

For Research Use

TakaRa

Probe qPCR Mix

Product Manual

v201710Da



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I. Introduction

Probe qPCR Mix is designed for probe-based qPCR. This product is also suitable for high-speed PCR. Probe qPCR Mix allows accurate target quantification and detection over a broad dynamic range and makes it possible to perform highly reproducible and reliable real-time PCR analyses. The product is supplied as a 2X premix to facilitate easy preparation of reaction mixtures. As the 2X premixed reagent contains Tli RNase H, a heat-resistant RNase H, it can minimize PCR inhibition by residual mRNA in reactions using cDNA templates. A combination of an optimized hot-start PCR enzyme, an anti-*Taq* antibody, and a buffer optimized for real-time PCR results in excellent suppression of non-specific amplification, high amplification efficiency, and high detection sensitivity in real-time PCR analyses.

Compatible Instrument Systems Include:

- Thermal Cycler Dice[™] Real Time System III (Cat. #TP950/TP970/TP980/TP990)^{*}
- Thermal Cycler Dice Real Time System // (Cat. #TP900/TP960)*
- Thermal Cycler Dice Real Time System *Lite* (Cat. #TP700/TP760)*
- Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System and StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)
- LightCycler 96/480 System (Roche Diagnostics)
- CFX96 Real-Time PCR Detection System (Bio-Rad)
- Smart Cycler System/Smart Cycler II System (Cepheid)
- * Not available in all geographic locations. Check for availability in your area.

II. Principle

This product uses a hot-start PCR enzyme for PCR amplification. PCR amplification products can be monitored in real time using a probe.

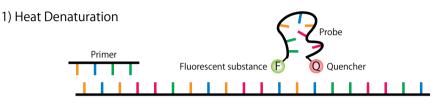
1. PCR

PCR is a technique used to amplify specific target sequences from minute amounts of DNA. By repeating three cycles of heat denaturation, primer annealing, and primer extension, the target fragment is amplified up to a million times by DNA polymerase within a short time.

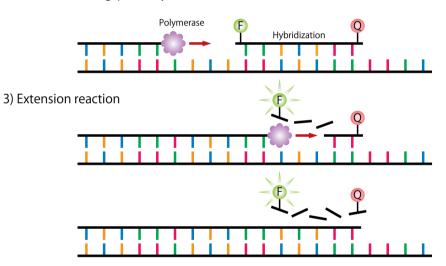
2. Fluorescence detection

Oligonucleotides modified with a 5' fluorophore (e.g., FAM) and a 3' quencher (e.g., TAMRA) are added to the reaction.

Under annealing conditions, the probe hybridizes in a sequence-specific manner to the template DNA. Fluorescence of the fluorophore is suppressed by the quencher. During the extension reaction, the $5' \rightarrow 3'$ exonuclease activity of *Taq* DNA polymerase degrades the hybridized probe, releasing quencher suppression and allowing fluorescence.



2) Primer annealing/probe hybridization



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III. Components (200 reactions, 50 μ l volume per reaction)

Probe qPCR Mix (2X) ^{*1}	1 ml x 5
ROX Reference Dye (50X) ^{*2}	200 µl
ROX Reference Dye II (50X) ^{*2}	200 µl

- *1 Contains PCR enzyme, dNTP Mixture, Mg²⁺ and Tli RNase H
- *2 Use when performing analyses with real-time PCR instruments that normalize fluorescent signals between wells, such as Applied Biosystems instruments.
 - Add ROX Reference Dye (50X) in a volume equivalent to 1/50 of the PCR reaction mixture when using the following Applied Biosystems systems:
 - 7300 Real-Time PCR System
 - StepOnePlus Real-Time PCR System
 - ♦ Add ROX Reference Dye II (50X) in a volume equivalent to 1/100 of the PCR reaction mixture when using the following Applied Biosystems systems:
 - 7500 Real-Time PCR System
 - 7500 Fast Real-Time PCR System
 - No ROX Reference Dye (50X) is required when using any of the following systems:
 - Thermal Cycler Dice Real Time System (Cat. #TP950/TP970/TP980/ TP990, TP900/TP960, TP700/TP760)*
 - LightCycler 96/480 System (Roche Diagnostics)
 - CFX96 Real-Time PCR Detection System (Bio-Rad)
 - Smart Cycler System/Smart Cycler II System (Cepheid)
 - * Not available in all geographic locations. Check for availability in your area.

