Cat. # RR055A

For Research Use

TaKaRa

PrimeScript™ One Step RT-PCR Kit Ver.2

Product Manual

v202108Da

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I. Description

PCR (Polymerase Chain Reaction) is a process that amplifies specific DNA regions using primers on either ends of a targeted DNA segment. PCR is a simple and powerful tool, but it cannot be used to amplify RNA directly. However, with RT-PCR, cDNA is first synthesized from RNA using reverse transcriptase, then targeted regions are amplified from the cDNA template using PCR. This method enables the application of the PCR process to RNA analysis. RT-PCR can be used for various applications including structural analysis of RNA, cDNA cloning, and analysis of gene expression at the RNA level.

The PrimeScript One Step RT-PCR Kit Ver.2 is designed for RT-PCR in a single tube. All components necessary for RT-PCR are mixed in one tube, and therefore it is unnecessary to add reagents at the midpoint of the procedure and the risk of contamination is minimized. This kit includes PrimeScript Reverse Transcriptase, an enzyme developed by Takara Bio based on M-MLV RTase to have excellent extension even for RNA templates containing higher-order structure, and the high efficiency hot-start PCR enzyme *TaKaRa Ex Taq*® HS. This kit contains PrimeScript 1 step Enzyme Mix (a highly optimized premix of PrimeScript RTase, *TaKaRa Ex Taq* HS, and RNase Inhibitor together with a stabilizing agent) and 2X 1 step buffer (a premix containing reaction buffer, dNTP mixture, and 1 step Enhancer Solution). These premix components allow easy preparation of reaction mixtures. The combination of PrimeScript RTase with a high efficiency hot-start PCR enzyme facilitates efficient production of RT-PCR products through efficient reverse transcription at 50℃ by permitting reaction conditions and reduction in non-specific amplification that can arise from mispriming or from primer-dimer during pre-cycling steps.

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

II. Components (50 reactions)

1.	PrimeScript 1 step Enzyme Mix	100 μl
2.	2X 1 step buffer	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 Upstream sense primer for Positive Control RNA
*2 Downstream anti-sense primer for Positive Control RNA

【Primer sequence】

Primer	Sequence
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. An approximately 1.4 kb fragment that includes the tetracycline resistance gene derived from pBR322 is present in this plasmid downstream from the SP6 promoter.

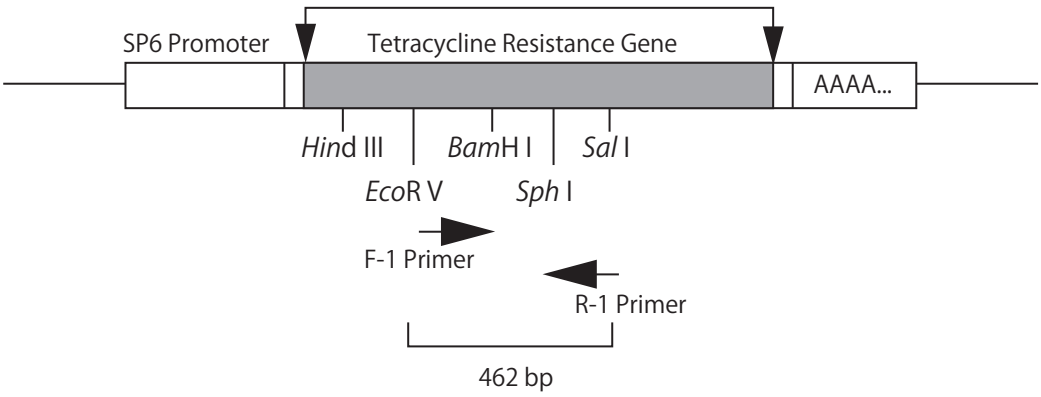


Figure 1. Amplification Products from Positive Control RNA with the Control F-1 and Control R-1 Primers

III. Materials Required but not Provided

DNA Thermal Cycler (authorized instruments)
e.g., TaKaRa PCR Thermal Cycler Dice™ *Touch* (Cat. #TP350)＊
TaKaRa PCR Thermal Cycler Dice Gradient (Cat. #TP600)＊, etc.

Agarose
e.g., Agarose L03 [TAKARA] (Cat. #5003/5003B)
PrimeGel™ Agarose PCR-Sieve (Cat. #5810A)＊, etc.

