Cat. # R026A

For Research Use

TaKaRa

PrimeScript™ II
High Fidelity One Step RT-PCR Kit

Product Manual

v202408Da

Table of Contents

I. Description

PCR (Polymerase Chain Reaction) is a reaction to amplify a specific DNA sequence using two primers that sandwich the target DNA region. However, by synthesizing cDNA from RNA by reverse transcription reaction and then PCR amplifying the target region (RT-PCR), the PCR method can be applied to RNA analysis. To date, this RT-PCR method has been reported to be applied in many fields, such as RNA structural analysis, efficient cDNA cloning, and expression analysis at the RNA level. PrimeScript II High Fidelity One Step RT-PCR Kit is a one-step RT-PCR kit that performs highly accurate RT-PCR in a single tube. PrimeScript II RTase, which has excellent elongation properties for full-length cDNA synthesis, is used for reverse transcriptase, and PrimeSTAR® GXL DNA Polymerase, which is highly accurate and shows excellent performance for PCR amplification of long and GC-rich cDNA, is optimized for one step RT-PCR. PCR is optimized for one-step RT-PCR.
As a result, it is now possible to rapidly and easily obtain accurate cDNA amplification products from a wide range of RNA concentrations for various targets, including long strands and GC-rich regions that are difficult to amplify, under high-speed conditions of 10 min for reverse transcription reaction and 10 sec/kb for PCR elongation time.

This kit exhibits the following features

- Targets can be accurately and conveniently amplified from RNA in a one-step reaction that reduces the risk of contamination.
- Fast reaction time of 10 min for reverse transcription reaction and 10 sec/kb for PCR elongation
- GC rich, supports long cDNA amplification
- 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.

II. Components (for 50 reactions)[*1]

1.	PrimeScript II RT Enzyme Mix	50 μl
2.	PrimeSTAR GXL for 1 step RT-PCR	200 μl
3.	2X One Step High Fidelity Buffer	625 μl x 2
4.	Control F-1 primer[*1] (20 μM)	10 μl
5.	Control R-1 primer[*2] (20 μM)	10 μl
6.	Positive Control RNA (2 x 10^5 copies/μl)	20 μl
7.	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

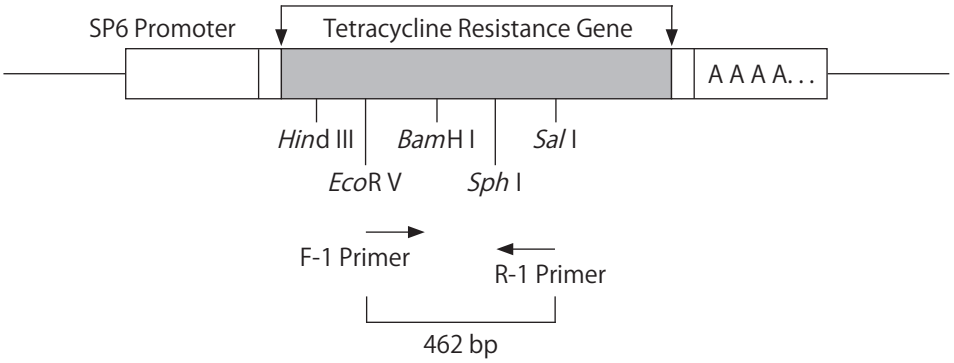


Figure 1. Amplification Products from Positive Control RNA with the Control F-1 and Control R-1 Primers

[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.

Materials Required but not Provided

1. Thermal cycler (authorized for use)
   e.g., TaKaRa PCR Thermal Cycler Dice™ Gradient (Cat. #TP600: discontinued)
   TaKaRa PCR Thermal Cycler Dice *Touch* (Cat. #TP350: discontinued)
2. Agarose
   e.g., Agarose L03 "TAKARA" (Cat. #5003/5003B)
   PrimeGel™ Agarose PCR-Sieve (Cat. #5810A)
3. Electrophoresis apparatus
   e.g., Mupid-2plus (Cat. #M-2P)
   Mupid-exU (Cat. #EXU-1)
4. Microcentrifuge
5. Micropipettes and tips (autoclaved)

III. Storage -20℃

IV. Principle

In PrimeScript II High Fidelity One Step RT-PCR Kit, cDNA synthesis from RNA is first performed by PrimeScript II RTase, followed by PCR amplification by PrimeSTAR GXL for 1 step RT-PCR in the same reaction system. The PrimeSTAR GXL for 1 step RT-PCR is used for PCR amplification.

