Cat. # R022A

For Research Use

TaKaRa

PrimeScript™ High Fidelity RT-PCR Kit

Product Manual

v1111Da

Table of Contents

I. Description

Polymerase chain reaction (PCR) amplifies specific DNA sequences using a pair of primers flanking the target DNA region. In principle, PCR cannot use RNA as a direct template. Nevertheless, it may be used to analyze RNA by synthesizing cDNA from RNA with reverse transcriptase followed by PCR amplification of the target region (RT-PCR). RT-PCR is used in many applications, including RNA structural analysis, high-efficiency cDNA cloning, and expression analysis at the RNA level.

The PrimeScript High Fidelity RT-PCR Kit is designed to synthesize and amplify cDNA from total RNA or mRNA with high fidelity.

This kit includes PrimeScript reverse transcriptase (RTase), developed by Takara Bio based on an M-MlV reverse transcriptase, and PrimeSTAR Max DNA Polymerase, which has the highest fidelity of any commercially available PCR enzyme. PrimeSTAR Max DNA Polymerase is formulated as a premix and offers high amplification efficiency. It also is compatible with a broad range of template quantity. Therefore, PrimeSTAR Max DNA Polymerase is exceptionally well suited for cDNA cloning studies that require high fidelity.

A 2 step RT-PCR protocol described in this manual is the standard protocol for this kit. A 2 step RT-PCR protocol has the following advantages:

- Efficient yield of RT-PCR amplification products while maintaining high fidelity
- Excellent extension, even with template RNAs likely to assume higher-order structures at the standard temperature for reverse transcription (42℃)
- Very broad tolerance for the amount of total RNA that may be used in the reaction, making the kit easy to use

This kit includes all reagents necessary for reverse transcription of RNA to cDNA followed by PCR amplification of cDNA.

In addition, using an optional protocol, this kit may also be used in 1 step RT-PCR.

II. Components (for 50 reactions)*1

(1)	PrimeScript RTase (for 2 step)	25 μl
(2)	5X PrimeScript Buffer	200 μl
(3)	RNase Inhibitor (40 U/μl)	25 μl
(4)	dNTP Mixture (10 mM each)	50 μl
(5)	Oligo dT Primer (2.5 μM)	50 μl
(6)	Random 6-mers (20 μM)	50 μl
(7)	PrimeSTAR Max Premix (2X)	625 μl x 2
(8)	Control F-1 Primer*2 (20 μM)	10 μl
(9)	Control R-1 Primer*3 (20 μM)	10 μl
(10)	Positive Control RNA (2 x 10^5 copies/μl)	20 μl
(11)	RNase Free dH_2O	1 ml

*1: For 50 reactions of 2 step RT-PCRs (20 μl RT reaction volume → 50 μl PCR volume)
*2: Upstream sense primer for the Positive Control RNA
*3: Downstream antisense primer for the Positive Control RNA

[Primer Sequences]

Primer	Sequence
Random 6 mers	pd $(N)_6$
Oligo dT Primer	dT-rich sequence, originally designed by Takara Bio
Control F-1 Primer	5'-CTGCTCGCTTCGCTACTTGGA-3'
Control R-1 Primer	5'-CGGCACCTGTCCTACGAGTTG-3'

[Positive Control RNA]
The Control RNA included in this kit is synthesized by *in vitro* transcription with SP6 RNA polymerase from a pSPTet3 plasmid template. The plasmid includes an approximately 1.4 kb DNA fragment encoding a pBR322-derived tetracycline-resistant gene. The tetracycline resistance cassette is inserted downstream from the SP6 promoter.

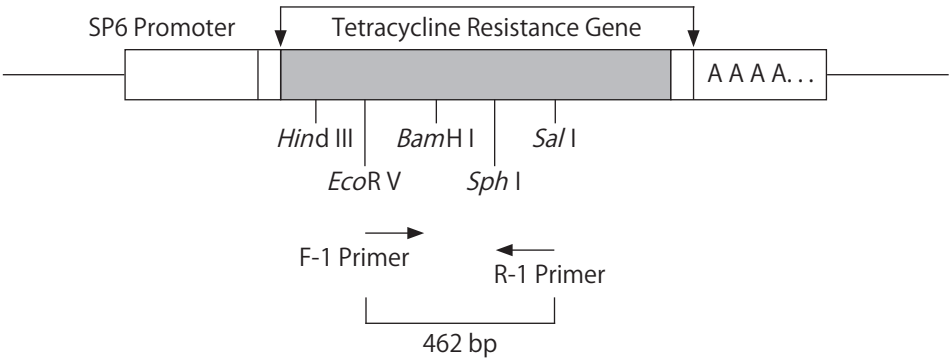


Figure 1. Control RNA: The amplification fragment generated using the control primers