IV. Materials Required but not Provided

- DNA amplification system for real-time PCR (authorized instruments)
- Reaction tubes or plates designed specifically for the qPCR instrument used
- PCR primers
- Probe for detection (Dual Labeled Probe, etc.)
- Sterile purified water
- Micropipette and tips (sterile, with filter)

V. Storage

Store at 4°C (stable for 6 months)

Every precaution should be taken to avoid contamination.

- 1. This product is shipped frozen at -20°C. Store the product at 4°C after receipt. Before use, gently invert tube to make sure reagent is completely dissolved and evenly mixed.
- 2. This product may be frozen at -20°C for long term storage. Once thawed, it should be stored at 4° C and used within 6 months.

VI. Features

- 1. This product allows rapid and accurate gene detection and quantification using real-time PCR.
- 2. The 2X premix enables easy pipetting.
- 3. Reduced sensitivity to contaminants that inhibit PCR allows for successful PCR reactions using templates purified using simpler methods.
- 4. The premix includes Tli RNaseH, a heat-resistant RNase H, that minimizes PCR inhibition by removing residual mRNA in input cDNA.

VII. Precautions Before Use

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

1. Before use, make sure the reagent is evenly mixed by gently inverting the tube several times without creating bubbles. Uneven reagent mixing will result in inadequate reactivity. Do not mix by vortexing.

When stored frozen at -20°C, Probe qPCR Mix (2X) may precipitate. To dissolve the precipitate completely, warm by hand or let stand at room temperature briefly, then invert the tube several times. Make sure reagent is evenly mixed before use.

- 2. Place reagent on ice immediately after it has thawed.
- 3. This product is not supplied with probe and primers.
- 4. Use fresh disposable tips to minimize potential cross-contamination between samples when preparing reaction mixtures or dispensing aliquots.

VIII. Protocol

1. Protocol Using the Thermal Cycler Dice Real Time System III, *II*, and *Lite*

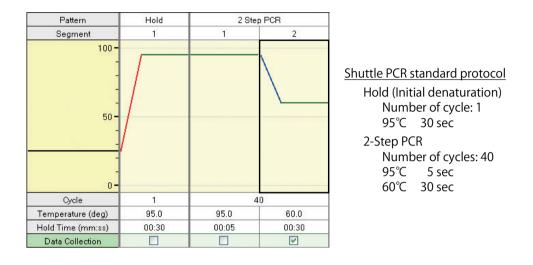
- * Follow the procedures provided in the manual for the respective Thermal Cycler Dice Real Time System.
 - 1. Prepare the PCR reaction mixture shown below.

<per reaction=""></per>		
Reagent	Volume	Final Conc.
Probe qPCR Mix (2X)	12.5 µl	1X
PCR Forward Primer (10 μ M)	0.5 µl	0.2 μM ^{*1}
PCR Reverse Primer (10 μ M)	0.5 µl	0.2 μ M ^{*1}
Probe ^{*2}	1 µl	
Template ^{*3}	2 µl	
Sterile purified water	8.5 µl	
Total	25 µl	

- *1 A final primer concentration of 0.2 μ M is most likely to yield good results. However, should further optimization be required, try adjusting primer concentrations in the range of 0.1 to 1.0 μ M.
- *2 The probe concentration varies depending on the real-time PCR instrument being used and the type of fluorescent label. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration. When using the Thermal Cycler Dice Real Time System, use a final concentration in the range of 0.1 to 0.5 μ M.
- *3 The optimal quantity depends on the copy number of the target in the template solution. Test serial dilutions to select the appropriate quantity. It is preferable to use no more than 100 ng of DNA template. When using cDNA (RT reaction mixture) as a template, the template volume should not exceed 10% of the PCR reaction mixture (e.g., no more than 2 μ I cDNA template solution for a 20 μ I PCR reaction).

