Cat. # RR092S
RR092A

For Research Use

TaKaRa

PrimeScript™ FAST RT reagent Kit with gDNA Eraser

Product Manual

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I. Description

PrimeScript FAST RT reagent Kit with gDNA Eraser is a specialized reverse transcription kit for real-time RT-PCR that includes a genomic DNA (gDNA) elimination reaction. Residual gDNA in the template RNA is eliminated by treatment at 42℃ for 2 min with gDNA Eraser, which has potent DNA degradation activity. A reverse-transcription reaction reagent with a component which completely inhibits DNA degradation activity is then added, and the reverse-transcription reaction proceeds for 10 min. Because the product comes with two premixes, one for genome elimination reagent and the other for reverse-transcription reactions, a simple protocol of adding a series of reagents to the RNA sample allows the reaction to proceed from genome elimination to cDNA synthesis without loss.

The kit is ideal for analysis by real-time RT-PCR in cases where genome residues are a problem, such as when an appropriate primer cannot be designed (for example, with a single-exon gene or gene without a large intron), when amplification of gDNA cannot be avoided because of the presence of pseudogenes or nonspecific amplification, etc.

cDNA obtained with this product can be used in real-time PCR assays performed by either the intercalator method or the probe-detection method. We suggest using the kit in combination with quantitative PCR reagents such as TB Green® *Premix Ex Taq*™ II Fast qPCR (Cat. #RR830S/A/B), TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820S/A/B), TB Green *Premix Ex Taq* (Tli RNaseH Plus) (Cat. #RR420S/A/B), Probe qPCR Mix (Cat. #RR391A/B), or Probe qPCR Mix, with UNG (Cat. #RR392A/B), depending on the purpose.

II. Components [40 reactions (RR092S) / 100 reactions (RR092A), 20 μl volume per reaction]

	Cat. # RR092S	RR092A
8X gDNA Eraser Premix*1	80 μl	200 μl
5X RT Premix*2	160 μl	400 μl
RNase Free H_2O	1 ml	1 ml x 2
EASY Dilution II (for Real Time PCR)*3, 4	1 ml	1 ml

*1 The components of the 8X gDNA Eraser Premix are necessary for the subsequent reverse-transcription reaction, so be sure to perform the gDNA elimination reaction. Contains RNase inhibitor.

*2 Contains dNTP mixture, Oligo dT Primer, and Random 6 mers.

*3 Use as dilution solution to make serial dilutions of total RNA or cDNA. Accurate dilution with water or TE is difficult, but EASY Dilution II (for Real Time PCR) makes accurate dilution down to low concentrations possible. Moreover, this buffer never affects the reactivity of reverse transcription or PCR. The diluted template solution can be used as-is for the template for the reverse-transcription or PCR reaction.

*4 Can be purchased separately [EASY Dilution II (for Real Time PCR) (Cat. #9451)].

III. Storage -20℃

IV. Materials Required but Not Provided

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

V. Features

1. Reaction proceeds from DNA elimination to cDNA synthesis in just 15 min.

2. Reaction performed with a simple protocol consisting of adding premixed reagents in sequence.

3. cDNA synthesized from template RNA without loss through a single-tube reaction.

4. All regions of RNA are uniformly synthesized when 5X RT Premix containing Random 6 mers and Oligo dT Primer is used as the primer for reverse transcription.

5. With EASY Dilution II (for Real Time PCR) (included), total RNA and cDNA after reverse transcription can be accurately diluted down to low concentrations, and it's possible to prepare real-time PCR calibration curves over a wide range.

VI. Precautions for Use

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

1. It is recommended that you use the following TB Green Premix series when performing a TB Green assay with cDNA solution prepared with this product as the template. Highly reliable results can be obtained by using TB Green Premix with this product.

   ・TB Green *Premix Ex Taq* II Fast qPCR (Cat. #RR830S/A/B)
   ・TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820S/A/B)
   ・TB Green *Premix Ex Taq* (Tli RNaseH Plus) (Cat. #RR420S/A/B)

   Note: Using this product in combination with TB Green Fast qPCR Mix (Cat. #RR430S/A/B) may result in poor reactivity and is not recommended.

2. Before using 8X gDNA Eraser Premix and 5X RT Premix, invert tube several times to mix and centrifuge gently.

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

VII. Protocol

1. gDNA elimination reaction

   Prepare the gDNA elimination reaction solution on ice as shown below.

   Mix together all the components other than the RNA sample. Prepare slightly more than needed, and add the RNA sample after dispensing into PCR tubes.

<Per reaction>

Reagent	Volume
8X gDNA Eraser Premix	2 μl
RNA sample*1	x μl
RNase Free H_2O	up to 16 μl
Total	16 μl

↓

42℃ 2 min (or room temperature, 5 min*2)
4℃

*1 In the next step, reverse transcription can be performed with up to approximately 1 μg of total RNA for a TB Green assay (intercalator method) and up to approximately 2 μg of total RNA for a probe assay in a 20 μl reverse-transcription reaction system. The reverse-transcription reaction can be scaled up, if necessary.