III. Materials Required but not Provided

- Thermal cycler (authorized for use)
  e.g, TaKaRa PCR Thermal Cycler Dice Gradient (Cat. #TP600)
  (not available in all geographic regions)
- Agarose gel
  e.g., Agarose L03 (Cat. #5003/5003B)
- Electrophoresis apparatus
  e.g., Mupid®-2plus (Cat. #AD110) or Mupid®-exU (Cat. #AD140)
- Microcentrifuge
- Micropipettes and tips (autoclaved)

IV. Storage

-20℃

Note : No decrease in activity of PrimeSTAR Max Premix (2X) has been shown over 25 freeze-thaw cycles. Nevertheless, take care to avoid excessive freeze-thaw cycles.

V. Fidelity of PrimeSTAR Max DNA Polymerase

Using GC-rich *Thermus thermophilus* HB8 genomic DNA as template, eight randomly selected regions (amplification size of approximately 500 bp each) were amplified by PCR using the DNA polymerases indicated below and cloned into a vector. Multiple colonies were selected for each target, and the amplified regions were sequenced. Sequence data were analyzed to determine the mutation frequency. The results show that the fidelity of PrimeSTAR Max DNA Polymerase is 10-fold higher than *Taq* DNA Polymerase and equivalent to or higher than that of PrimeSTAR HS DNA Polymerase or company A' s high-fidelity enzyme. This protocol offers precise fidelity. PrimeSTAR Max DNA Polymerase can be relied on for PCR when fidelity is critical.

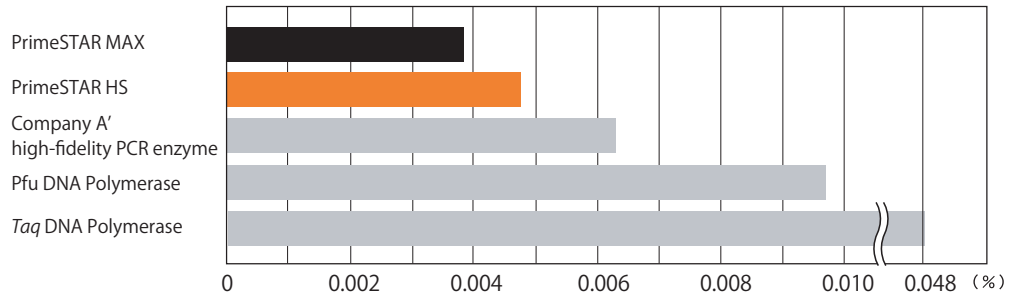


Figure 2. Fidelity comparison of various enzymes
With amplification by PrimeSTAR Max DNA Polymerase, only 9 errors occurred in 230,129 bases analyzed.

VI. RNA Sample Preparation

This kit is designed to perform cDNA synthesis from RNA template, followed by cDNA amplification. Highly pure RNA samples are essential to the success of cDNA synthesis. In addition, it is critical to inhibit the action of intracellular RNase and to prevent RNase contamination.

When preparing RNA samples, avoid RNase contamination from perspiration or saliva. Wear clean disposable gloves. Designate a laboratory bench exclusively for preparing RNA samples.

Use disposable plastic equipment. All glassware should be treated as follows:

(1) Treat with 0.1% diethyl pyrocarbonate (DEPC) at 37℃ for 12 hours
(2) Autoclave at 120℃ for 30 min to remove the residual DEPC.
The use of RNase-OFF (RNase decontamination solution) (Cat. #9037) is recommended for removal of RNase on laboratory benches, apparatuses, and tubes. Use only materials (plastic or glass) designated exclusively for RNA experiments.