2. Start the reaction.

The recommended protocol for PCR reactions is the shuttle PCR standard protocol below. Try this protocol first and optimize PCR conditions as necessary. (See "PCR Conditions" on page 17.)



Note:

The DNA polymerase in this product is a hot-start PCR enzyme that includes an anti-*Taq* antibody that inhibits polymerase activity. The initial denaturation step prior to PCR should be 95°C 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 curves and plot a standard curve if absolute quantification will be performed.

2. Protocol Using the Applied Biosystems 7300 Real-Time PCR System or StepOnePlus Real-Time PCR System

* Follow the procedures provided in the manual for the respective instrument.

1. Prepare the PCR reaction mixture shown below.

<per reaction=""></per>			
Reagent	Volume	Volume	Final Conc.
Probe qPCR Mix (2X)	10 µl	25 µl	1X
PCR Forward Primer (10 μ M)	0.4 µl	1 µl	0.2 μM ^{*1}
PCR Reverse Primer (10 μ M)	0.4 µl	1 µ l	0.2 μ M ^{*1}
Probe ^{*2}	0.8 µl	2 µl	
ROX Reference Dye (50X) ^{*3}	0.4 µI	1μ l	1X
Template ^{*4}	2 µI	4 μl	
Sterile purified water	6μl	16 µl	
Total	20 μl ^{*5}	50 μl ^{*5}	

- *1 A final primer concentration of 0.2 μ M is likely to yield good results. However, if further optimization is required, adjust the primer concentration in the range of 0.1 - 1.0 μ M.
- *2 The probe concentration varies depending on the real-time PCR instrument being used and the type of fluorescent label. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration.
- *3 Use the ROX Reference Dye at a final concentration of 1X.
- *4 The optimal quantity depends on the copy number of the target in the template solution. Test serial dilutions to select the appropriate quantity. It is preferable to use no more than 100 ng of DNA template. When using cDNA (RT reaction mixture) as a template, the template volume should not exceed 10% of the PCR reaction mixture (e.g., no more than 2 μ l cDNA template solution for a 20 μ l PCR reaction).
- *5 Adjust according to the recommended volume for the instrument.

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2. Start the reaction.

The recommended protocol for PCR reactions is the shuttle PCR standard protocol below. Try this protocol first and optimize PCR conditions as necessary. (See "PCR Conditions" on page 17.)

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<7300 Real-Time PCR System>
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Shuttle PCR standard protocol Stage 1: Initial denaturation Number of cycle: 1 95°C 30 sec Stage 2: PCR

Number of cycles: 40 95°C 5 sec 60°C 31 sec

<StepOnePlus Real-Time PCR System>

Shuttle PCR standard protocol Fast Mode Holding Stage Number of cycle: 1 95°C 20 sec Cycling Stage Number of cycles: 40 95°C 1 sec

60°C 20 sec

Note:

The DNA polymerase in this product is a hot-start PCR enzyme that includes an anti-*Taq* antibody that inhibits polymerase activity. The initial denaturation step prior to PCR should be 95°C 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 curves and plot a standard curve if absolute quantification will be performed.

3. Protocol Using the Applied Biosystems 7500/7500 Fast Real-Time PCR System

- * Follow the procedures provided in the manual for the respective instrument.
 - <Per reaction> Volume Volume Final Conc. Reagent 10 µl 25 µl 1X Probe aPCR Mix (2X) $0.2 \ \mu M^{*1}$ PCR Forward Primer (10 μ M) 0.4 µl 1μ 0.4 µl PCR Reverse Primer (10 μ M) 0.2 μM^{*1} 1μ Probe*2 0.8 µl 2 µl ROX Reference Dye II (50X) 0.2 μl 0.5 μl $0.5X^{*3}$ Template*4 2 µl 4 μl Sterile purified water 16.5 µl 6.2 µl 20 µl^{*5} 50 μl^{*5} Total
 - 1. Prepare the PCR reaction mixture shown below.