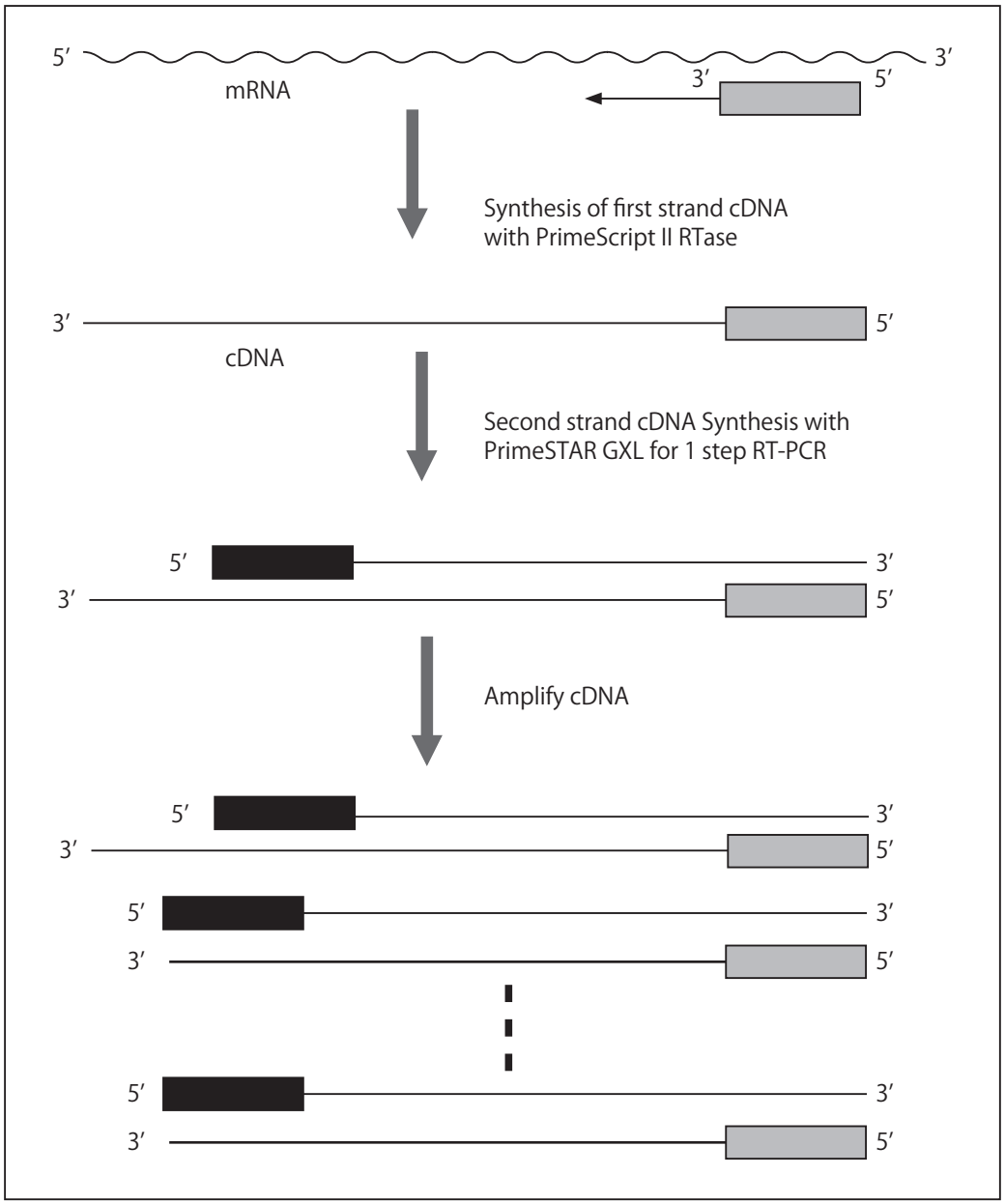


Figure 2. Principle of PrimeScript II High Fidelity One Step RT-PCR Kit

V. Features

Template RNA	From all species
Amplification product length	Amplification of 8 kb cDNA products has been confirmed
Reverse transcriptase	PrimeScript II RTase (used at 45℃)
DNA Polymerase	PrimeSTAR GXL for 1 step RT-PCR
RNase Inhibitor	Required (included in PrimeScript II RT Enzyme Mix)
Primers for 1st strand cDNA synthesis	Requires sequence-specific downstream primer (anti-sense primer for PCR) (Do not use an oligo dT primer or random primers.)
Protocol	RT and PCR carried out sequentially in a single tube

VI. Precautions for Use

Notes: Read these precautions before use and follow them when using this product.

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. PrimeScript II RT Enzyme Mix and PrimeSTAR GXL for 1 step RT-PCR should be mixed gently without foaming. Gently spin down the solution prior to pipetting. 50% glycerol-containing enzymes are highly viscous, so pipet carefully and slowly.

3. 2X One Step High Fidelity Buffer should be vortexed well and centrifuged gently before use.

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

5. Avoid freezing and thawing the Positive Control RNA as much as possible to prevent degradation. Dispensing into small aliquots for storage is recommended. Store Positive Control RNA aliquots at -70 to -80℃ .

6. Use new disposable pipette tips to avoid contamination between samples for transferring reagent.

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.

VII. Protocol

1. Prepare the following mixture.

Reagents	Volume	Final conc.
2X One Step High Fidelity Buffer	25 μl	1X
PrimeScript II RT Enzyme Mix	1 μl	
PrimeSTAR GXL for 1 step RT-PCR	4 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	x μl[*3]	
(or Positive Control RNA	1 μl)	
RNase Free dH_2O	up 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 10 to 1,000 ng (recommended).

