: No template control
2: HeLa total RNA 20 pg
3: HeLa total RNA 200 pg
4: HeLa total RNA 2 ng
5: HeLa total RNA 20 ng
M: pHY Marker 200 ng

(2) Figure 2A - C shows the detection of human *GAPDH* using real-time PCR. When 10-fold dilutions of each of the cDNAs were used as a template (Figure 2A), the background was observed to be somewhat increased with CellAmp Whole Transcriptome Amplification Kit (Real Time), but target gene detection with a lower background was possible with the Ver.2 kit. When the cDNA was diluted 40-fold, the target gene was readily detected even with CellAmp Whole Transcriptome Amplification Kit (Real Time) (Figure 2B). Comparison of the Ct values shown in Figure 2C indicate that the Ver.2 kit provides superior amplification efficiency.

CellAmp Whole Transcriptome Amplification Kit (Real Time)



CellAmp Whole Transcriptome Amplification Kit (Real Time), Ver.2, Cat. #3734

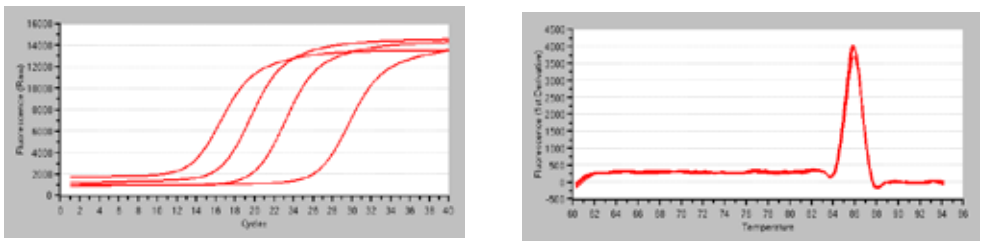
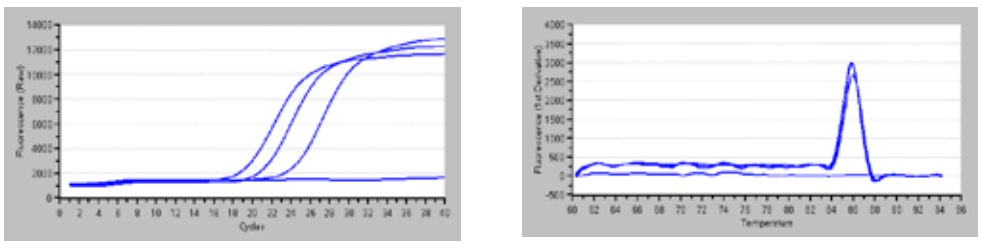


Figure 2A. Use of the 10-fold diluted cDNA amplification product as template

CellAmp Whole Transcriptome Amplification Kit (Real Time)



CellAmp Whole Transcriptome Amplification Kit (Real Time), Ver.2, Cat. #3734

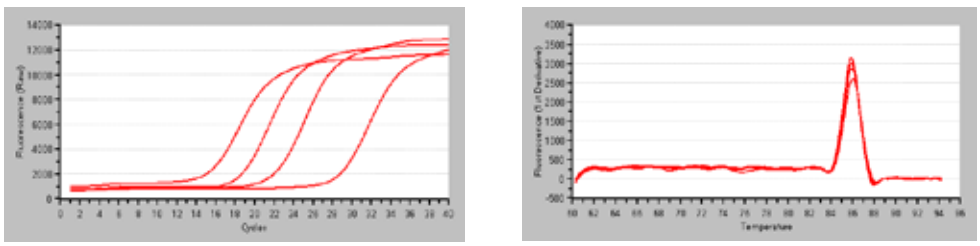
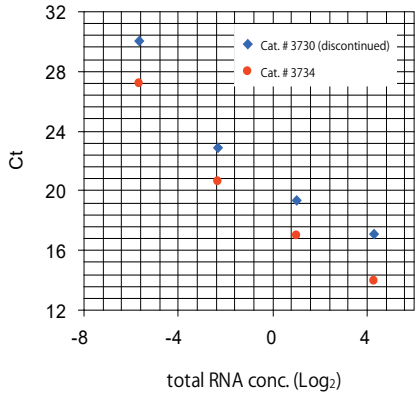


Figure 2B. Use of the 40-fold diluted cDNA amplification product as template



Instrument: Thermal Cycler Dice Real Time System
Reagent: TB Green *Premix Ex Taq* II (Perfect Real Time)
Target: Human *GAPDH*

Figure 2C. Comparison of Ct values (10-fold diluted cDNA)

2. Amplification of cDNA from cells

[Method]

Mouse 3T3 cells were suspended in PBS to a concentration of 2 x 10^6 cells/ml. A serial dilution was prepared (final dilution 2 x 10^3 cells/ml), and 0.5 µl aliquots of the dilutions (corresponding to 1, 10, 100, and 1,000 cells) were used with this kit for cDNA amplification.