- *1 A final primer concentration of 0.2 μ M is likely to yield good results. However, if further optimization is required, adjust the primer concentration in the range of 0.1 - 1.0 μ M.
- *2 The probe concentration varies depending on the real-time PCR instrument being used and the type of fluorescent label. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration.
- *3 Use the ROX Reference Dye II at a final concentration of 0.5X.
- *4 The optimal quantity depends on the copy number of the target in the template solution. Test serial dilutions to select the appropriate quantity. It is preferable to use no more than 100 ng of DNA template. When using cDNA (RT reaction mixture) as a template, the template volume should not exceed 10% of the PCR reaction mixture (e.g., no more than 2 μ l cDNA template solution for a 20 μ l PCR reaction).
- *5 Adjust according to the recommended volume for the instrument.

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2. Start the reaction.

The recommended protocol for PCR reactions is the shuttle PCR standard protocol below. Try this protocol first and optimize PCR conditions as necessary. (See "PCR Conditions" on page 17.)

<7500 Real-Time PCR System>

Shuttle PCR standard protocol Stage 1: Initial denaturation Number of cycle: 1 95°C 30 sec Stage 2: PCR Number of cycles: 40 95°C 5 sec 60°C 34 sec

<7500 Fast Real-Time PCR System>

Shuttle PCR standard protocol

Fast Mode Holding Stage Number of cycle: 1

95℃ 20 sec Cycling Stage Number of cycles: 40

> 95℃ 3 sec 60℃ 30 sec

Note:

The DNA polymerase in this product is a hot-start PCR enzyme that includes an anti-*Taq* antibody that inhibits polymerase activity. The initial denaturation step prior to PCR should be 95°C 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 curves and plot a standard curve if absolute quantification will be performed.

4. Protocol Using the LightCycler 96 System

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* Follow the procedures provided in the manual for the LightCycler 96 System.

1. Prepare the PCR reaction mixture shown below.

<per reaction=""></per>		
Reagent	Volume	Final Conc.
Probe qPCR Mix (2X)	10 µl	1X
PCR Forward Primer (10 μ M)	0.4 µl	0.2 μM ^{*1}
PCR Reverse Primer (10 μ M)	0.4 µl	0.2 μ M ^{*1}
Probe ^{*2}	0.8 µl	
Template ^{*3}	2 µl	
Sterile purified water	6.4 µl	
Total	20 µl	

*1 A final primer concentration of 0.2 μ M is likely to yield good results. However, if further optimization is required, adjust the primer concentration in the range of 0.1 - 1.0 μ M.

- *2 The probe concentration varies depending on the real-time PCR instrument being used and the type of fluorescent label. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration.
- *3 The optimal quantity depends on the copy number of the target in the template solution. Test serial dilutions to select the appropriate quantity. It is preferable to use no more than 100 ng of DNA template. When using cDNA (RT reaction mixture) as a template, the template volume should not exceed 10% of the PCR reaction mixture (e.g., no more than 2 μ l cDNA template solution for a 20 μ l PCR reaction).

2. Start the reaction.

The recommended protocol for PCR reactions is the shuttle PCR standard protocol below. Try this protocol first and optimize PCR conditions as necessary. (See "PCR Conditions" on page 17.)

Shuttle PCR standard protocol

Hold (Initial denaturation) Number of cycle: 1 95°C 30 sec 2-Step PCR Number of cycles: 40 95°C 5 sec 60°C 30 sec

Note:

The DNA polymerase in this product is a hot-start PCR enzyme that includes an anti-*Taq* antibody that inhibits polymerase activity. The initial denaturation step prior to PCR should be 95°C 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 curves and plot a standard curve if absolute quantification will be performed.

5. Protocol Using the the LightCycler 480 System

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* Follow the procedures provided in the manual for the LightCycler 480 System.

1. Prepare the PCR reaction mixture shown below.

Reagent	Volume	Final Conc.
Probe qPCR Mix (2X)	10 µl	1X
PCR Forward Primer (10 μ M)	0.4 µl	0.2 μM^{*1}
PCR Reverse Primer (10 μ M)	0.4 µl	0.2 μM^{*1}
Probe ^{*2}	0.8 µl	
Template ^{*3}	2 µl	
Sterile purified water	6.4 µl	
Total	20 µl	

*1 A final primer concentration of 0.2 μ M is likely to yield good results. However, if further optimization is required, adjust the primer concentration in the range of 0.1 - 1.0 μ M.