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

Standard condition

45℃ [*4]	10 min	
94℃	2 min	
↓		
98℃	10 sec	⎫
60 or 55℃ [*5]	15 sec	⎬ 30 cycles
68℃	10 sec/kb [*6]	⎭

For Positive Control RNA (For the control reaction, 462 bp is amplified.)

45℃	10 min	
94℃	2 min	
↓		
98℃	10 sec	⎫
60℃	15 sec	⎬ 30 cycles
68℃	10 sec	⎭

*4 Reverse transcription reaction temperature
PrimeScript II RTase (included in PrimeScript II RT Enzyme Mix) has improved priming specificity, allowing reverse transcription reactions from downstream primers that require specificity to be performed at 45℃ without the risk of RNA degradation or enzyme inactivation. In the case of amplification of long strands exceeding 6 kb, the amount of cDNA synthesis may be improved by extending the reverse transcription reaction time to 15 min.

*5 Annealing temperature
If the primer's Tm value (calculated by the formula below) exceeds 55℃
→ Set at 60℃
If the primer's Tm value (calculated by the formula below) is less than 55℃
→ Set at 55℃
Tm value calculation
Tm value (℃) = [(number of A, T) x 2] + [(number of G, C) x 4] - 5

*6 Set the extension time at 10 sec. per kb. However, if it is less than 1 kb, set it to 10 sec.

If the above settings do not yield favorable results, consider the following.

< When smear or extra band appears >
(1) Try raising the annealing temperature by 2℃ up to 63℃.
(2) If the primer Tm value is less than 50℃, try annealing at 50 - 55℃.

< When the target product is not amplified (low) >
(1) Adjust the template volume to the recommended conditions.
(2) Increase the number of PCR cycles to 40 - 50 cycles and perform the reaction.
(3) Try lowering the annealing temperature by 2℃.

Note: In PrimeSTAR GXL for 1 step RT-PCR, dUTP cannot be used in place of dTTP (significantly reduces activity). Also, avoid using primers containing inosine.