Electrophoresis Apparatus

Microcentrifuge

Micropipettes and pipette tips (autoclaved)

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

IV. Storage

-20℃

V. Principle

This kit allows reverse transcription from RNA to cDNA using PrimeScript RTase and subsequent PCR amplification using *TaKaRa Ex Taq* HS in the same tube.

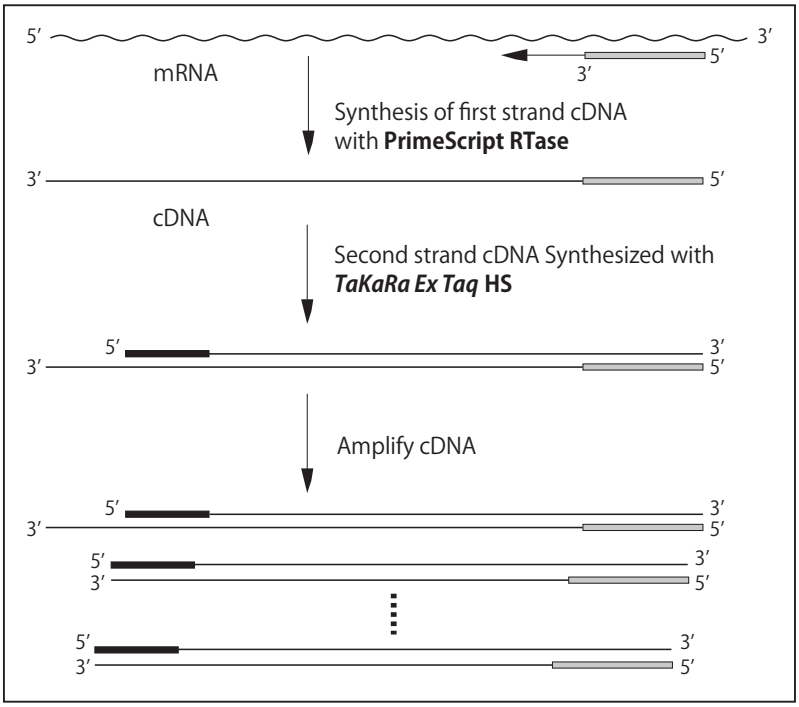


Figure 2. Principle of PrimeScript One Step RT-PCR Kit Ver.2

VI. Specifications

Template RNA	From all species
Amplification product length	Amplification of 8 kb cDNA products has been confirmed
PrimeScript 1 step Enzyme Mix	・PrimeScript RTase ・DNA Polymerase (*TaKaRa Ex Taq* HS) ・RNase Inhibitor
2X 1 step buffer	・Reaction buffer ・dNTP Mixture (final conc. 400 μM) ・One Step Enhancer Solution
Primer for 1st strand cDNA synthesis	Requires sequence-specific downstream primer (Do not use an oligo dT primer or random primers.)
Protocol	RT and PCR carried out sequentially in a single tube

VII. Precautions for Use

Please read and observe the following precautions before using this kit.

(1) Prepare a master mix for at least 10 reactions (minimum) when mixing reagents for PCR. Preparation of a master mix minimizes loss and error due to pipetting, allowing the reagents to be dispensed more accurately. This, in turn, 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 well and then centrifuge it briefly just before use.

(4) Keep the enzyme at -20℃ until just before use. Return to the freezer immediately after use.

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

(6) Always use fresh disposable tips when dispensing reagents to avoid cross-contamination.

(7) A sequence-specific primer is required for the reverse transcription reaction with this kit. Do not use random primers or an oligo dT primer.

VIII. Protocol

1. Prepare the reaction mixture

Reagent	Volume	Final conc.
PrimeScript 1 step Enzyme Mix	2 μl	
2X 1 step buffer	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. Set the reaction tube in a thermal cycler, and perform RT-PCR under the following conditions.

Standard Conditions

(A) When using 3 step PCR

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

(B) When using 2 step PCR

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

For Positive Control RNA*

50℃	30 min	
94℃	2 min	
↓		
94℃	30 sec	30 cycles
60℃	30 sec	
72℃	1 min	

* For the control reaction, 462 bp is amplified.