- *2 The probe concentration varies depending on the real-time PCR instrument being used and the type of fluorescent label. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration.
- *3 The optimal quantity depends on the copy number of the target in the template solution. Test serial dilutions to select the appropriate quantity. It is preferable to use no more than 100 ng of DNA template. When using cDNA (RT reaction mixture) as a template, the template volume should not exceed 10% of the PCR reaction mixture (e.g., no more than 2 μ l cDNA template solution for a 20 μ l PCR reaction).

2. Start the reaction.

The recommended protocol for PCR reactions is the shuttle PCR standard protocol below. Try this protocol first and optimize PCR conditions as necessary. (See "PCR Conditions" on page 17.)

Shuttle PCR standard protocol

Hold (Initial denaturation) Number of cycle: 1 95°C 30 sec 2-Step PCR Number of cycles: 40 95°C 5 sec 60°C 30 sec

Note:

The DNA polymerase in this product is a hot-start PCR enzyme that includes an anti-*Taq* antibody that inhibits polymerase activity. The initial denaturation step prior to PCR should be 95° C 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 curves and plot a standard curve if absolute quantification will be performed.

6. Protocol Using the CFX96 Real-Time PCR Detection System

* Follow the procedures provided in the manual for the CFX96 Real-Time PCR Detection System.

- <Per reaction> Volume Final Conc. Reagent Probe qPCR Mix (2X) 10 µl 1X 0.2 μM^{*1} PCR Forward Primer (10 μ M) 0.4 µl 0.2 μM^{*1} PCR Reverse Primer (10 μ M) 0.4 µl Probe^{*2} 0.8 µl Template*3 2 µl Sterile purified water 6.4 µl Total 20 µl
- 1. Prepare the PCR reaction mixture shown below.

*1 A final primer concentration of 0.2 μ M is likely to yield good results. However, if further optimization is required, adjust the primer concentration in the range of 0.1 - 1.0 μ M.

- *2 The probe concentration varies depending on the real-time PCR instrument being used and the type of fluorescent label. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration.
- *3 The optimal quantity depends on the copy number of the target in the template solution. Test serial dilutions to select the appropriate quantity. It is preferable to use no more than 100 ng of DNA template. When using cDNA (RT reaction mixture) as a template, the template volume should not exceed 10% of the PCR reaction mixture (e.g., no more than 2 μ I cDNA template solution for a 20 μ I PCR reaction).

2. Start the reaction.

The recommended protocol for PCR reactions is the shuttle PCR standard protocol below. Try this protocol first and optimize PCR conditions as necessary. (See "PCR Conditions" on page 17.)

Shuttle PCR standard protocol

Hold (Initial denaturation) Number of cycle: 1 95°C 30 sec 2-Step PCR Number of cycles: 40 95°C 5 sec 60°C 30 sec

Note:

The DNA polymerase in this product is a hot-start PCR enzyme that includes an anti-*Taq* antibody that inhibits polymerase activity. The initial denaturation step prior to PCR should be 95° C 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 curves and plot a standard curve if absolute quantification will be performed.

7. Protocol Using the Smart Cycler II System

<Per reaction>

* Follow the procedures provided in the manual for the Smart Cycler System.

1. Prepare the PCR reaction mixture shown below.

Reagent	Volume	Final Conc.
Probe qPCR Mix (2X)	12.5 µl	1X
PCR Forward Primer (10 μ M)	0.5 µl	0.2 μ M ^{*1}
PCR Reverse Primer (10 μ M)	0.5 µl	0.2 μ M ^{*1}
Probe ^{*2}	1 µl	
Template ^{*3}	2 µl	
Sterile purified water	8.5 µl	
Total	25 µl	

*1 A final primer concentration of 0.2 μ M is likely to yield good results. However, if further optimization is required, adjust the primer concentration in the range of 0.1 - 1.0 μ M.