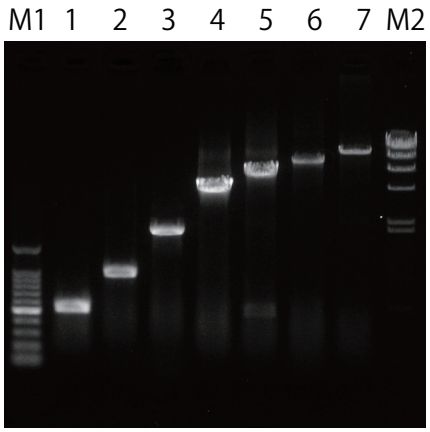
VIII. Experimental Examples

1. Confirmation of amplified chain length

[Methods] Using human heart total RNA, mouse heart total RNA or HL60 cell total RNA as a template (1 μg/50 μl PCR reaction), target genes of various lengths were amplified by one-step RT-PCR.

PCR Condition:

45℃	10 min (15 min when a size is 12 kb)	
94℃	2 min	
↓		
98℃	10 sec	] 30 cycles
55℃	15 sec (60℃ for 8 kb and 12 kb)	
68℃	10 sec/kb	



Target	Size	(Template)
1 : TFR	0.5 kb	(total RNA from HL60)
2 : Dystrophin	1 kb	(total RNA from human heart)
3 : Dystrophin	2 kb	(total RNA from human heart)
4 : TFR	4.4 kb	(total RNA from HL60)
5 : Dystrophin	6 kb	(total RNA from mouse heart)
6 : Dystrophin	8 kb	(total RNA from mouse heart)
7 : Dystrophin	12 kb	(total RNA from mouse heart)

M1 : 100 bp DNA Ladder
M2 : λ-*Hin*d III digest

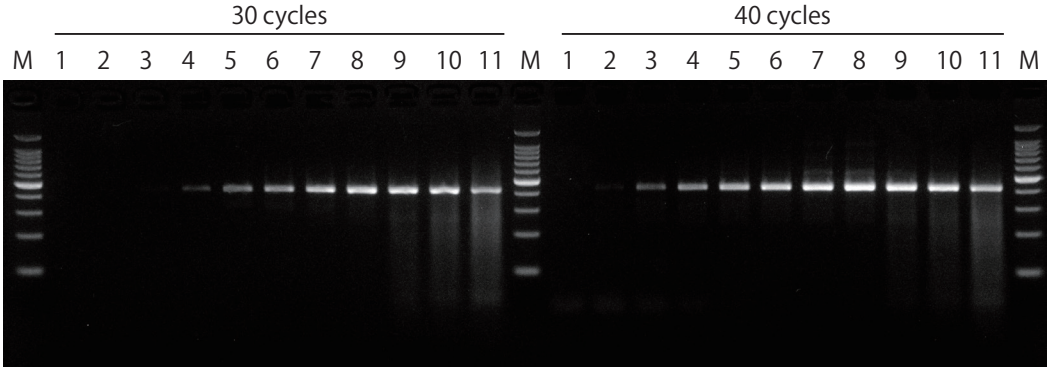
[Result] Amplification for products 0.5 - 12 kb was confirmed.

2. Measurement of detection sensitivity

[Methods] The detection sensitivity was measured by one-step RT-PCR targeting 428 bp of the GAPDH gene using various amounts of HL60 cell total RNA as a template.

PCR Condition:

45℃	10 min	
94℃	2 min	
↓		
98℃	10 sec.	┐
55℃	15 sec	├ 30 or 40 cycles
68℃	10 sec	┘



Template amount (HL60 cell total RNA)

1 : 10 fg	7 : 10 ng
2 : 100 fg	8 : 100 ng
3 : 1 pg	9 : 1 μg
4 : 10 pg	10 : 2 μg
5 : 100 pg	11 : 4 μg
6 : 1 ng	M : 100 bp DNA Ladder

[Results] Detection of the target gene was observed using10 pg (30 cycles) or 100 fg (40 cycles) of total RNA as template. In addition, a good reaction was also observed when 4 μg of total RNA was used, confirming that the allowable range of template amounts that can be used for the reaction is wide.

