

Cat. # R022A

For Research Use

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# TAKARA

## PrimeScript™ High Fidelity RT-PCR Kit

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Product Manual

v202107Da

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## 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 in 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°C)
- 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 $\mu$ l
2	5X PrimeScript Buffer	200 $\mu$ l
3	RNase Inhibitor (40 U/ $\mu$ l)	25 $\mu$ l
4	dNTP Mixture (10 mM each)	50 $\mu$ l
5	Oligo dT Primer (2.5 $\mu$ M)	50 $\mu$ l
6	Random 6-mers (20 $\mu$ M)	50 $\mu$ l
7	PrimeSTAR Max Premix (2X)	625 $\mu$ l x 2
8	Control F-1 Primer* 2 (20 $\mu$ M)	10 $\mu$ l
9	Control R-1 Primer* 3 (20 $\mu$ M)	10 $\mu$ l
10	Positive Control RNA (2 x 10 <sup>5</sup> copies/ $\mu$ l)	20 $\mu$ l
11	RNase Free dH <sub>2</sub> O	1 ml

\* 1 For 50 reactions of 2 step RT-PCRs  
(20  $\mu$ l RT reaction  $\rightarrow$  50  $\mu$ l PCR)

\* 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) <sub>6</sub>
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.

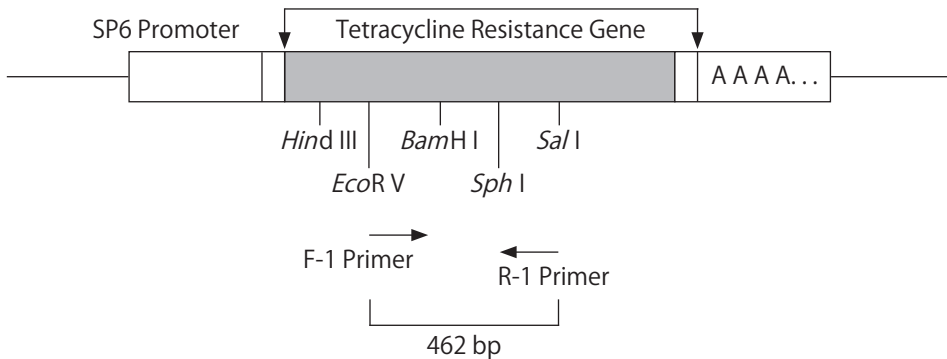


Figure 1. Control RNA: The amplification fragment generated using the control primers

III. Materials Required but not Provided

- Thermal cycler (authorized for use)  
e.g., TaKaRa PCR Thermal Cycler Dice Gradient (Cat. #TP600)
- Agarose  
e.g., Agarose L03 「TAKARA」 (Cat. #5003/5003B)  
PrimeGel™ Agarose PCR-Sieve (Cat. #5810A)
- Electrophoresis apparatus  
e.g., Mupid-One (Cat. #O1-01)  
Mupid-2plus (Cat. #M-2P)  
Mupid-exU (Cat. #EXU-1)
- Microcentrifuge
- Micropipettes and tips (autoclaved)

IV. Storage -20°C

**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 over 10-fold higher than *Taq* DNA Polymerase and equivalent to or higher than that of PrimeSTAR HS DNA Polymerase or company A high-fidelity enzyme. This protocol offers precise fidelity. PrimeSTAR Max DNA Polymerase can be useful for PCR when fidelity is critical.

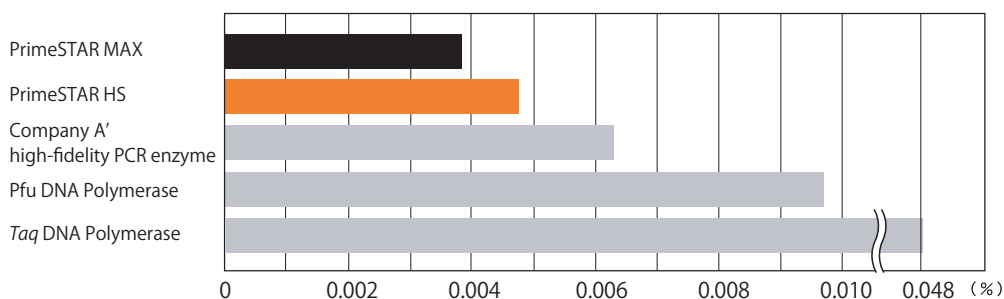


Figure 2. Fidelity of various PCR enzymes

By amplification with PrimeSTAR Max DNA Polymerase, only 9 errors occurred in 230,129 bases analyzed.