[Preparation of RNA]

When preparing highly purified total RNA samples from cultured cells or tissue samples, it is convenient to use NucleoSpin® RNA II (Clontech Cat. #740955.10/.50/.250).
When preparing mRNA from total RNA, *Oligotex™-dT30 <Super>* (Cat. #W9021) or *Oligotex™-dT30 <Super>* mRNA Purification Kit (From Total RNA) (Cat. #9086) allows rapid and efficient recovery of mRNA. (Cat. #W9021 and Cat. #9086 are not sold in all geographic regions. Check for availability.)

VII. Principle

The standard protocol for the PrimeScript High Fidelity RT-PCR Kit (2 step RT-PCR) comprises two steps:

1) Synthesis of cDNA from RNA by PrimeScript RTase
2) PCR amplification of cDNA by PrimeSTAR Max DNA Polymerase using an aliquot of the RT reaction mixture as the template

Primers that can be used for synthesis of cDNA from RNA include Random 6 mers, Oligo dT Primer, or a target-specific primer.

When conducting 1 step RT-PCR according to the optional protocol, synthesize cDNA from RNA with a reaction mixture containing both PrimeScript RTase and PrimeSTAR Max DNA Polymerase, followed by PCR amplification.
In 1 step RT-PCR, use the antisense PCR primer for synthesizing cDNA from RNA.

VIII. Features

■ 2 step RT-PCR (Standard Protocol)

RNA template	General
Amplified product length	Excellent amplification of 6 kb products
Reverse transcriptase	PrimeScript RTase (optimal temperature: 42℃)
DNA Polymerase	PrimeSTAR Max DNA Polymerase (2X premix)
RNase Inhibitor	Required (supplied in this kit)
Primer for 1st strand cDNA synthesis	Random 6 mers, Oligo dT Primer, or specific downstream primer of a target gene

■ 1 step RT-PCR (Optional Protocol)

RNA template	General
Product length	Successful amplification of 6 kb product
Reverse transcriptase	PrimeScript RTase (use at 50℃)
DNA Polymerase	PrimeSTAR Max DNA Polymerase (2X premix)
RNase Inhibitor	Required (supplied in this kit)
Primer for 1st strand cDNA synthesis	Specific downstream primer of a target gene. Not compatible with Random Primer or Oligo dT Primer.
Protocol	Perform RT and PCR sequentially in 1 tube

IX. Precautions

This section describes precautions for using this kit. Read them before use.

(1) For convenience, prepare master mixes of reaction mixture sufficient for up to 10 reactions. Preparing master mixes reduces pipetting losses and facilitates accurate reagent dispensing, which minimizes data variations between experiments.

(2) Centrifuge the PrimeScript RTase, RNase Inhibitor, or PrimeSTAR Max Premix (2X) briefly to collect the liquid at the bottom of the tube. Before pipetting each reagent, mix gently without introducing bubbles. These enzymes and the inhibitor are provided in 50% glycerol solution, and are highly viscous. Slow and careful pipetting is required.

(3) Keep enzymes and the inhibitor at -20℃ until just before use. Return to -20℃ storage immediately after use.

(4) Avoid freezing and thawing the positive control RNA as much as possible to prevent degradation. Dispensing the positive control RNA into small aliquots for storage is recommended. Store positive control RNA aliquots at -70℃ to -80℃.

(5) Use fresh disposable tips when dispensing reagents to minimize the risk of cross-contamination between samples.

[Primer Selection for 2 step RT-PCR]

For the primer in reverse transcription, you may select from Random 6 mers, Oligo dT Primer, or a specific downstream primer of a target DNA depending on the experimental purposes. Any of these three selections are suitable for short mRNAs without a hairpin structure. For selection criteria, see below.

Oligo dT Primer

Suitable only for reverse transcription of mRNAs with poly (A) tails.
(Note: Prokaryotic RNAs, eukaryotic rRNAs and tRNAs, and some eukaryotic mRNAs lack poly (A) tails.)

Random 6 mers

Best used for reverse transcription of longer RNAs and RNAs with a hairpin structure. Random 6 mers may also be used to reverse transcribe all classes of RNAs, including rRNA, mRNA, and tRNA.

Specific downstream primer

Since it is necessary to synthesize oligonucleotides having a sequence complementary to the template, knowledge of the target sequence is required.