IX. Preparation of RNA Sample

This kit is designed to perform reverse transcription of RNA to cDNA and subsequent amplification. It is important to use a high purity RNA sample for greater yields of cDNA. Therefore, it is essential to inhibit the activity of RNase in the cells and to prevent contamination of RNase derived from equipment and solutions used. Additional precautions should be taken during the sample preparation, such as using clean disposable gloves, dedicating a workspace exclusively for RNA preparation, and avoiding unnecessary talking during operation to prevent contamination of RNase from sweat or saliva.

[Equipment]

Disposable plastic equipment should be used. For glass tools, treat with the following procedure prior to use.

(1) Treat the glass tools with 0.1% diethylpyrocarbonate (DEPC) at 37℃ for 12 hours.
(2) Autoclave at 120℃ for 30 min to remove DEPC.

It is recommended that RNase-OFF® (Cat. #9037) be used for RNase removal from table, instruments, tubes, and others. It is also recommended that all equipment be used exclusively for RNA preparation.

[Preparation of RNA samples]

For preparation of high purity total RNA from cultured cells or tissue samples, the spin column type NucleoSpin RNA (Cat. #740955.10/.50/.250) or RNAiso Plus (Cat. #9108/9109), a reagent that simplifies the AGPC method, are useful. NucleoSpin RNA Blood (Cat. #740200.10/.50) can also be used for extraction from blood.

X. Electrophoresis, Cloning, and Restriction Enzyme Treatment of Amplified Products

1. Electrophoresis
   TAE Buffer is recommended for agarose gel electrophoresis of amplified products that are obtained with this kit; use of TBE Buffer may result in DNA band patterns that are enlarged at the bottom of the gel.

2. Cloning of amplification products
   Most PCR products amplified with this kit have blunt-end termini. Accordingly, they can be cloned directly into blunt-end vectors. If necessary, phosphorylate the amplified products before cloning. Use of Mighty Cloning Reagent Set (Blunt End) (Cat. #6027) is recommended for cloning into a blunt-end vector. If you want to clone into T-vectors, add dA to the 3′ end of the amplified product with Mighty TA-cloning Reagent Set (Cat. #6019)*.

   * Not available in all geographic regions. Please check availability in your area.

3. Restriction enzyme reaction
   Prior to performing restriction enzyme digestion of amplified PCR products, remove all
   traces of proteins from the reaction mixture by phenol/chloroform extraction. Particularly for 3′-protruding restriction enzymes such as *Pst* I,
   the 3′-protruding termini produced by these enzymes may be deleted by 3′ → 5′ exonuclease activity of PrimeSTAR GXL for 1 step RT-PCR, if residual polymerase remains present in the restriction digest reaction.

XI. Related Products

PrimeScript™ II 1st strand cDNA Synthesis Kit (Cat. #6210A/B)
PrimeScript™ 1st strand cDNA Synthesis Kit (Cat. #6110A/B)
PrimeScript™ II Reverse Transcriptase (Cat. #2690A/B/C)*
PrimeScript™ Reverse Transcriptase (Cat. #2680A/B/C)
PrimeSTAR® Max DNA Polymerase (Cat. #R045A/B)
PrimeSTAR® GXL DNA Polymerase (Cat. #R050A/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)
PrimeScript™ High Fidelity RT-PCR Kit (Cat. #R022A/B)
PrimeScript™ II High Fidelity RT-PCR Kit (Cat. #R023A/B)*
Agarose L03 "TAKARA" (Cat. #5003/5003B)
PrimeGel™ Agarose PCR-Sieve (Cat. #5810A)
RNase-OFF® RNase Contamination Removal Solution (Cat. #9037)
NucleoSpin RNA (Cat. #740955.10/.50/.250)*
RNAiso Plus (Cat. #9108/9109)
NucleoSpin RNA Blood (Cat. #740200.10/.50)*
Mighty TA-cloning Reagent Set for PrimeSTAR® (Cat. #6019)*
Mighty Cloning Reagent Set (Blunt End) (Cat. #6027)

* Not available in all geographic regions. Please check availability in your area.