Cat. # RR047A

For Research Use

TaKaRa

PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time)

Product Manual

v201903Da

Table of Contents

I. Description

In order to accurately perform gene expression analysis, it is necessary to detect cDNA in samples without contamination from genomic DNA. By designing primers on exon regions spanning introns, it is possible in many cases to avoid amplification derived from genomic DNA. However, there may be cases where a suitable primer cannot be designed, as with a gene with a single exon or a gene without a long intron. In addition, it may be difficult to avoid unexpected amplification from genomic DNA due to non-specific amplification or the existence of a pseudo-gene. RNA samples can be pretreated with DNase I, but this treatment involves deactivating and eliminating DNase I, which may lead to degradation or loss of RNA.
PrimeScript RT reagent Kit with gDNA Eraser is a reverse-transcription kit for real-time RT-PCR (RT-qPCR) that includes a genomic DNA elimination reaction. cDNA synthesis from RNA can be achieved without sample loss in a rapid reaction that is complete in less than 20 min. Genomic DNA is eliminated by treatment for 2 min at 42℃ with gDNA Eraser, which has potent DNA degradation activity. Then a reverse-transcription reaction reagent is added that includes a component which completely inhibits DNA degradation activity, and the reverse-transcription reaction proceeds for 15 min.
The cDNA obtained using this product can be used with either intercalator qPCR assay or probe qPCR assay. Please use in combination with quantitative PCR reagent such as TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B)＊1, TB Green *Premix Ex Taq* (Tli RNaseH Plus) (Cat. #RR420A/B)＊1, and Probe qPCR Mix (Cat. #RR391A)＊2.

＊1 We have begun the process of changing the names for Takara Bio's intercalator-based real-time PCR (qPCR) products to the "TB Green series". These products can be used the same way as before, as only the names are changing. Catalog number and product performance are unaffected by this transition.

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

II. Components (100 reactions, 20 μl per reaction)

1.	gDNA Eraser	100 μl
2.	5X gDNA Eraser Buffer＊3	200 μl
3.	PrimeScript RT Enzyme Mix I＊4	100 μl
4.	5X PrimeScript Buffer 2 (for Real Time)＊5	400 μl
5.	RT Primer Mix＊6	400 μl
6.	RNase Free dH_2O	1 ml x 2
7.	EASY Dilution (for Real Time PCR)＊7	1 ml

＊3 Because 5X gDNA Eraser Buffer is needed for the subsequent reverse-transcription reaction, please be sure to perform the genomic DNA elimination reaction.

＊4 Contains RNase inhibitor.

＊5 Contains dNTP mixture.

＊6 Contains Oligo dT Primer and Random 6 mers.

＊7 To be used when producing serial dilutions of total RNA and cDNA. EASY Dilution (for Real Time PCR) makes it possible to obtain a precise dilution down to very low concentrations. Moreover, this solution will not affect reverse-transcription or PCR reactivity. You can use the diluted template solution directly in reverse-transcription and PCR reactions.
EASY Dilution (for Real Time PCR) may also be purchased separately (Cat. #9160).

Note: Please use EASY Dilution (for Real Time PCR) in combination with Takara Bio's real-time PCR Reagents. Compatibility with products from other manufacturers has not yet been verified.

III. Materials Required but not Provided

- Thermal cycler (or 37℃ water bath, 42℃ water bath and 85℃ heat block)
- 0.2 ml and 1.5 ml microtubes (for reverse-transcription reaction)
- Micropipettes and tips (autoclaved)

IV. Storage -20℃

V. Features

1. With gDNA Eraser, you can eliminate genomic DNA in just 2 min.

2. Template cDNA for real-time PCR can be efficiently synthesized in just 15 min. This kit is best suited for 2 step RT-PCR.

3. All regions of RNA can be uniformly synthesized when an RT Primer Mix combining Random 6 mers and Oligo dT Primer is used as the primer for reverse transcription.