*2 If the reaction takes place at room temperature, it can be allowed to go for up to 30 min without adverse effect.

2. Reverse-transcription reaction

   On ice, add 4 μl of 5X RT Premix to each tube of reaction solution from Step 1.

   Mix well by gently pipetting the solution up and down, then perform the reverse-transcription reaction.

<Per reaction>

Reagent	Volume
Reaction solution from Step 1	16 μl
5X RT Premix	4 μl
Total	20 μl

↓

37℃ 10 min
85℃ 5 sec
4℃ *3

*3 For long-term storage of cDNA synthesis products, store at -20℃ or lower.

Note: When incorporating a reverse-transcription reaction solution into real-time PCR, the volume of the reverse-transcription reaction solution should not exceed 10% of the volume of the PCR reaction solution.

VIII. Real-time PCR

The following is an example of the protocol for performing real-time PCR with TB Green *Premix Ex Taq* II Fast qPCR (Cat. #RR830S/A/B) after performing a reverse-transcription reaction with a reverse-transcription reaction with PrimeScript FAST RT reagent Kit with gDNA Eraser.

Note: Please follow the procedures in the manual provided with each respective instrument.

1. Prepare the PCR reaction solution shown below.

<Per reaction>

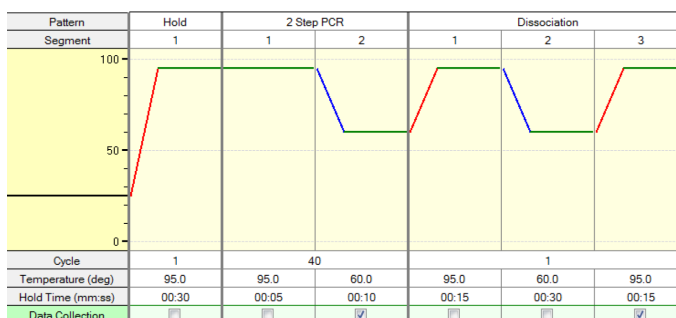
Reagent	Volume	Final conc.
TB Green *Premix Ex Taq* II Fast qPCR (2X)	12.5 μl	1X
PCR Forward Primer (10 μM)	1.0 μl	0.4 μM[*1]
PCR Reverse Primer (10 μM)	1.0 μl	0.4 μM[*1]
cDNA template (reverse-transcription reaction solution)[*2]	≤ 2.5 μl	
Sterile purified water	x μl	
Total	25.0 μl	

*1 In many cases, good results can be obtained with a final primer concentration of 0.4 μM, but when there are problems with reactivity, please consider an optimal concentration within the range of 0.2 - 1.0 μM.

*2 The amount of cDNA template (reverse-transcription reaction solution) to be added should not be more than 10% of the volume of the PCR reaction solution.

2. Begin the reaction.
It is recommended that you perform the PCR reaction according to the following two-step PCR standard protocol. Try this protocol first, and then optimize the PCR conditions as necessary. If the reaction is difficult with two-step PCR, such as with a primer with a low Tm value, perform three-step PCR instead.

Two-step PCR standard protocol



Hold (Initial Denaturation)
Cycle：1
95℃ 30 sec
Two-Step PCR
Cycles：40
95℃ 5 sec
60℃ 10 sec
Dissociation

3. After the reaction ends, check the amplification curve and melting curve. Prepare a calibration curve if quantitation is to be performed.

For details about the analysis method, refer to the instruction manual of the real-time PCR instrument.

IX. Related Products

PrimeScript™ RT reagent Kit (Perfect Real Time) (Cat. #RR037A/B)
PrimeScript™ RT Master Mix (Perfect Real Time) (Cat. #RR036A/B)
TB Green® *Premix Ex Taq*™ II Fast qPCR (Cat. #RR830S/A/B)
TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820S/A/B)
TB Green® *Premix Ex Taq*™ (Tli RNaseH Plus) (Cat. #RR420S/A/B)
TB Green® *Premix Ex Taq*™ GC (Perfect Real Time) (Cat. #RR071A/B)*
TB Green® Premix DimerEraser™ (Perfect Real Time) (Cat. #RR091A/B)*
Probe qPCR Mix (Cat. #RR391A/B)
Probe qPCR Mix, with UNG (Cat. #RR392A/B)
EASY Dilution II (for Real Time PCR) (Cat. #9451)
Thermal Cycler Dice™ Real Time System IV (Cat. #TP1000/TP1010/TP1030)*
Thermal Cycler Dice™ Real Time System III (Cat. #TP950/TP970/TP980/TP990)*
NucleoSpin RNA (Cat. #740955.10/.50/.250)*
RNAiso Plus (Cat. #9108/9109)

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

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