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AmpEasy™ Cells cDNA Kit

Description

AmpEasy™ Cells cDNA Kit is designed for high-speed preparation of first-strand cDNA directly from cultured cells without RNA purification. By using AmpEasy™ Cells cDNA Kit, RNA in the cell lysate can be directly converted to cDNA. The cDNA synthesized accurately represents the cellular gene expression profile, making it highly suited for use in real-time RT-PCR. The whole process, from cells to cDNA, can be completed in less than 30 minutes. When AmpEasy™ Cells cDNA Kit is used together with optimized PairFast™ Real-Time PCR Mastermix for real-time PCR, several cell samples can be easily processed and analyzed within a few hours. This allows analysis of a large number of differentially treated cultures much faster and simpler.

Features

- High-speed preparation of first-strand cDNA directly from cultured cells without RNA purification.
- No RNA purification is required, minimizing pipetting tasks and saving plenty of time.
- Just less than 30 minutes, from cells to cDNA can be completed.
- Sensitive in detecting low-abundance transcripts.
- Highest cDNA yields of specific and long cDNA synthesis (~2.1KB).

Applications

- Ideal for performing reverse transcription reactions on small number of cells.
- Ideal for analysis of large numbers of differentially treated cultures.
- RT-PCR, primer extension, and 3' and 5' RACE
- Gene-expression analysis

Quality Control

Specificity and reproducibility of AmpEasy™ Cells cDNA Kits are tested in reproducibility assay: parallel 20µl reactions containing 2ul of human total RNA from embryonic kidney cell lysate and 0.5 µM primers, specific for d(T)18. After 35 cycles, B2M transcript is detected. The length of cDNA achieved is verified as 248 bp by electrophoresis and DNA sequencing.

Shipping and Storage Conditions

AmpEasy™ Cells cDNA Kit is shipped on dry ice. Buffer W should be stored at room temperature (15–25℃). All other components of AmpEasy ™ Cells cDNA Kit should be stored immediately upon receipt at -20℃ in a constant temperature freezer. With proper storage, AmpEasy™ Cells cDNA Kit can be stored for up to 12 months without showing any deduction in performance and quality.

Product Components and Storage Conditions

Cat. No.:	Storage Temperature	RCC050	RCC100
Product Name:		AmpEasy™ Cells cDNA Kit	
Size (20 µl/reaction):		50 preps	100 preps
Buffer Set (part 1 of 2):			
Buffer W	Room temperature	25 ml	50 ml
Buffer C	-20℃	2.5 ml	5 ml
Reverse-Transcription Set (part 2 of 2):			
RealScript™ Reverse Transcriptase#	-20℃	100 ul	200 ul
2X First-Strand Reaction Mix*	-20℃	0.5 ml	1 ml
Oligo (dT) primer (10 µM)	-20℃	50 ul	100 ul
RNase-Free Water	-20℃	1 ml	1 ml

Contains RNase inhibitor.

* Includes Mg^{2+} and dNTPs.

Principle and procedure

AmpEasy™ Cells cDNA Kit procedure comprises only 3 steps: cell wash, cell lysis, and Reverse Transcription.

Cell Wash

Cultured cells are briefly washed with Buffer W to remove cell-culture medium, extracellular material released by living cells, and intracellular material released by any dead, lysed cells. Removal of extracellular contaminants is important, since they can interfere with reverse transcription and subsequent quantification by real-time RT-PCR.

Cell Lysis

After the wash with Buffer W, the cultured cells are then lysed for 5 minutes using Buffer C. This buffer also stabilizes the cellular RNA and blocks inhibitors of reverse transcription. This allows efficient synthesis of first-strand cDNA from RNA that accurately reflects the in vivo gene expression profile. When human cultured cells are analyzed, the optimal cell concentration is $5x10^4$ cells (50 µl of Buffer C is required).

Reverse Transcription

After cell lysis, the cell lysate is ready for reverse transcription using Reverse-Transcription Set included in the kit. The RT reaction takes place at 42℃ and is then inactivated at 95℃. No additional steps for RNA denaturation, primer annealing, and RNase H digestion are required in the procedure. The cDNA produced is ready to be used in the subsequent PCR.