4. Protocols are prepared for use with intercalator (TB Green) qPCR assay and probe qPCR assay. Please select the protocol based on the assay method to be used for real-time PCR.

   There are the following differences between the protocols for TB Green qPCR assay and probe qPCR assay.
   - Amount of RT Primer Mix used in reverse-transcription reaction
   - Amount of total RNA capable of using in reverse-transcription reaction

5. A standard curve must be generated for the quantification of real-time RT-PCR. To generate a standard curve, it is important to dilute the total RNA and reverse-transcribed cDNA precisely to low concentrations. However, dilution with water or TE buffer can narrow the range of the curve due to template instability at low concentrations. Using EASY Dilution (for Real-Time PCR) results in more accurate measurements at low template concentrations, facilitating the creation of a broad-range standard curve.

VI. Precautions for Use

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

1. When performing a TB Green real-time PCR assay with cDNA resulting from this protocol, use TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820A/B) or TB Green *Premix Ex Taq* (Tli RNaseH Plus) (Cat. #RR420A/B) in order to obtain highly reliable results. DO NOT use TB Green Fast qPCR Mix (Cat. #RR430A/B) for real-time PCR, as it may result in an abnormal reaction.

2. Prepare sufficient Master Mix (mixture of RNase Free dH_2O, buffer, and enzyme) for up to 10 reactions to minimize reagent loss due to pipetting. Minimizing the number of times kit reagents are dispensed will result in more accurate dispensing and less variation in experimental data.

3. Briefly centrifuge gDNA Eraser and PrimeScript RT Enzyme Mix I before use and allow the reagent to settle to the bottom of the tube. Pipette the enzyme slowly and carefully, since it is in 50% glycerol solution and high in viscosity.

4. Vortex the 5X gDNA Eraser Buffer and 5X PrimeScript Buffer 2 (for Real Time) well and centrifuge briefly before use.

5. When dispensing the reagent, be sure to use a new disposable tip to avoid contamination between samples.

VII. Protocol

1. Genomic DNA elimination reaction

Prepare the genomic DNA elimination reaction solution on ice.

Prepare a master mix for components other than the RNA sample in a volume sufficient for the number of reactions plus 2. Dispense an appropriate volume of master mix (total volume minus total RNA volume) into a microtube and then add the RNA sample.

<Per reaction>

Reagent	Volume
5X gDNA Eraser Buffer	2.0 μl
gDNA Eraser	1.0 μl
total RNA＊1	
RNase Free dH_2O	
Total	10.0 μl

↓

42℃ 2 min (or room temperature, 5 min)＊2

Store at 4℃

＊1 Up to 1 μg of total RNA for intercalator (TB Green) qPCR assay and up to 2 μg of total RNA for probe qPCR assay can be used in a 20 μl reverse-transcription reaction.

＊2 If the reaction is to take place at room temperature, then allow the reaction to go for about 30 min.

2. Reverse-transcription reaction

Prepare the reverse-transcription reaction solution on ice.

Prepare a master mix in a sufficient volume for the number of reactions plus 2. The master mix should contain all components except the genomic DNA elimination reaction solution. Add 10 μl of master mix to the reaction solution＊3 from step 1 and then mix gently. Proceed immediately with the reverse-transcription reaction.

【 for intercalator (TB Green) qPCR assay 】

<Per reaction>

Reagent	Volume	
Reaction solution from Step 1	10.0 μl	
5X PrimeScript Buffer 2 (for Real Time)	4.0 μl	Master mix 10 μl
PrimeScript RT Enzyme Mix I	1.0 μl	
RT Primer Mix＊4	1.0 μl	
RNase Free dH_2O	4.0 μl	
Total	20.0 μl＊5	

↓

37℃ 15 min＊6

85℃ 5 sec

Store at 4℃＊7

【 for probe qPCR assay 】

<Per reaction>

Reagent	Volume	
Reaction solution from Step 1	10.0 μl	
5X PrimeScript Buffer 2 (for Real Time)	4.0 μl	Master mix 10 μl
PrimeScript RT Enzyme Mix I	1.0 μl	
RT Primer Mix＊4	4.0 μl	
RNase Free dH_2O	1.0 μl	
Total	20.0 μl＊5	