## VI. RNA Preparation

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

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

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

- (1) Treat glassware with 0.1% diethyl pyrocarbonate (DEPC) at 37°C for 12 hours
- (2) Autoclave it at 120°C 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 and apparatuses. Use only materials (plastic or glass) designated exclusively for RNA experiments.

### [Preparation of RNA]

When preparing highly purified total RNA from cultured cells or tissue, it is convenient to use NucleoSpin RNA (Cat. #740955.10/.50/.250).

When preparing poly A<sup>+</sup> RNA 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 poly A<sup>+</sup> RNA.

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

## VII. Principle

The standard protocol for the PrimeScript High Fidelity RT-PCR Kit is 2 step RT-PCR below.

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

Primers for cDNA synthesis can be selected from Random 6 mers, Oligo dT Primer and a target-specific primer.

The 1 step RT-PCR (optional protocol) is performed cDNA synthesis, followed by PCR amplification in reaction mixture containing both PrimeScript RTase and PrimeSTAR Max DNA Polymerase.

In 1 step RT-PCR, the antisense PCR primer is used for synthesizing cDNA from RNA.

## VIII. Features

### ■ 2 step RT-PCR (Standard Protocol)

RNA template	General
Amplified product length	6 kb products
Reverse transcriptase	PrimeScript RTase (Reaction at 42°C)
DNA Polymerase	PrimeSTAR Max DNA Polymerase (2X premix)
RNase Inhibitor	Add (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
Amplified Product length	6 kb product
Reverse transcriptase	PrimeScript RTase (Reaction at 50°C)
DNA Polymerase	PrimeSTAR Max DNA Polymerase (2X premix)
RNase Inhibitor	Add (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 briefly PrimeScript RTase, RNase Inhibitor and PrimeSTAR Max Premix (2X) to collect the liquid at the bottom of the tube. Before pipetting each reagent, mix gently without introducing bubbles. As these enzymes and the inhibitor are provided are highly viscous, pipette slowly and carefully.
- (3) Keep the enzymes and the inhibitor at -20°C until just before use. Return to -20°C storage immediately after use.
- (4) Avoid freezing and thawing the Positive Control RNA as much as possible to prevent degradation. Dispensing it into small aliquots for storage is recommended. Store Positive Control RNA aliquots at -70°C to -80°C.
- (5) Use new 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. Selection criteria is 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, 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 standard protocol is 2 step RT-PCR.

For 1 step RT-PCR, see section XII. Optional Protocol (1 step RT-PCR).

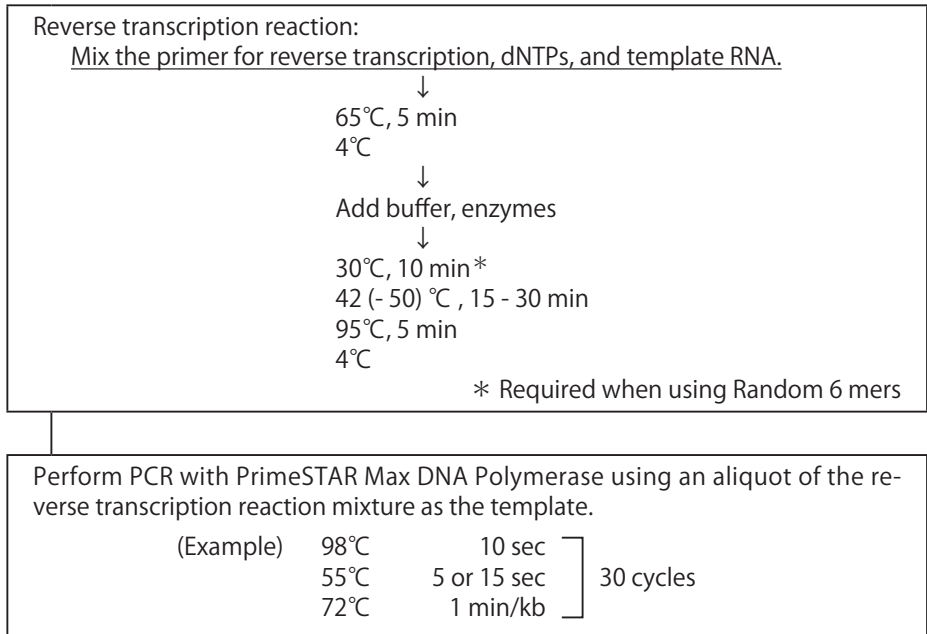


Figure 3. Flowchart of 2 step RT-PCR

**[2 step RT-PCR (Standard Protocol)]**

**A. RNA denaturation and reverse transcription**

A-1. Prepare the reaction mixture in a PCR tube as shown below:

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)* <sup>1</sup>	1 μl
Template RNA * <sup>2</sup> (or Positive Control RNA	2 μl [4 x 10 <sup>5</sup> copies])
RNase Free dH <sub>2</sub> O	to 10 μl

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

\* 2 Up to 8 μl of template RNA may be used. The total RNA is recommended to use 100 ng to 1 μg, and the maximal amount is up to 3 μg.



A-2. Set a tube in a Thermal Cycler, then incubate at the following condition:

65°C, 5 min  
4°C

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

A-3. Prepare mix reagents as follows.

Reagent	Amount
The mixture from A-2	10 $\mu$ l
5X PrimeScript Buffer	4 $\mu$ l
RNase Inhibitor (40 U/ $\mu$ l)	0.5 $\mu$ l
PrimeScript RTase (for 2 step)	0.5 $\mu$ l
RNase Free dH <sub>2</sub> O	5 $\mu$ l
Total	20 $\mu$ l

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

30°C            10 min \*3  
42°C (~ 50°C)    15 - 30 min  
95°C            5 min \*4  
4°C

- \*3 Add this step when using Random 6 mers. This step facilitates annealing of Random 6 mers with template RNA at 42°C (to 50°C), thereby improving the efficiency of reverse transcription.
- \*4 The enzyme is inactivated at this step. When amplifying a long cDNA, inactivate the enzyme at 70°C for 15 min to avoid damaging the 1st strand cDNA.

**Note:** In general, perform reactions at 42°C, because PrimeScript RTase exhibits a strong extension activity even when RNA template has higher-order structures.

When a specific downstream primer is used as the reverse transcription primer, nonspecific amplification products may result from mispriming. In this case, a reaction at 50°C may improve the results.

**B. PCR**

B-1. Prepare the reaction mixture as follows:

Reagent	Amount	Final Conc.
PrimeSTAR Max Premix (2X)	25 $\mu$ l	1X
Upstream Primer (20 $\mu$ M) <sup>*5</sup> (sense)	0.5 $\mu$ l	0.2 $\mu$ M
Downstream Primer (20 $\mu$ M) <sup>*6</sup> (antisense)	0.5 $\mu$ l	0.2 $\mu$ M
Reverse transcription mixture from A-4	$\leq$ 5 $\mu$ l	
Sterile purified water	to 50 $\mu$ l	

\*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 at the condition below.

Standard reaction		] 30 cycles	For Positive Control <sup>*7</sup>		] 30 cycles
98°C	10 sec		98°C	10 sec	
55°C	5 or 15 sec	55°C	5 sec		
72°C	1 min/kb	72°C	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    Recommend at 98°C for 5 - 10 sec. Set for 10 - 15 sec if denaturation is at 94°C
- Annealing time            If primer Tm\* is  $\geq$  55°C, set to 5 sec  
                                      If primer Tm\* is < 55°C, set to 15 sec

\* How to calculate Tm:

$$T_m (\text{°C}) = [(the\ number\ of\ A\ and\ T) \times 2] + [(the\ number\ of\ G\ and\ C) \times 4] - 5$$

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.

**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:

- With a primer having a Tm of  $\geq$  70°C, 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°C.
  - (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°C.

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

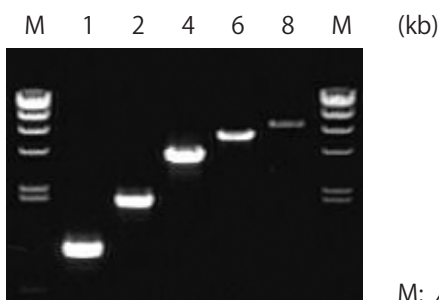
### XI. Experimental Examples (2 Step RT-PCR)

- (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  $\mu$ l reaction containing 800 ng total RNA. The RT reaction mixture (equivalent to 200 ng total RNA) was used as template in 50  $\mu$ l PCR reactions using the following conditions:

PCR conditions:	98°C	10 sec	} 30 cycles
	55°C	15 sec	
	72°C	1 min/kb	

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

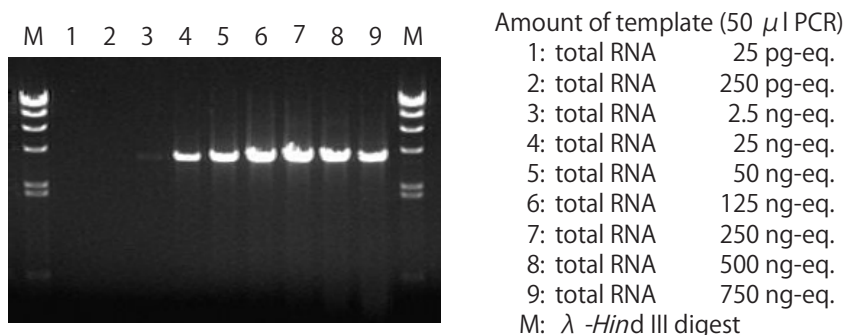


- (2) RT-PCR efficiency at various template concentrations:

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

PCR conditions:	98°C	10 sec	} 30 cycles
	55°C	15 sec	
	72°C	4 min	

[Result] The presence of up to 3  $\mu$ g total RNA per RT reaction (20  $\mu$ l 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  $\mu$ g per 20  $\mu$ l RT reaction by setting the extension time for PCR to 2 min/kb.



**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]**

1. Prepare the reaction mixture as follows.

Reagent	Amount	Final Conc.
PrimeSTAR Max Premix (2X)	25 $\mu$ l	1X
PrimeScript RTase (for 2 step)	0.5 $\mu$ l	
RNase Inhibitor (40 U/ $\mu$ l)	0.5 $\mu$ l	
Upstream Primer (20 $\mu$ M)* <sup>1</sup> (sense)	1 $\mu$ l	0.4 $\mu$ M
Downstream Primer (20 $\mu$ M)* <sup>2</sup> (antisense)	1 $\mu$ l	0.4 $\mu$ M
Template RNA * <sup>3</sup>		
RNase Free dH <sub>2</sub> O	to 50 $\mu$ l	

\* 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  $\mu$ l of Positive Control RNA).

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

Standard reaction

50°C	30 min	
94°C	2 min	
↓		
98°C	10 sec	} 30 cycles
55°C	5 or 15 sec	
72°C	1 min/kb	

Positive Control RNA

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

50°C	30 min	
94°C	2 min	
↓		
98°C	10 sec	} 30 cycles
55°C	5 sec	
72°C	30 sec	

**[PCR conditions for 1 step RT-PCR]**

- Denaturation condition      Recommend at 98°C for 5 - 10 sec Set to 10 - 15 sec if the denaturation is at 94°C
- Annealing time                If primer T<sub>m</sub>\* is  $\geq$  55°C, set to 5 sec  
If primer T<sub>m</sub>\* is < 55°C, set to 15 sec

\* T<sub>m</sub> (°C) = [(the number of A and T) x 2] + [(the number of G and C) x 4] - 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°C.
  
- 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°C.

Note: 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)

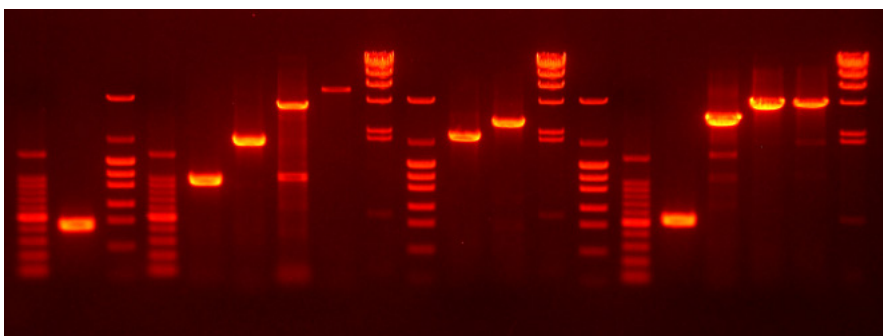
(1) Amplification of products of various sizes:

[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°C	30 min	
	94°C	2 min	
	↓		
	98°C	10 sec	}
	55°C	5 or 15 sec	
	72°C	1 min/kb	

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

M1 1 M2 M1 2 3 4 5 M3 M2 6 7 M3 M2 M1 8 9 10 11 M3



1: GAPDH	428 bp	7 : CCND2	2.8 kb
2: Dystrophin	1 kb *	8 : TFR	500 bp
3: Dystrophin	2 kb *	9 : TFR	3 kb
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	

