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AmpEasy™ Direct RT-PCR Kit

Description

AmpEasy™ Direct RT-PCR Kit is designed for high-speed preparation of first-strand cDNA directly from cultured cells without RNA purification. By using AmpEasy™ Direct RT-PCR Kit, RNA in the cell lysate can be directly converted to cDNA and subsequently analyzed using the PCR reagents included in the kit. The whole process, from cells to cDNA and from cDNA to PCR, can be completed in less than 1.5 hours. AmpEasy™ Direct RT-PCR Kit accelerates and streamlines real-time PCR analysis of cultured cells. 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.
- Whole process including PCR can be completed in less than 1.5 hours.
- Immediate startup using optimized reagents and protocols.
- Sensitive in detecting low-abundance transcripts.
- Highest cDNA yields of specific and long cDNA synthesis (~2.1KB).

Applications

- Ideal for performing reverse transcription reactions and PCR on small number of cells.
- Ideal for analysis of large numbers of differentially treated cultures.
- Gene-expression analysis

Quality Control

Specificity and reproducibility of AmpEasy™ Direct RT-PCR 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™ Direct RT-PCR Kit is shipped on dry ice. Buffer W should be stored at room temperature (15–25℃). All other components of AmpEasy™ Direct RT-PCR Kit should be stored immediately upon receipt at -20℃ in a constant temperature freezer and protected from light. Avoid repeated freeze–thaw cycles. With proper storage and handled correctly, AmpEasy™ Direct RT-PCR 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 Temp.	DRP050	DRP100
Product Name:		AmpEasy™ Direct RT-PCR Kit	
Size:		50 preps	100 preps
Buffer Set (part 1 of 3):			
Buffer W	Room Temp.	25 ml	50 ml
Buffer C	-20℃	2.5 ml	5 ml
Reverse-Transcription Set (part 2 of 3):			
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
PCR Set (part 3 of 3) :			
2X RealSens HotStart DNA Polymerase Mastermix	-20℃	1.25 ml	2.5 ml

Contains RNase inhibitor.

* Includes Mg^{2+} and dNTPs.

Principle and procedure

AmpEasy™ Direct RT-PCR Kit procedure comprises only 3 steps: cell wash, cell lysis, and two-step RT-PCR.

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 RT and subsequent PCR.

Cell Lysis

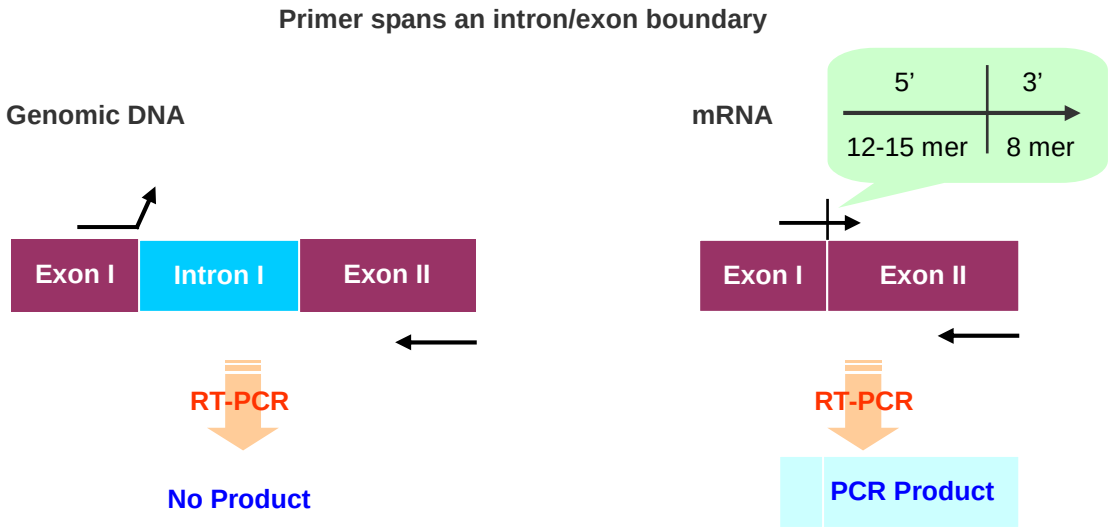
After the wash with Buffer W, the cultured cells are then lysed for 5 minutes using Buffer C. This buffer also stabilizes the cellular RNA and blocks inhibitors of reverse transcription. This allows efficient synthesis of first-strand cDNA from RNA that accurately reflects the in vivo gene expression 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.

Primer Design

Since removal of genomic DNA is not included in AmpEasy™ Direct RT-PCR 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.



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.
5. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

Step 1: Cell Wash

1. Transfer the cells (5×10^4) 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: Two-Step RT-PCR

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 PCR. For long-term storage, store reverse-transcription reactions at –20℃.

PCR

Our protocol is for a reaction size of 25ul. This protocol serves only as a guideline for PCR amplification. Optional reaction conditions may vary and must be individual determined.

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

Component	Volume/ Reaction	Final Concentration
RealSens HotStart DNA Polymerase Mastermix	12.5 µl	1X
Forward Primer (10µM)	0.5 µl	0.2µM
Reversed Primer (10µM)	0.5 µl	0.2µM
Sterilized ddH_2O	Add to 23.0µl	

2. Mix above components thoroughly by pipetting up and down and dispense the 23µl of mixture into PCR tubes or plates.
3. Add 2µl of the cDNA and mix carefully by pipetting up and down.

4. Suggested reaction parameters are as below.

Segment	Number of Cycles	Temperature	Time
1	1	95℃	10 minutes
2	40	95℃	30 seconds
		50~68℃ #	30 seconds
		72 ℃ *	30 seconds
3	1	72 ℃	7 minutes
4	1	4 ℃	∞

Optimal annealing temperature is depending on user's primer sequences.

* For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.

5. Place the PCR tubes or PCR plates in the thermal cycle and start the RT-PCR program.

Troubleshooting Guide

1. Inappropriate cell numbers used
Try different numbers of cells per well/tube. Carry out the AmpEasy™ Direct RT-PCR 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.