↓

37℃ 15 min＊6

85℃ 5 sec

Store at 4℃＊7

＊3 When adding reagents individually (instead of preparing a master mix), add RNase Free dH_2O and 5X PrimeScript Buffer 2 (for Real Time) to the reaction solution from Step 1 first and mix. Add RT Primer Mix and PrimeScript RT Enzyme Mix after the activity of gDNA Eraser has been completely inhibited. Gently mix the reaction solution and then perform the reverse-transcription reaction.

＊4 If RT Primer Mix is used, cDNA can be synthesized efficiently along the full length of mRNA. It is also possible to use an Oligo dT primer and Gene Specific Primer that have been prepared separately. Use the following amount of each primer.

Oligo dT primer	50 pmol/20 μl reaction system
Gene Specific Primer	5 pmol/20 μl reaction system

＊5 The scale of the reverse-transcription reaction can be increased as necessary.

＊6 When Gene Specific Primer is used, please allow the reverse-transcription reaction to proceed at 42℃ for 15 min. If non-specific amplification occurs in PCR, improvement may be achieved by changing the reverse-transcription temperature to 50℃.

＊7 When storing the cDNA synthesis product for long periods of time, please store at or below -20℃.

Note:

- The RT Primer Mix volume in the RT reaction for intercalator (TB Green) qPCR assay is 1 μl, while the RT Primer Mix volume in the RT reaction for probe qPCR assay is 4 μl.
- If the reverse-transcription reaction solution is to be introduced to a real-time PCR system, the reverse-transcription reaction solution volume should correspond to 10% or less of the PCR reaction solution volume.

VIII. Real-Time PCR

The following protocol is for real-time PCR using TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820A). The RT reaction solution is prepared using this kit (see Section VII).

【 Method using the Thermal Cycler Dice™ Real Time System 】

1. Prepare the PCR reaction mixture shown below.

<Per reaction>

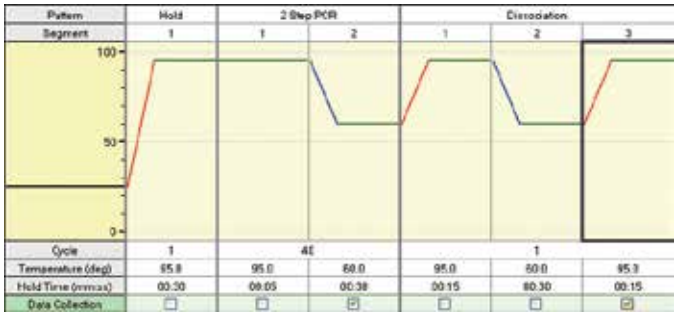
Reagent	Volume	Final conc.
TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (2X)	12.5 μl	1X
PCR Forward Primer (10 μM)	1.0 μl	0.4 μM＊1
PCR Reverse Primer (10 μM)	1.0 μl	0.4 μM＊1
RT reaction solution (cDNA)＊2	2.0 μl	
Sterile purified water	8.5 μl	
Total	25 μl	

＊1 Good results are mostly obtained with a final primer concentration of 0.4 μM, but when there is a problem with reactivity, it is best to consider an optimal concentration in the range of 0.2 - 1.0 μM.

＊2 It is preferable to use a quantity of cDNA corresponding to 10 pg - 100 ng of total RNA template. In addition, the volume of RT reaction solution should correspond to 10% or less of the PCR reaction solution volume.

2. Start the reaction.

Shuttle PCR standard protocol (below) is recommended. Try this protocol first, and optimize the reaction condition if needed. When the shuttle protocol is difficult due to a primer with low T_m value, etc., try a 3 step PCR protocol.