Protocol

Important notes before starting:

1. Optimal cell concentration is 5x10^4 cells. Suggested concentration is 1x 10^4 cells to 1x 10^5 cells.
2. If the expected PCR product size is larger than 500 bp, it is recommended to include 0.5 µl RNase inhibitor (40 unit/µl) in 50 µl of the Buffer C. (Refer to Cat. No. YRI001 RNAarmor™ RNase Inhibitor)
3. Do not add RNase inhibitor and dNTPs to the reverse-transcription reaction. RNase inhibitor and dNTPs are included in RealScript™ Reverse Transcriptase and 2X First-Strand Reaction Mix respectively.
4. Additional items to be prepared by the users: 1.5ml RNase-free plastic tubes, ice, heating block or water bath (capable of reaching 95℃), vortexer and micro centrifuge.

Step 1: Cell Wash

1. Transfer the cells (5 x 10^4) to a 1.5 ml microcentrifuge tube and harvest by centrifugation for 5 minutes at 4,000 rpm at room temperature.
2. Remove the supernatant completely and resuspend the cells in 500 µl of Buffer W.
3. Centrifugation for 5 minutes at 4,000 rpm at room temperature.

Step 2: Cell Lysis

1. Remove the supernatant completely and resuspend the cells in 50 µl of Buffer C.
2. Vortex 5 seconds and incubate at room temperature for 5 minutes.

Step 3: Reverse Transcription

1. Add the following components to a sterile microtube on ice:

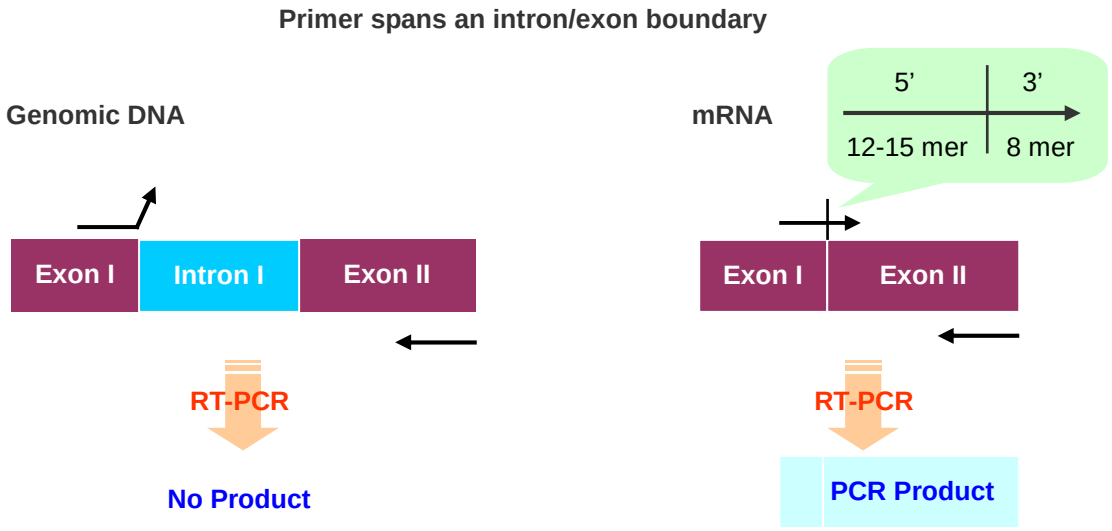
Component	Volume/ Reaction
Reverse-Transcription Set	
RealScript™ Reverse Transcriptase	2 µl
2X First-Strand Reaction Mix	10 µl
Oligo (dT) primer (10 µM)	1 µl
RNase-Free Water	5 µl
Template RNA	
Cell Lysate	2 µl
Total Volume	**20 µl**

2. Reverse Transcription: Incubate the mircrotube at 42℃ for 5 minutes.
3. Inactivation: Incubate the mircrotube at 95℃ for 10 minutes.
4. Store reverse-transcription reactions on ice and proceed directly with real-time PCR. For long-term storage, store reverse-transcription reactions at –20℃.