IX. PCR Conditions

● Annealing temperature

Set the PCR annealing temperature to 60℃ for Positive Control RNA, but change conditions for sequence-specific primers for experimental samples. For experimental samples, determine the optimal annealing temperature by testing temperatures in a range from 55 - 65℃. If necessary, investigate a broader range (45 - 65℃).

● Extension step

The duration of the extension step depends on the size 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. For cloning in a T-Vector, use the Mighty TA-cloning Kit (Cat. #6028).＊ 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).

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

X. Experimental Examples

(1) Using PrimeScript One Step RT-PCR Kit Ver.2, either human heart total RNA or HL60 cell total RNA was used as a template. Target genes of various lengths were amplified using the following protocol.

Target gene	total RNA used
Dystrophin	Human Heart
Transferrin receptor (TFR)	HL60 cell
Cyclin D2 (CCND2)	HL60 cell

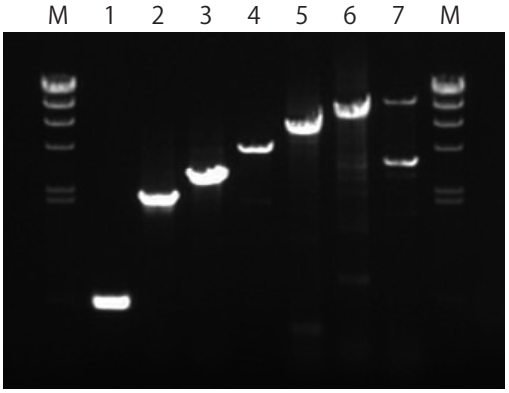
PCR Conditions:

<u>0.5 - 6 kb</u>

50℃	30 min	
94℃	2 min	
↓		
94℃	30 sec	┐
55℃	30 sec	┘ 28 cycles
72℃	1 min/kb	

<u>8 - 12 kb</u>

50℃	30 min	
94℃	2 min	
↓		
98℃	10 sec	┐
68℃	8 or 12 min	┘ 28 cycles



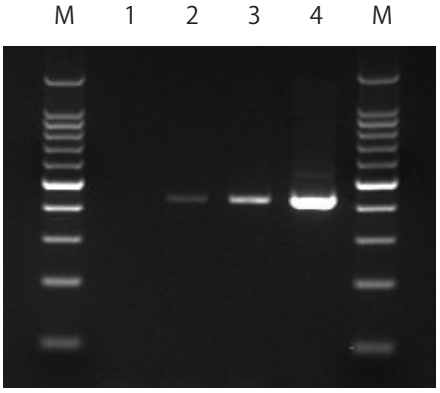
1 : TFR 0.5 kb
2 : CCND2 2.1 kb
3 : CCND2 2.8 kb
4 : TFR 4.4 kb
5 : Dystrophin 6 kb
6 : Dystrophin 8 kb
7 : Dystrophin 12 kb
M: *λ-Hin*d III digest

Excellent extension and amplification was observed for the various target and template types. Products ranged in length from 0.5 - 8 kb.

(2) Using HL60 total RNA as template, the limit of detection of GAPDH gene was measured using this kit according the recommended protocol.

Target: GAPDH 428 bp
RT-PCR conditions:

50℃ 30 min
94℃ 2 min
↓
94℃ 30 sec ┐
55℃ 30 sec ├ 40 cycles
72℃ 1 min ┘



Amount of template (total RNA)
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 total RNA as template.

XI. RNA Sample Preparation

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 the preparation of RNA.

【 Equipment 】

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® (RNase decontamination solution) (Cat. #9037) is recommended for removing RNase from the lab bench, instruments, tubes, etc. Glassware, pipettes, plastic tips and tubes, and other materials used for RNA experiments should be dedicated for use only with RNA.

【 Preparation of RNA sample 】

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 of the highest purity possible.
When preparing high-purity total RNA from cell cultures or tissue samples, NucleoSpin RNA (Cat. #740955.10/.50/.250) or the AGPC method reagent RNAiso Plus (Cat. #9108/9109)＊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™ One Step RT-PCR Kit Ver.2 (Dye Plus) (Cat. #RR057A/B)
PrimeScript™ RT-PCR Kit (Cat. #RR014A/B)
PrimeScript™ 1st strand cDNA Synthesis Kit (Cat. #6110A/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)
Mighty Cloning Reagent Set (Blunt End) (Cat. #6027)
Mighty TA-cloning Kit (Cat. #6028)*

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