Shuttle PCR standard protocol

Hold (initial denaturation)
Cycle：1
95℃ 30 sec
2 Step PCR
Cycles：40
95℃ 5 sec
60℃ 30 - 60 sec
Dissociation

Note:
TaKaRa Ex Taq® HS used in this product is a hot-start PCR enzyme utilizing an anti-*Taq* antibody that suppresses polymerase activity. The initial denaturation step prior to PCR should be at 95℃ for 30 sec. Longer heat treatment may decrease enzyme activity and affect amplification efficiency and quantification.

3. After the reaction is complete, check the amplification and melting curves and plot a standard curve if a quantitative assay will be performed.
When using Thermal Cycler Dice Real Time System, please refer to its instruction manual for analytical methods.

【 Method using the Applied Biosystems 7300/7500 Real-Time PCR System 】

＊ Please follow the manual for each model.

1. Prepare the PCR reaction mixture shown below.

<Per reaction>

Reagent	Volume	Volume	Final conc.
TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (2X)	10 μl	25 μl	1X
PCR Forward Primer (10 μM)	0.8 μl	2 μl	0.4 μM＊1
PCR Reverse Primer (10 μM)	0.8 μl	2 μl	0.4 μM＊1
ROX Reference Dye or Dye II (50X)＊3	0.4 μl	1 μl	1X
RT reaction solution (cDNA solution)＊2	2 μl	4 μl	
Sterile purified water	6 μl	16 μl	
Total	20 μl＊4	50 μl＊4	

＊1 Good results are mostly obtained with a final primer concentration of 0.4 μM, but when there is a problem with reactivity, it is best to consider an optimal concentration in the range of 0.2 - 1.0 μM.

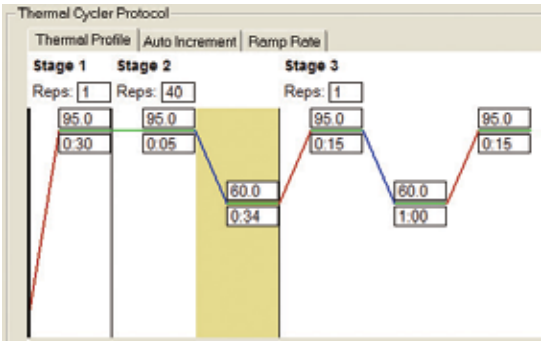
＊2 In a 20 μl reaction volume, it is preferable to use a quantity of cDNA corresponding to 10 pg - 100 ng of total RNA template. In addition, the volume of RT reaction solution should correspond to 10% or less of the PCR reaction solution volume.

＊3 The concentration for ROX Reference Dye II (50X) is lower than that for ROX Reference Dye (50X). When performing analysis with 7500 Real-Time PCR System, use ROX Reference Dye II (50X). With 7300 Real-Time PCR System, use ROX Reference Dye (50X).

＊4 Prepare in accordance with the recommended volume for each instrument.

2. Start the reaction.

Shuttle PCR standard protocol (below) is recommended. Try this protocol first, and optimize the reaction condition if needed. When the shuttle protocol is difficult due to a primer with low T_m value, etc., try a 3 step PCR protocol.



Shuttle PCR standard protocol

Stage 1: Initial denaturation
Reps: 1
95℃ 30 sec

Stage 2: PCR reaction
Reps: 40
95℃ 5 sec
60℃ 31 sec, 34 sec＊

Dissociation Stage

＊ Set to 31 sec for 7300, and 34 sec for 7500.

Note:
TaKaRa Ex Taq HS used in this product is a hot-start PCR enzyme utilizing an anti-*Taq* antibody that suppresses polymerase activity. The initial denaturation step prior to PCR should be at 95℃ for 30 sec. Longer heat treatment may decrease enzyme activity and affect amplification efficiency and quantification.

3. After the reaction is complete, check the amplification and melting curves and plot a standard curve if a quantitative assay will be performed.

