Cat. # RR057A

For Research Use

TaKaRa

PrimeScript™
One Step RT-PCR Kit Ver.2 (Dye Plus)

Product Manual

v202108Da

Table of Contents

I. Description

Although RNA cannot be used directly as a template for PCR, amplification of a target region can be performed after synthesizing cDNA from RNA using reverse transcriptase (RT-PCR).

The PrimeScript One Step RT-PCR Kit Ver.2 (Dye Plus) is used for one-step RT-PCR in a single tube. There is no need to add reagents during the reaction, minimizing the risk of contamination. Furthermore, electrophoresis loading dyes (blue and yellow) are included in the premix to allow direct analysis by electrophoresis. The bright green color of the reaction mixture allows visual confirmation of gel loading.

The kit includes PrimeScript RTase, a reverse transcriptase that provides superior elongation, and *TaKaRa Ex Taq*® HS, a PCR enzyme formulated for hot start PCR that provides high yields. The reaction components are optimized to provide the same level of performance as conventional RT-PCR systems that lack dyes and loading buffer.

Additionally, reaction preparation and experimental workflow are made simple by using the premixed PrimeScript 1 step Enzyme Mix, which includes PrimeScript RTase, *TaKaRa Ex Taq* HS, and RNase Inhibitor along with a stabilizer. This premix is highly optimized for one-step RT-PCR. Another premix, 2X 1 step Buffer (Dye Plus), includes buffer, dNTP mixture, 1 step Enhancer Solution, dye, and a high-density agent to facilitate gel loading at electrophoresis of PCR product.

Advantages of the PrimeScript One Step RT-PCR Kit Ver.2 (Dye Plus) include:

- High yields of RT-PCR amplification products
- Simple preparation of reactions and direct loading on electrophoresis gels
- Efficient reverse transcription at 50℃, which minimizes the possibility of nonspecific amplification
- Prevention of nonspecific amplification due to pre-cycle mispriming and primer dimer formation during reaction preparation

This kit includes all of the reagents necessary for the synthesis of cDNA from RNA by reverse transcription and amplification of cDNA by PCR.

II. Components (50 reactions)

(1) PrimeScript 1 step Enzyme Mix	100 μl
(2) 2X 1 step Buffer (Dye Plus)	625 μl x 2
(3) Control F-1 Primer*1 (20 μM)	20 μl
(4) Control R-1 Primer*2 (20 μM)	20 μl
(5) Positive Control RNA (2 x 10^5 copies/μl)	20 μl
(6) RNase Free dH_2O	625 μl x 2

*1 Positive Control RNA upstream sense primer
*2 Positive Control RNA downstream anti-sense primer

[Primer sequences]

Control F-1 Primer 5′ -CTGCTCGCTTCGCTACTTGGA-3′
Control R-1 Primer 5′ -CGGCACCTGTCCTACGAGTTG-3′

[Positive Control RNA]

The Positive Control RNA was synthesized by *in vitro* transcription using SP6 RNA polymerase. Plasmid pSPTet3 was used as template. A fragment of approximately 1.4 kb, that includes the tetracycline resistance gene derived from pBR322, is present in this plasmid downstream from the SP6 promoter.

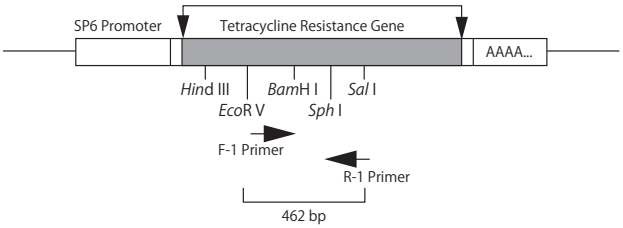


Figure 1. Amplification Products from Positive Control RNA with the Control F-1 and Control R-1 Primers

III. Materials Required but not Provided

1. Thermal cycler (authorized instruments)
   TaKaRa PCR Thermal Cycler Dice™ *Touch* (Cat. #TP350)*
   TaKaRa PCR Thermal Cycler Dice Gradient (Cat. #TP600)*, etc.
2. Agarose gel
   Agarose L03 [TAKARA] (Cat. #5003/5003B)
   PrimeGel™ Agarose PCR-Sieve（Cat. #5810A）, etc.
3. Electrophoresis system
   Mupid-2plus (Cat. #M-2P)*
   Mupid-exU (Cat. #EXU-1)*, etc.
4. Microcentrifuge
5. Micropipettes and sterile tips

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

IV. Storage

-20℃

V. Principle

With the PrimeScript One Step RT-PCR Kit Ver.2 (Dye Plus), cDNA is synthesized from RNA using PrimeScript RTase, and then PCR amplification is carried out in the same reaction tube using *TaKaRa Ex Taq* HS.