In 1 step RT-PCR (optional protocol), the specific downstream primer (the antisense primer in PCR) alone may be used. Note that 1 step RT-PCR is not compatible with Oligo dT Primer or Random 6 mers.

X. Protocol (2 step RT-PCR)

The recommended standard protocol is 2 step RT-PCR.
This section provides procedures for 2 step RT-PCR. For 1 step RT-PCR, see section XII. Optional Protocol (1 step RT-PCR).

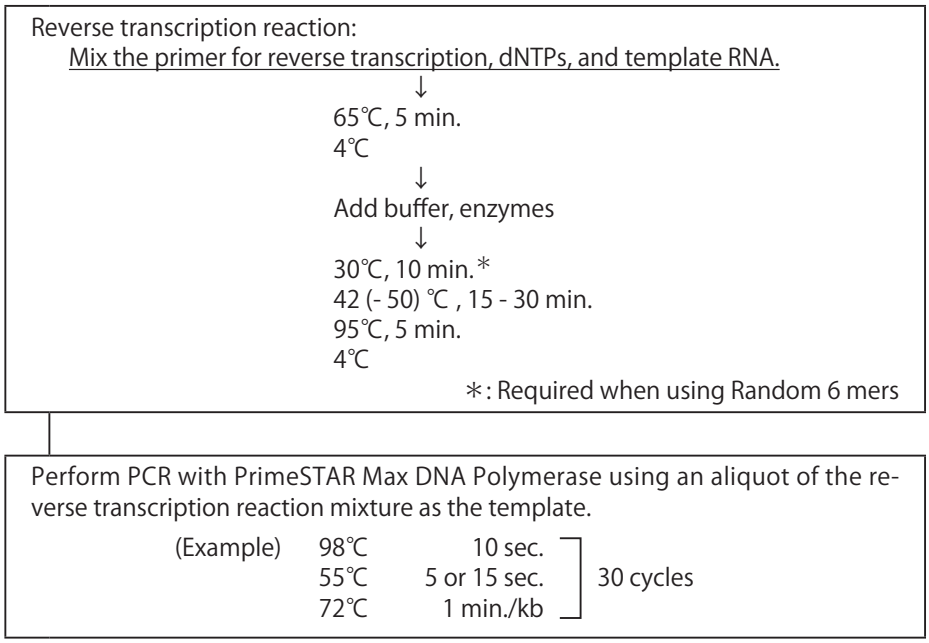


Figure 3. Flowchart of 2 step RT-PCR

[2 step RT-PCR (Standard Protocol)]

A. Template RNA denaturation and reverse transcription

A-1. Prepare the reaction mixture as shown:

Reagent	Amount
dNTP Mixture (10 mM each)	1 μl
Oligo dT Primer (2.5 μM) or Random 6 mers (20 μM) or Specific Primer (2 μM)*1	1 μl
Template RNA*2 (or Positive Control RNA	Use 2 μl [4 x 10^5 copies] for control RNA)
RNase Free dH_2O	to 10 μl

*1: Select the primer type from Oligo dT Primer, Random 6 mers, or the specific downstream primer (for Control RNA, use R-1 Primer). For primer selection criteria, see section IX. Precautions.

*2: Up to 8 μl of template RNA may be used. For total RNA template, up to 3 μg RNA can be used (recommended: 100 ng to 1 μg).

A-2. Set tubes containing the mixture in a Thermal Cycler, then perform denaturation and annealing using the following program:

65℃, 5 min.
4℃

Important: This denaturation/annealing process facilitates reverse transcription by efficient denaturation of template RNA and efficient, specific annealing of a primer to template RNA.

A-3. After denaturation and annealing, mix reagents as follows.

Reagent	Amount
The denatured and annealed reaction mixture from A-2	10 μl
5X PrimeScript Buffer	4 μl
RNase Inhibitor (40 U/μl)	0.5 μl
PrimeScript RTase (for 2 step)	0.5 μl
RNase Free dH_2O	5 μl
Total	20 μl

A-4. Set the tubes in a Thermal Cycler and perform reverse transcription using the following program:

(30℃	10 min.)＊3
42℃ (- 50℃)	15 - 30 min.
95℃	5 min.＊4
4℃	

＊3 : Perform this step when using Random 6 mers for reverse transcription. This process facilitates annealing of Random 6 mers with template RNA at 42℃ (to 50℃), thereby improving the efficiency of reverse transcription.