IX. Experimental Examples

A. Removal of genomic DNA

[Method]

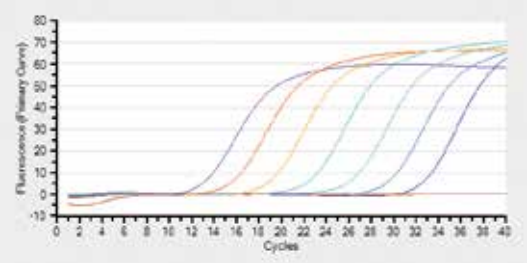
cDNA synthesis was performed on total RNA with added genomic DNA, using this product (Cat. #RR047A) or PrimeScript RT Master Mix (Perfect Real Time) (Cat. #RR036A), according to each product's protocol.
The resulting cDNA was used for qPCR analysis.

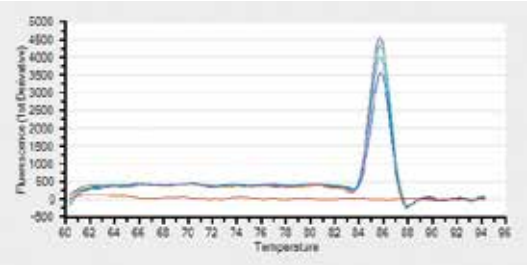
- Template : total RNA from HL60 cells
  (0 pg, 1 pg, 10 pg, 100 pg, 1 ng, 10 ng, 100 ng, and 1 μg)
  + 1 ng of human genomic DNA
- qPCR : TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820A)
- Template volume in the qPCR reaction : 2 μl of each RT reaction mixture
- Target gene : *RPLP1*
- Primer : Original design
- Instrument : Thermal Cycler Dice Real Time System *II* (Cat. #TP900)

[Results]

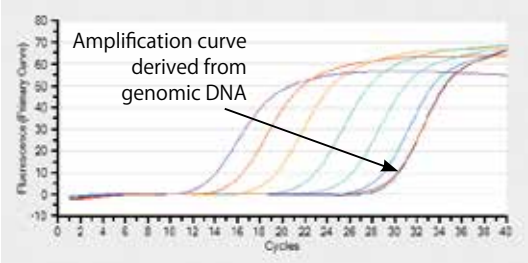
The genomic DNA added to the total RNA sample was completely removed by the gDNA Eraser in this product, yielding amplification curves only from cDNA.

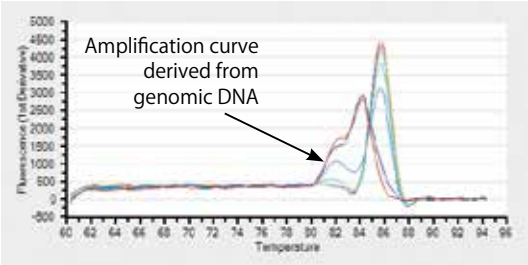
RR047A





RR036A





B. Comparison of cDNA synthesis efficiency

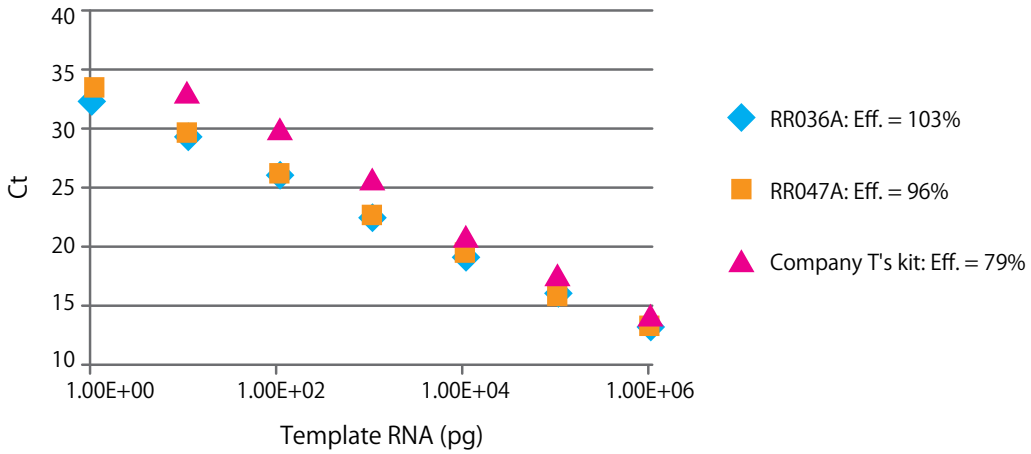
[Method]