M2: pHY Marker  
M3: λ -Hind III digest

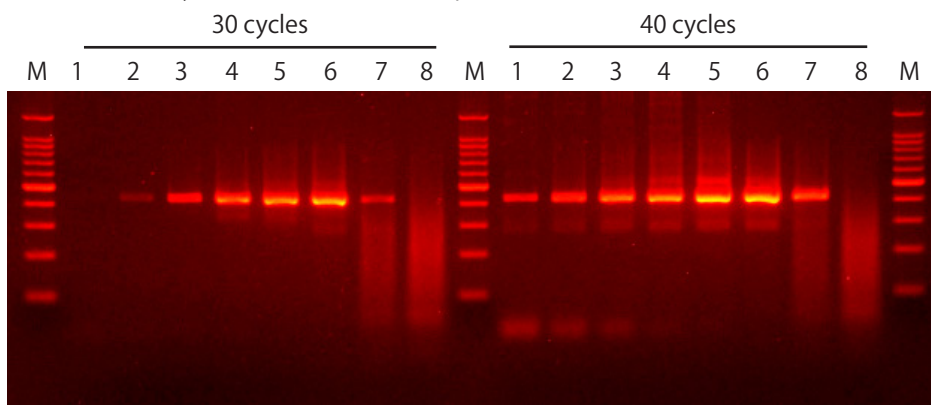
\* Human heart total RNA was the template for these samples. For all others, HL-60 cell total RNA was used.

(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°C	30 min	
	94°C	2 min	
	↓		
	98°C	10 sec	} 30 or 40 cycles
	55°C	15 sec	
	72°C	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)	5:	10 ng	
1:	1 pg	6:	100 ng
2:	10 pg	7:	1 μg
3:	100 pg	8:	2 μg
4:	1 ng	M:	100 bp DNA Ladder

**XIV. Electrophoresis, Cloning, and Sequencing**

- (1) Electrophoresis of amplified products  
The use of TAE Buffer is recommended for gel electrophoresis of amplified products with this kit. Using TBE Buffer may lead to band spreading during electrophoresis.
- (2) Cloning of amplified products  
Most of the amplified products with this kit are blunt-ended. Therefore, the PCR products can 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) is recommended.
- (3) Restriction enzyme treatment  
Before digesting amplified products with restriction enzymes purify the DNA by phenol/chloroform extraction or NucleoSpin Gel and PCR Clean-up (Cat. #740609/.10/.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 deletion of 3' overhangs during restriction enzyme digestion.
- (4) Direct sequencing  
PrimeSTAR Max DNA Polymerase has 3' → 5' exonuclease activity. Purify the DNA by phenol/chloroform extraction or NucleoSpin Gel and PCR Clean-up before direct sequencing.

**XV. Related Products**

PrimeScript™ Reverse Transcriptase (Cat. #2680A/B/C)\*  
PrimeSTAR® Max DNA Polymerase (Cat. #R045A/B)  
PrimeScript™ RT-PCR Kit (Cat. #RR014A/B)\*  
PrimeScript™ One Step RT-PCR Kit Ver. 2 (Cat. #RR055A/B)\*  
PrimeScript™ One Step RT-PCR Kit Ver.2 (Dye Plus) (Cat. #RR057A/B)  
TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600)  
TaKaRa PCR Thermal Cycler Dice™ *Touch* (Cat. #TP350)  
Agarose L03 「TAKARA」 (Cat. #5003/5003B)  
PrimeGel™ Agarose PCR-Sieve (Cat. #5810A)  
RNase-OFF® (RNase decontamination solution) (Cat. #9037)  
NucleoSpin RNA (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 Gel and PCR Clean-up (Cat. #740609/.10/.50/.250)  
PrimeScript™ II High Fidelity RT-PCR Kit (Cat. #R023A/B)  
PrimeScript™ II High Fidelity One Step RT-PCR Kit (Cat. #R026A/B)  
RNAiso Plus (Cat. #9108/9109)

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

PrimeSTAR is a registered trademarks of Takara Bio Inc.

RNase-OFF is a registered trademark of PureBiotech, LLC.

PrimeScript, PrimeGel, and Thermal Cycler Dice are trademarks of Takara Bio Inc.

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**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.

Takara products may not be resold or transferred, modified for resale or transfer, or used to manufacture commercial products without written approval from Takara Bio Inc.

If you require licenses for other use, please contact us by phone at +81 77 565 6972 or from our website at [www.takarabio.com](http://www.takarabio.com).

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