＊4 : The enzyme is inactivated at this step. When amplifying a long target, inactivate the enzyme at 70℃ for 15 min to avoid nicking or otherwise damaging the 1st strand cDNA.

Note: In general, perform reactions at 42℃, because PrimeScript RTase exhibits a strong extension activity even when RNA template assumes higher-order structures.
When a specific downstream primer is used as the reverse transcription primer, nonspecific amplification products may result from mispriming. In these situations, a reaction temperature of 50℃ may improve the results.

B. PCR

B-1. Prepare the reaction mixture as follows:

Reagents	Amount	Final Conc.
PrimeSTAR Max Premix (2X)	25 μl	1X
Upstream Primer (20 μM)*5 (sense)	0.5 μl	0.2 μM
Downstream Primer (20 μM)*6 (antisense)	0.5 μl	0.2 μM
Reverse transcription mixture from A-4	≦ 5 μl	
Sterile distilled water	to 50 μl	

*5 : Use F-1 Primer for Positive Control RNA
*6 : Use R-1 Primer for Positive Control RNA

B-2. Set tubes containing the mixture in a Thermal Cycler and start PCR using the appropriate program.

General reaction conditions

98℃	10 sec.	30 cycles
55℃	5 or 15 sec.	
72℃	1 min./kb	

For Positive Control*7

98℃	10 sec.	30 cycles
55℃	5 sec.	
72℃	30 sec.	

*7 : In PCR using Positive Control RNA as template, a 462 bp product is amplified whether using Oligo dT primer, Random 6mer, or R-1 primer for RT reactions.

[PCR Conditions]

- Denaturation condition — Recommended: 98℃ for 5 - 10 sec. Set the time to 10 - 15 sec. if denaturation is to take place at 94℃.
- Annealing temperature — Try 55℃ first.
- Annealing time — If primer Tm* is ≧55℃, set to 5 sec.
  If primer Tm* is < 55℃, set to 15 sec.

* : How to calculate Tm:

$Tm\ (℃) = 2\ (NA + NT) + 4\ (NC + NG) - 5$

This equation is applicable for primers no more than 25 nucleotides in length. For primers longer than 25 nucleotides, set the annealing time to 5 sec.

※ PrimeSTAR Max DNA Polymerase has very high priming efficiency. Perform the reaction with an annealing time of 5 or 15 sec. A longer annealing time may cause smearing of PCR products.

If the above conditions do not yield good results, try the following:

- With a primer having a Tm of ≧70℃, try 2 step PCR (shuttle PCR).
- If smearing and/or extra bands appear:
  (1) Shorten the annealing time; for instance, anneal for 5 sec. instead of 15 sec.
  (2) If the annealing time is already set to 5 sec., try raising the annealing temperature to 58 - 63℃.
  (3) Switch to 2 step PCR.
- If little or no amplification of the target is observed:
  (1) Lengthen the annealing time; for instance, anneal for 15 sec. instead of 5 sec.
  (2) Lower the annealing temperature to 50 - 53℃.
- Do not substitute dUTP for dTTP when using PrimeSTAR Max DNA Polymerase, as enzyme reactivity will decline markedly. Do not use primers containing inosine.

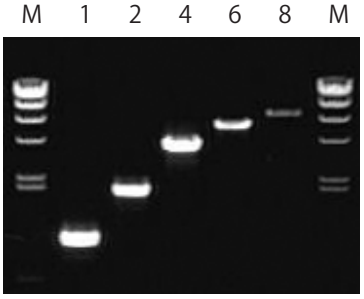
XI. Experimental Example (2 Step RT-PCR)

(1) Amplification of products of various sizes:
Human heart total RNA was used as a template to amplify various lengths of Dystrophin gene cDNA by 2 step RT-PCR.