cDNA synthesis was performed on total RNA with this product (Cat. #RR047A), PrimeScript RT Master Mix (Perfect Real Time) (Cat. #RR036A), or Company T's kit, according to each product's protocol. The resulting cDNA was used for qPCR analysis.

- Template : total RNA from HL60 cells
  (0 pg, 1 pg, 10 pg, 100 pg, 1 ng, 10 ng, 100 ng, and 1 μg)
- qPCR : TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820A)
- Template volume in the qPCR reaction : 2 μl of each RT reaction mixture
- Target gene : *RPLP1*
- Primer : Original design
- Instrument : Thermal Cycler Dice Real Time System *II* (Cat. #TP900)

[Results]

This product has the same cDNA synthesis efficiency as PrimeScript RT Master Mix (Perfect Real Time) and a higher efficiency than Company T's kit. Additionally, this product can complete the reaction more quickly than the other two kits.



	RR047A	Company T's kit
DNA removal	42℃ 2 min	37℃ 5 min
RT reaction	37℃ 15 min	37℃ 15 min 50℃ 5 min
	85℃ 5 sec	98℃ 5 min
Total reaction time	~ 17 min	~ 30 min

(Our comparison data)

X. Appendix

1. Preparation of RNA Samples

It is important to use highly pure RNA samples for better cDNA yield. It is essential to inhibit contaminant RNase activity in RNA preparations, and also to prevent RNase derived from equipment and solutions used. Extra precautions should be taken during sample preparation, including use of clean disposable gloves and a table used exclusively for RNA preparation, and avoiding RNase contamination from operator sweat or saliva.

A. Equipment

Disposable plastic equipment should be used. Glass tools should be treated with the following procedure prior to use.

(1) Hot-air sterilization (180℃, 60 min)
(2) Sterilize glassware in 0.1% diethyl pyrocarbonate (DEPC) solution at 37℃ for 12 hours and then autoclave (120℃, 30 min) to remove any residual DEPC.

It is recommended that all the equipment be used exclusively for RNA preparation.

B. Reagents

All reagents to be used in this experiment must be prepared using tools which were treated as described (Hot-air sterilization (180℃, 60 min) or DEPC treatment), and all purified water must be treated with 0.1% DEPC and autoclaved.

All reagents and purified water should be used exclusively for RNA experiments.

C. Method of Preparation for RNA Samples

Use of highly purified RNA obtained by GTC (Guanidine thiocyanate) or other comparable method is recommended. RNA isolation kits such as RNAiso Plus (Cat. #9108/9109) can also be used for isolating high purity total RNA. The purified RNA sample should be dissolved in sterilized purified water or sterilized TE buffer.

XI. Related Products

TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B)
TB Green® *Premix Ex Taq*™ (Tli RNaseH Plus) (Cat. #RR420A/B)
Probe qPCR Mix (Cat. #RR391A/B)*
EASY Dilution (for Real Time PCR) (Cat. #9160)
Thermal Cycler Dice™ Real Time System III (Cat. #TP950/TP970/TP980/TP990)＊
Thermal Cycler Dice™ Real Time System *II* (Cat. #TP900/TP960)＊
Thermal Cycler Dice™ Real Time System *Lite* (Cat. #TP700/TP760)＊
PrimeScript™ RT reagent Kit (Perfect Real Time) (Cat. #RR037A)
PrimeScript™ RT Master Mix (Perfect Real Time) (Cat. #RR036A)＊
RNAiso Plus (Cat. #9108/9109)

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