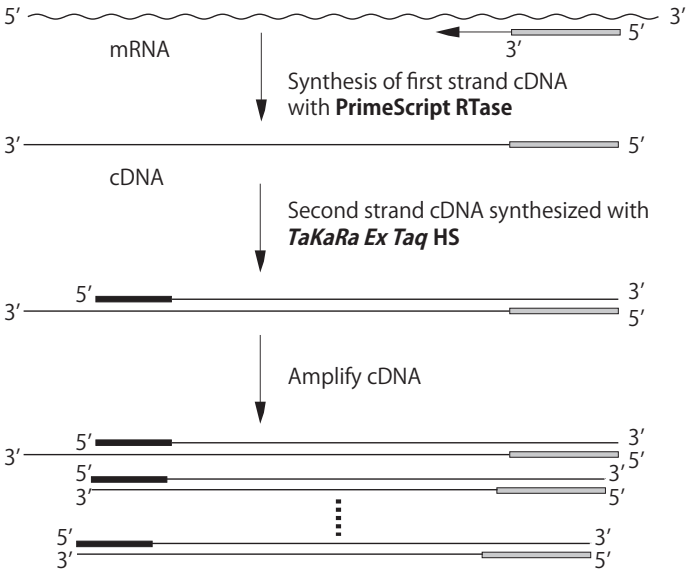


Figure 2. Principle of the PrimeScript One Step RT-PCR Kit Ver.2 (Dye Plus)

VI. Specifications

RNA template	From all species
Amplification product length	Amplification of 8 kb cDNA products has been confirmed
PrimeScript 1 step Enzyme Mix components	・Reverse transcriptase (PrimeScript RTase) ・DNA Polymerase (*TaKaRa Ex Taq* HS) ・RNase Inhibitor
2X 1 step Buffer (Dye Plus) components	・Reaction buffer ・dNTP Mixture (final concentration 400 μM) ・1 Step Enhancer Solution ・Dye and density agent
1st strand cDNA Synthesis primer	Requires sequence-specific downstream primer (do not use oligo dT primer or random primer)
Protocol	RT and PCR carried out sequentially in a single tube

VII. Precautions Before Use

Read the following precautions when using this kit.

(1) For the reaction solution, prepare master mix for the required number of reactions plus a few extra. Preparation of a master mix minimizes losses and error due to pipetting, allowing the reagents to be dispensed more accurately. This reduces experimental variability.

(2) Before using PrimeScript 1 step Enzyme Mix, briefly centrifuge the tube to collect the reagent at the bottom of the tube. Additionally, since PrimeScript 1 step Enzyme Mix contains 50% glycerol and is highly viscous, pipette it slowly and carefully.

(3) Vortex the 2X 1 step Buffer (Dye Plus) and briefly centrifuge it just before use.

(4) Store the PrimeScript 1 step Enzyme Mix at -20℃ until just before use. Return the reagent to -20℃ immediately after use.

(5) To avoid degradation of the Positive Control RNA, avoid unnecessary freeze-thaw cycles. We recommend storing aliquots at -70 to -80℃.

(6) Always use fresh disposable tips to avoid any potential cross-contamination between samples when preparing or dispensing reaction mixtures.

(7) Use a specific primer for reverse transcription reactions performed with this kit. Do not use random primers or Oligo dT primer.

VIII. Protocol

1. Prepare the reaction mixture shown below.

Reagent	Volume (per reaction)	Final concentration
PrimeScript 1 step Enzyme Mix	2 μl	
2X 1 step Buffer (Dye Plus)	25 μl	
Upstream Primer (20 μM)＊1 (sense)	1 μl	0.4 μM
Downstream Primer (20 μM)＊2 (anti-sense)	1 μl	0.4 μM
Template RNA	X μl＊3	
(or Positive Control RNA	1 μl)	
RNase Free dH_2O	to 50 μl	

＊1 F-1 Primer for Positive Control RNA
＊2 R-1 Primer for Positive Control RNA
＊3 When using total RNA, add no more than 1 μg.