The resulting cDNA amplification product was diluted 10-fold, and 2 µl aliquots of the dilution were used as template for real-time PCR.

[Results]

Figure 3 shows the detection of 3 gene targets at varying expression levels using real-time PCR. For *GAPDH*, Ct correlated with cell number in the range of 1 - 1,000 cells. For genes expressed at lower levels (*Ywhaz* and *Tfrc*), *Ywhaz* could be detected within the range of 10 - 1,000 cells, and *Tfrc* from 100 - 1,000 cells.

The cell number that could used as starting material varied according to the cell type.

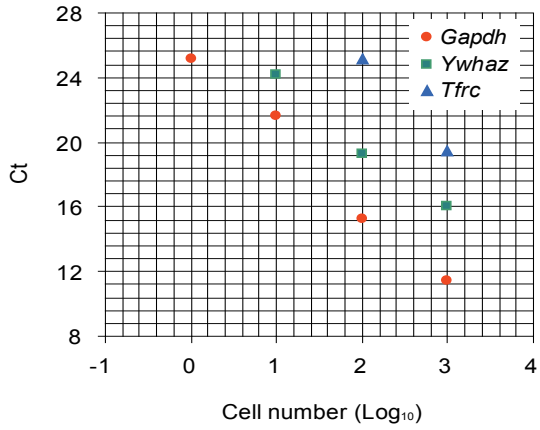


Figure 3. Real-time PCR analysis expression level correlates with starting cell number

Instrument: Thermal Cycler Dice Real Time System
Reagent: TB Green *Premix Ex Taq* II (Perfect Real Time)
Target: Mouse *GAPDH*, *Ywhaz*, or *Tfrc*

3. Amplification of cDNA from single cells

[Method]

Lysis Buffer was added to 0.2 ml microtubes, which were then put on ice.
8 individual HeLa cells were isolated with a capillary pipette, and each cell was transferred into an aliquot of Lysis Buffer in a tube. cDNA amplification was then carried out using the kit, and the resulting cDNA amplification product was diluted 10-fold.
2 μl aliquots of the dilution were used as template for real-time PCR.

[Results]

Figure 4 shows the detection of 3 different target genes by real-time PCR.

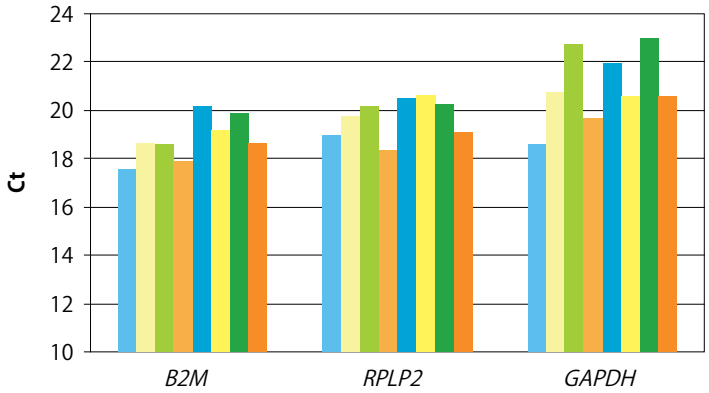


Figure 4. cDNA amplification (n=8) from single cells

Instrument: Thermal Cycler Dice Real Time System
Reagent: TB Green *Premix Ex Taq* II (Perfect Real Time)
Target: Human *B2M*, *RPLP2*, *GAPDH*

All of the target genes were detected. There was some variance in the Ct values for each gene, but correlations were observed across the 3 different target genes. The result may reflect differences in the content of each mRNA in the individual cells.

VIII. References

1) Kurimoto K, *et al*. Global single-cell cDNA amplification to provide a template for representative high-density oligonucleotide microarray analysis. Kurimoto K, Yabuta Y, Ohinata Y, Saitou M. *Nature Protocol*. (2007) **2**(3): 739-752.
2) Brady G and Iscove NN. Construction of cDNA libraries from single cells. *Methods Enzymol*. (1993) **225**: 611-623.

IX. Related Products

TB Green® *Premix Ex Taq*™ II (Tli RNase H Plus) (Cat. #RR820A/B)
Probe qPCR Mix (Cat. #RR391A/B)
Thermal Cycler Dice™ Real Time System III (Cat. #TP950/TP970/TP980/TP990)*
TaKaRa Ex Taq® Hot Start Version (Cat. #RR006A/B)
TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600)*
TaKaRa PCR Thermal Cycler Dice™ *Touch* (Cat. #TP350)*

* Not available in all geographic regions. Check for availability in your area.