Appendix

Primer Design

Since removal of genomic DNA is not included in AmpEasy™ Cells cDNA Kit, genomic DNA will be amplified if inappropriate primer or probe is used. Detection of contaminating genomic DNA can be eliminated with specially designed primers as below.



Real-Time PCR

In quantitative, real-time, two-step RT-PCR, cDNA is first synthesized by reverse transcription. An aliquot of the finished reverse-transcription reaction is then used for PCR. Reverse transcription and PCR are performed sequentially in 2 separate reaction tubes.

1. Once reverse transcription is done, add an aliquot of each finished reverse-transcription reaction to real-time PCR mix. (Note: No more than 1/10 of the final PCR volume should derive from the finished reverse-transcription reaction. For example, for a 20 µl PCR assay, use ≦2 µl of the finished reverse-transcription reaction.)
2. Carry out real-time PCR as recommended by the supplier. We recommend using following kits:

RealSens™ Real-Time PCR Mastermix (For SYBR Green System), Cat. No. RT301/RT302/RT303.
RealSens™ Real-Time PCR Mastermix (For SYBR Green System w/ ROX), Cat. No. RT401/RT402.
RealSens™ Real-Time PCR Mastermix (For Probe System), Cat. No. RT501/RT502/RT503.
RealSens™ Real-Time PCR Mastermix (For Probe System w/ ROX), Cat. No. RT601/RT602/RT603.
PairFast™ Real-Time PCR Mastermix (For SYBR Green System, w/ ROX), Cat. No. RT701/RT702.
PairFast™ Real-Time PCR Mastermix (For Probe System, w/ ROX), Cat. No. RT801/RT802/RT803.

PCR

An aliquot of the finished reverse-transcription reaction is ready to be analyzed by performing PCR.

1. Once reverse transcription is done, add an aliquot of each finished reverse-transcription reaction to PCR mix. (Note: No more than 1/10 of the final PCR volume should derive from the finished reverse-transcription reaction. For example, for a 20 µl PCR assay, use ≦2 µl of the finished reverse-transcription reaction.)
2. Carry out PCR as recommended by the supplier. We recommend using following kits:
   RealSens™ HotStart DNA Polymerase Mastermix (2X), Cat. No. RT101(w/o dye)/ RTD101(w/ dye).
   RealSens™ HotStart DNA Polymerase Mastermix (5X), Cat. No. RT102(w/o dye)/ RTD102(w/ dye).

Troubleshooting Guide

No PCR product or unexpected PCR products (problems occurring during reverse transcription)
1. Inappropriate cell numbers used
Try different numbers of cells per well/tube. Carry out the AmpEasy™ Cells cDNA Kit procedure and determine which cell number gives optimal PCR results. Please be cautious that RNase in the sample might not be completely inactivated when high numbers of cells are lysed.
2. Cells not washed with Buffer C or Cells treated with incorrect volume of Buffer C
Cell lysate may contain inhibitors of RT-PCR. Be sure to remove intracellular and extracellular contaminants by using Buffer C. When human cultured cells are analyzed, the optimal cell concentration is $5x10^4$ cells. 50 µl of Buffer C is required for $5x10^4$ cells.
3. Incorrect setup of reverse transcription reaction
Be sure to set up the reaction on ice.
4. High volume of reverse transcription reaction is added to PCR or real-time PCR
To prevent the reduction amplification efficiency and the linearity of the reaction, the volume of reverse transcription reaction added should not exceed 10% of the final PCR volume to prevent the reduction in PCR efficiency.
5. Inappropriate temperature of reverse transcription reaction
Reverse transcription should be carried out at 42℃ . The temperature range of 42-50℃ is acceptable if different temperature is required. Temperatures over 50℃ is not recommended as it might reduce the activity of RealScript™ Reverse Transcriptase and therefore affect the cDNA yield.
6. RNA denaturation
Denaturation of the template RNA is not necessary. If denaturation was performed, the integrity of the RNA may be affected.
No PCR product or unexpected PCR products (problems occurring during PCR
Refer to the instructions for the PCR or real-time PCR kit you are using.