- *2 The probe concentration varies depending on the real-time PCR instrument being used and the type of fluorescent label. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration. When using the Smart Cycler System/Smart Cycler II System, use a final concentration in the range of 0.1 0.5 μ M.
- *3 The optimal quantity depends on the copy number of the target in the template solution. Test serial dilutions to select the appropriate quantity. It is preferable to use no more than 100 ng of DNA template. When using cDNA (RT reaction mixture) as a template, the template volume should not exceed 10% of the PCR reaction mixture (e.g., no more than 2.5 μ l cDNA template solution for a 25 μ l PCR reaction).

2. Start the reaction.

The recommended protocol for PCR reactions is the shuttle PCR standard protocol below. Try this protocol first and optimize PCR conditions as necessary. (See "PCR Conditions" on page 17.)

Shuttle PCR standard protocol

Stage 1: Initial denaturation Cycle: 1 95℃ 30 sec Stage 2: 2-Step PCR Repeat: 40 95℃ 5 sec 60℃ 20 sec

Note:

The DNA polymerase in this product is a hot-start PCR enzyme that includes an anti-*Taq* antibody that inhibits polymerase activity. The initial denaturation step prior to PCR should be 95° C 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 curves and plot a standard curve if absolute quantification will be performed.

8. PCR Conditions

Initial Denaturation

Step	Temperature	Time	Detection	Comment
Initial denaturation	95℃	30 sec	Off	Generally, 95°C for 30 sec is sufficient for initial denaturation in most cases, even with difficult to denature templates such as circular plasmids and genomic DNAs. This procedure may be extended to 1 - 2 min at 95°C depending on template condition. Prolonged denaturation may inactivate the enzyme. Therefore, do not perform denaturation for more than 2 min.

Shuttle PCR (2-step PCR) number of cycles: 30 - 45 cycles

Step	Temperature	Time	Detection	Comment
Denaturation	95℃	3 - 5 sec	Off	Generally the amplification product size for real-time PCR does not exceed 300 bp. Therefore, 95°C for about 3 - 5 sec is usually sufficient.
Annealing/ Extension	56 - 64℃	20 - 30 sec (31, 34 seconds)*	On	When optimizing reaction conditions, evaluate results using annealing/ extension temperature in the range of 56 - 64°C. If poor reactivity occurs, in- creasing incubation time for this step may improve results.

* Some instruments do not allow a detection step setting of 30 sec or less.

Set the Applied Biosystems 7300 Real-Time PCR System for 31 sec or longer. Set the Applied Biosystems 7500 Real-Time PCR System for 34 sec or longer.

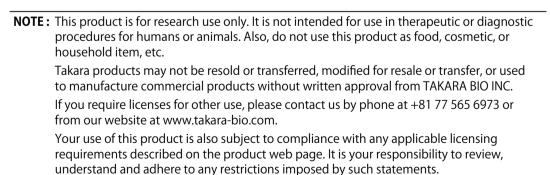
IX. Related Products

PrimeScript[™] RT reagent Kit (Perfect Real Time) (Cat. #RR037A/B) PrimeScript[™] RT reagent Kit with gDNA Eraser (Perfect Real Time) (Cat. #RR047A/B) PrimeScript[™] RT Master Mix (Perfect Real Time) (Cat. #RR036A/B) One Step PrimeScript[™] RT-PCR Kit (Perfect Real Time) (Cat. #RR064A/B)*¹ TB Green[™] Premix Ex Taq[™] II (Tli RNaseH Plus) (Cat. #RR820A/B)*² TB Green[™] Fast qPCR Mix (Cat. #RR430A/B)*^{1, 2} TB Green[™] Premix Ex Taq[™] (Tli RNaseH Plus) (Cat. #RR420A/B)*² One Step TB Green[™] PrimeScript[™] PLUS RT-PCR Kit (Perfect Real Time) (Cat. #RR096A/B)*^{1, 2}

Thermal Cycler Dice[™] Real Time System III (Cat. #TP950/TP970/TP980/TP990)*1 Thermal Cycler Dice[™] Real Time System // (Cat. #TP900/TP960)*1 Thermal Cycler Dice[™] Real Time System *Lite* (Cat. #TP700/TP760)*1

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

Thermal Cycler Dice, TB Green, Premix Ex Taq, and PrimeScript are trademarks of TAKARA BIO INC.



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