[Method]: Reverse transcription using oligo dT Primer was performed in a 20 μl reaction containing 800 ng total RNA. The RT reaction mixture (equivalent to 200 ng total RNA) was used as template in 50 μl PCR reactions using the following conditions:

PCR conditions:	98℃	10 sec.	30 cycles
	55℃	15 sec.	
	72℃	1 min./kb	

[Result]: Excellent extension and amplification were observed for products 6 kb in length and shorter.



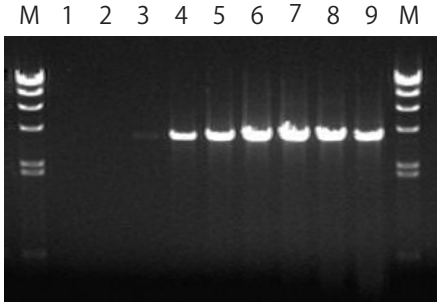
M: λ -*Hin*d III digest

(2) RT-PCR efficiency at various template concentrations:

[Method] Total RNA of HL-60 cells (100 pg, 1 ng, 10 ng, 100 ng, 200 ng, 500 ng, 1 μg, 2 μg, and 3 μg) was used as template in reverse transcriptions using Oligo dT Primer in 20 μl reactions. An aliquot of the mixture (5 μl) was used in PCR to amplify a 4 kb region of transferrin receptor (TFR).

PCR conditions:	98℃	10 sec.	30 cycles
	55℃	15 sec.	
	72℃	4 min.	

[Result] The presence of up to 3 μg total RNA per RT reaction (20 μl RT reaction volume) resulted in excellent amplification of the TFR gene, showing no inhibition of amplification.
It is possible to increase the amount of total RNA used in the RT reaction up to 6 μg per 20 μl RT reaction by setting the extension time for PCR to 2 min./kb.



Amount of template (50 μl PCR volume)
1: total RNA 25 pg-eq.
2: total RNA 250 pg-eq.
3: total RNA 2.5 ng-eq.
4: total RNA 25 ng-eq.
5: total RNA 50 ng-eq.
6: total RNA 125 ng-eq.
7: total RNA 250 ng-eq.
8: total RNA 500 ng-eq.
9: total RNA 750 ng-eq.
M: λ -*Hin*d III digest

XII. Optional Protocol (1 Step RT-PCR)

This kit may be used for 1 step RT-PCR by modification of the standard protocol, as described here.

[1 step RT-PCR (Optional Protocol)]

1. Prepare the reaction mixture as follows.

Reagent	Amount	Final Conc.
PrimeSTAR Max Premix (2X)	25 μl	1X
PrimeScript RTase (for 2 step)	0.5 μl	
RNase Inhibitor (40 U/μl)	0.5 μl	
Upstream Primer (20 μM)*1 (sense)	1 μl	0.4 μM
Downstream Primer (20 μM)*2 (antisense)	1 μl	0.4 μM
Template RNA*3		
RNase Free dH_2O	to 50 μl	

*1 : Use F-1 Primer for Positive Control RNA
*2 : Use R-1 Primer for Positive Control RNA
*3 : Use 20 - 200 ng for total RNA (or use 1 μl of Positive Control RNA).

2. Set tubes in a Thermal Cycler and start RT-PCR using the program described below.

General reaction conditions

50℃	30 min.	
94℃	2 min.	
	↓	
98℃	10 sec.	┐
55℃	5 or 15 sec.	├ 30 cycles
72℃	1 min./kb	┘

Positive Control RNA In the control reaction, a 462 bp product is amplified.

50℃	30 min.	
94℃	2 min.	
	↓	
98℃	10 sec.	┐
55℃	5 sec.	├ 30 cycles
72℃	30 sec.	┘

[PCR conditions for 1 step RT-PCR]

- Denaturation condition — Recommended: 98℃ for 5 - 10 sec. Set to 10 - 15 sec. if the denaturation is to take place at 94℃.
- Annealing temperature — Try 55℃ first.
- Annealing time — If primer Tm* is ≧ 55℃, set to 5 sec.
  If primer Tm* is < 55℃, set to 15 sec.

* : Tm (℃) = 2 (NA + NT) + 4 (NC + NG) − 5
This equation can be used for primers of no more than 25 nucleotides in length. For primers longer than 25 nucleotides, set the annealing time to 5 sec.