2. Place the reaction tube in a thermal cycler, and perform RT-PCR under the following conditions.

Standard conditions

(A) 3 step PCR

50℃	30 min	
94℃	2 min	
↓		
94℃	30 sec	┐
55 - 65℃	30 sec	├ 25 - 30 cycles
72℃	1 min/kb	┘

(B) 2 step PCR

50℃	30 min	
94℃	2 min	
↓		
98℃	10 sec	┐
68℃	1 min/kb	┘ 25 - 30 cycles

For Positive Control RNA＊

50℃	30 min	
94℃	2 min	
↓		
94℃	30 sec	┐
60℃	30 sec	├ 25 cycles
72℃	1 min	┘

＊ In the control reaction, 462 bp is amplified.

3. After PCR is complete, run an aliquot (approximately 5 μl) on an agarose electrophoresis gel.

Note: When running 5 μl of the reaction on a 1% Agarose L03 gel, the blue dye runs at approximately 3 - 5 kb, and the yellow dye runs at ≦50 bp.

IX. PCR Conditions

- Denaturation conditions

  Denaturation conditions vary depending on the thermal cycler and tubes used for PCR. Denaturation for 5 - 10 sec at 98℃ or 20 - 30 sec at 94℃ is recommended.

- Annealing temperature

  Set the PCR annealing temperature to 60℃ for Positive Control RNA, but change it to the optimal temperature for sequence-specific primers used with experimental samples. Determine this optimal annealing temperature by testing temperatures ranging from 55 to 65℃. If necessary, investigate a broader range (45 - 65℃).

- Extension time

  The extension time depends on the length of the amplified product. When using *TaKaRa Ex Taq* HS at 72℃, use 1 minute per 1 kb as an estimate.

- Number of cycles

  For a small amount of cDNA, use 40 - 50 cycles.

- A single A nucleotide is added to the 3′ end of nearly all PCR products synthesized with this kit. Consequently, PCR products can be used directly for TA cloning. Alternatively, blunting and phosphorylation may be performed so that products can be cloned into a blunt-end vector. For cloning in a blunt-end vector, use the Mighty Cloning Reagent Set (Blunt End) (Cat. #6027).

X. Experimental Examples

(1) Using either total RNA derived from mouse heart or total RNA derived from HL60 cells as a template, one-step RT-PCR amplification of target genes of various lengths was carried out with this kit according to the recommended protocol.

Target gene	**Total RNA** ＊
Dystrophin	From mouse heart
Transferrin receptor (TFR)	From HL60 cells
Cyclin D2 (CCND2)	From HL60 cells

＊ 1 μg total RNA used

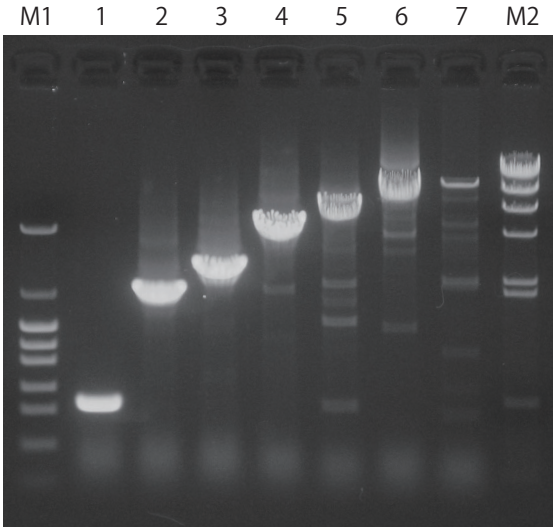
RT-PCR conditions:

<u>0.5 - 6 kb</u>

50℃	30 min	
94℃	2 min	
↓		
94℃	30 sec	30 cycles
55℃	30 sec	
72℃	1 min/kb	

<u>8 - 12 kb</u>

50℃	30 min	
94℃	2 min	
↓		
98℃	10 sec	30 cycles
68℃	1 min/kb	

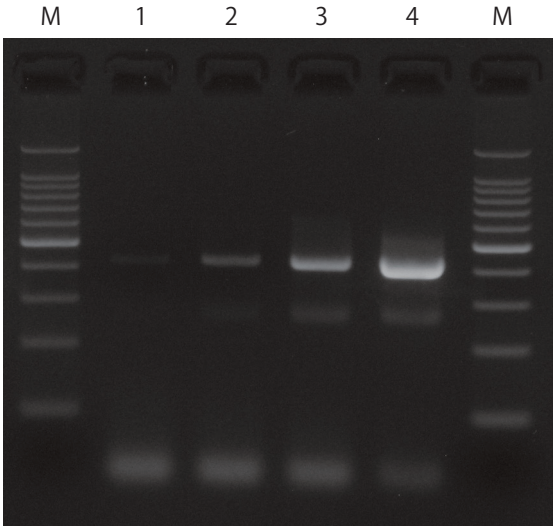


1: TFR 0.5 kb
2: CCND2 2.1 kb
3: CCND2 2.8 kb
4: TFR 4.4 kb
5: Dystrophin 6 kb
6: Dystrophin 8 kb
7: Dystrophin 12 kb
M1: pHY Marker
M2: λ-*Hin*d III digest

Excellent extension and amplification was observed for products ranging in size from 0.5 - 8 kb.

(2) Using HL60 total RNA as template, the limit of detection for the *GAPDH* gene was tested under the following conditions.

Target:	GAPDH	428 bp	
RT-PCR condition:	50℃	30 min	
	94℃	2 min	
	↓		
	94℃	30 sec	40 cycles
	55℃	30 sec	
	72℃	1 min	



Template (total RNA) amounts
1: 0.01 pg
2: 0.1 pg
3: 1 pg
4: 10 pg
M: 100 bp DNA Ladder

Detection of the target gene was observed using 0.1 pg of total RNA as template.

XI. Preparation of RNA Samples

The kit is used for synthesis of cDNA from RNA and PCR amplification of a target gene. For successful cDNA synthesis, it is essential to obtain highly pure RNA. Great care must be taken to inhibit RNase from both endogenous and external sources.

To prevent RNase contamination (e.g., from sweat or saliva introduced while handling and preparing the RNA), take measures such as avoiding unnecessary talking, wearing clean disposable gloves, and using a dedicated laboratory bench for preparing RNA.

Use RNase-free disposable plastic tips and tubes. Treat any glassware used during RNA preparation and RT-PCR as described below.

(1) Treat glassware for 12 hours with a solution of a 0.1% diethyl pyrocarbonate (DEPC) at 37℃.
(2) Autoclave the glassware at 120℃ for 30 min to remove any residual DEPC.

In addition, RNase-OFF® (Cat. #9037) is recommended for removing RNase from lab benches, instruments, tubes, etc.

Glassware, pipettes, plastic tips and tubes, and other materials used for RNA experiments should be dedicated for use only with RNA.

[RNA Sample Preparation Methods]

Since RT-PCR usually requires only small amounts of RNA, common purification methods are usually sufficient. However, we recommended that the guanidine thiocyanate (GTC) method be used if possible. In general, RNA should be the highest purity possible.

When preparing high purity total RNA from cultured cells or tissue samples, NucleoSpin RNA (Cat. #740955.10/.50/.250) or the AGPC method simplified reagent RNAiso Plus (Cat. #9108/9109)＊can be used. For blood samples, NucleoSpin RNA Blood (Cat. #740200.10/.50) or RNAiso Blood (Cat. #9112/9113) can be used.

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

XII. Related Products

PrimeScript™ Reverse Transcriptase (Cat. #2680A/B/C)
PrimeScript™ II Reverse Transcriptase (Cat. #2690A/B/C)＊
PrimeScript™ RT-PCR Kit (Cat. #RR014A/B)
PrimeScript™ High Fidelity RT-PCR Kit (Cat. #R022A/B)
PrimeScript™ II High Fidelity RT-PCR Kit (Cat. #R023A/B)＊
PrimeScript™ One step RT-PCR Kit Ver.2 (Cat. #RR055A/B)
PrimeScript™ 1st strand cDNA Synthesis Kit (Cat. #6110A/B)
PrimeScript™ II 1st strand cDNA Synthesis Kit (Cat. #6210A/B)＊
TaKaRa PCR Thermal Cycler Dice™ *Touch* (Cat. #TP350)＊
TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600)＊
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)
NucleoSpin RNA Blood (Cat. #740200.10/.50)
RNAiso Plus (Cat. #9108/9109)＊
RNAiso Blood (Cat. #9112/9113)＊
Mighty Cloning Reagent Set (Blunt End) (Cat. #6027)

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