Note: PrimeSTAR Max DNA Polymerase has very high priming efficiency. Perform the reaction with an annealing time of 5 or 15 sec. A longer annealing time may cause smearing of PCR products.

If the above conditions do not yield good results, try the following:

- If smearing and/or extra bands appear:
  (1) Shorten the annealing time; for instance, anneal for 5 sec. instead of 15 sec.
  (2) If the annealing time is already set to 5 sec., try raising the annealing temperature to 58 - 63℃.

- If little or no amplification of the target is observed:
  (1) Adjust the amount of template to the recommended levels.
  (2) Increase the number of PCR cycles to 40 - 50.
  (3) Lengthen the annealing time; for instance, anneal for 15 sec. instead of 5 sec.
  (4) Lower the annealing temperature to 50 - 53℃.

- Do not substitute dUTP for dTTP when using PrimeSTAR Max DNA Polymerase, as enzyme reactivity will decline markedly. Do not use primers containing inosine.

XIII. Experimental Example (1 step RT-PCR)

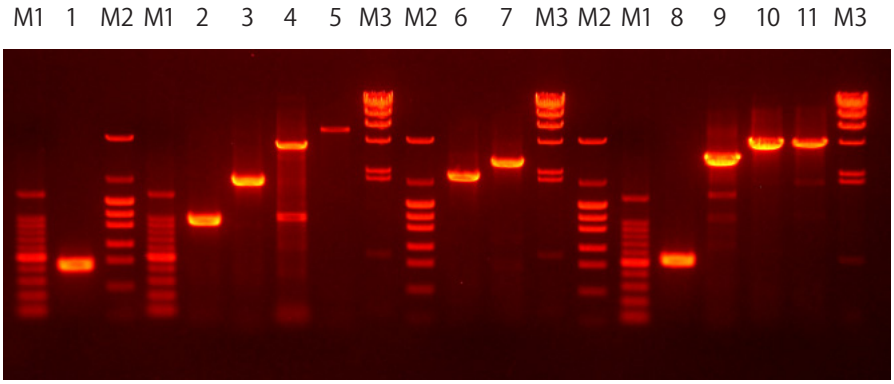
(1) Amplification of products of various sizes:

[Method] Human heart total RNA or HL-60 cell total RNA (100 ng/50 μl reaction) was used as a template to amplify target cDNAs of various lengths, as indicated below, in a 1 step RT-PCR.

Reaction conditions:

50℃	30 min.	
94℃	2 min.	
↓		
98℃	10 sec.	┐
55℃	5 or 15 sec.	├ 30 cycles
72℃	1 min./kb	┘

[Result] Amplification of products in the range of 0.5 - 6 kb was confirmed.



1: GAPDH	428 bp	7: CCND2	2.8 kb	M2: pHY Marker
2: Dystrophin	1 kb *	8: TFR	500 bp	M3: λ -*Hin*d III digest
3: Dystrophin	2 kb *	9: TFR	3 kb	* : Human heart total RNA was the template for these samples. For all others, HL-60 cell total RNA was used.
4: Dystrophin	4 kb *	10: TFR	4 kb	
5: Dystrophin	6 kb *	11: TFR	4.4 kb	
6: CCND2	2.1 kb	M1: 100 bp DNA Ladder		

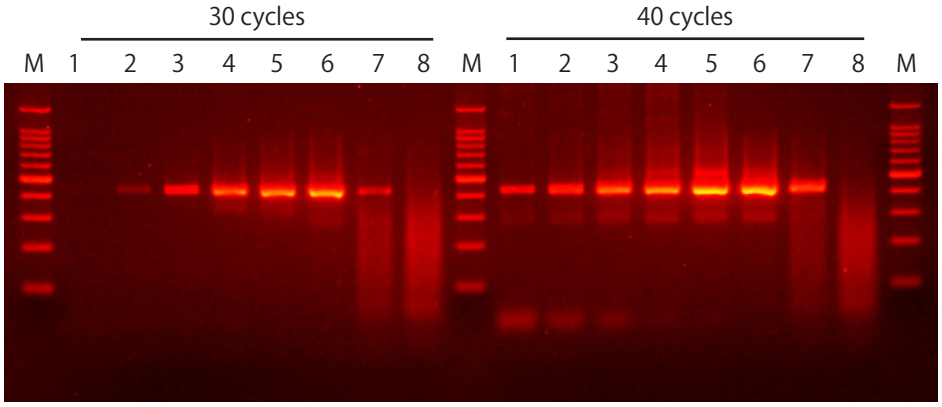
(2) Detection sensitivity

[Method]: With various amounts of HL-60 cell total RNA as the template, 1 step RT-PCRs were performed to measure the detection sensitivity for a 428-bp region of the GAPDH gene.

Reaction conditions:

50℃	30 min.	
94℃	2 min.	
↓		
98℃	10 sec.	┐
55℃	15 sec.	├ 30 or 40 cycles
72℃	30 sec.	┘

[Result] Successful detection was achieved with as little as 10 pg (30 cycles) or 1 pg (40 cycles) of total RNA as template.



Amount of template (total RNA)

1:	1 pg
2:	10 pg
3:	100 pg
4:	1 ng
5:	10 ng
6:	100 ng
7:	1 μg
8:	2 μg

M: 100 bp DNA Ladder

XIV. Electrophoresis, Cloning, and Sequencing

(1) Electrophoresis of amplification products

The use of TAE Buffer is recommended for electrophoresis of amplification products obtained with this kit. Using TBE Buffer may lead to band spreading during electrophoresis, resulting in unsatisfactory electrophoresis results.

(2) Cloning of amplification products

Most of the amplification products obtained with this kit are blunt-ended. Therefore, the PCR products may be cloned directly (subject to phosphorylation when necessary) into blunt-end vectors. For cloning into blunt-end vectors, Mighty Cloning Reagent Set (Blunt End) (Cat. #6027) may be used.

To clone into T-vectors, the addition of dA to the 3′ ends is required. Use the Mighty TA-cloning Reagent Set for PrimeSTAR (Cat. #6019) (not available in all geographic regions).

(3) Restriction enzyme treatment

Before digesting amplification products with restriction enzymes, remove proteins by phenol/chloroform extraction or NucleoSpin® Extract II (Clontech Cat. #740609.50/.250). Particularly with 3′ overhang restriction enzymes (e.g., *Pst* I), residual 3′ → 5′ exonuclease activity of PrimeSTAR Max DNA Polymerase would result in the removal of 3′ overhangs during restriction enzyme digestion.

(4) Direct sequencing

PrimeSTAR Max DNA Polymerase has 3′ → 5′ exonuclease activity. Remove protein by phenol/chloroform extraction or NucleoSpin® Extract II (Cat. #740609.50/.250) before direct sequencing.

XV. Related Products

PrimeScript® Reverse Transcriptase (Cat. #2680A/B/C)＊
PrimeSTAR® Max DNA Polymerase (Cat. #R045A)
PrimeScript® RT-PCR Kit (Cat. #RR014A/B)＊
PrimeScript® One Step RT-PCR Kit Ver. 2 (Cat. #RR055A)＊
TaKaRa PCR Thermal Cycler Dice® Gradient (Cat. #TP600)＊
Agarose L03 (Cat. #5003)
RNase-OFF™ (RNase decontamination solution) (Cat. #9037)
NucleoSpin® RNA II (Clontech Cat. #740955.10/.50/.250)
Oligotex™-dT30 < Super > (Cat. #W9021A/B)＊
Oligotex™-dT30 < Super > mRNA Purification Kit (From Total RNA) (Cat. #9086)＊
Mighty TA-cloning Reagent Set for PrimeSTAR® (Cat. #6019)＊
Mighty Cloning Reagent Set (Blunt End) (Cat. #6027)
NucleoSpin® Extract II (Clontech Cat. #740609.50/.250)

＊: Not available in all geographic regions

NOTICE TO PURCHASER：LIMITED LICENSE

[P1] PCR Notice
Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,789,224, 5,618,711, 6,127,155 and claims outside the US corresponding to expired US Patent No. 5,079,352. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claims, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

[L15] Hot Start PCR
Licensed under U.S. Patent No. 5,338,671 and 5,587,287, and corresponding patents in other countries.

[M54] PrimeSTAR® HS DNA Polymerase
This product is covered by the claims of U.S. Patent No. 7,704,713 and its foreign counterparts.

NOTE : This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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