Cat. # RR430S
RR430A

For Research Use

TaKaRa

# TB Green® Fast qPCR Mix

Product Manual

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.

v201903Da

# Table of Contents

## I. Description

TB Green Fast qPCR Mix is designed for intercalator-based real-time PCR using TB Green for detection. It is supplied at a 2X concentration and includes TB Green at a concentration appropriate for real-time monitoring, making it easy to prepare reaction mixtures. The 2X premixed reagent also contains Tli RNase H, a heat-resistant RNase H that minimizes PCR inhibition by degrading residual mRNA when using cDNA as a template. The combination of an improved enzyme (a mutant-type *Taq* DNA polymerase optimized for high-speed reactions) and a reaction mixture composition optimized for hot-start reactions, allows high amplification efficiency and detection sensitivity. This product is suitable for high-speed PCR and enables accurate detection and quantification of targets, making it possible to obtain highly reproducible and reliable real-time PCR results over a wide dynamic range. The improved enzyme and reaction mixture combination also enables a high resistance to PCR inhibitory substances and an ability to handle relatively long and GC-rich targets.

**This product can be used with the following instruments:**

- 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)*
- Applied Biosystems 7300/7500 Real-Time PCR System, 7500 Fast Real-Time PCR System, StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)
- LightCycler 96/LightCycler 480 System (Roche Diagnostics)
- CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories)
- Smart Cycler System/Smart Cycler II System (Cepheid)

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

## II. Principle

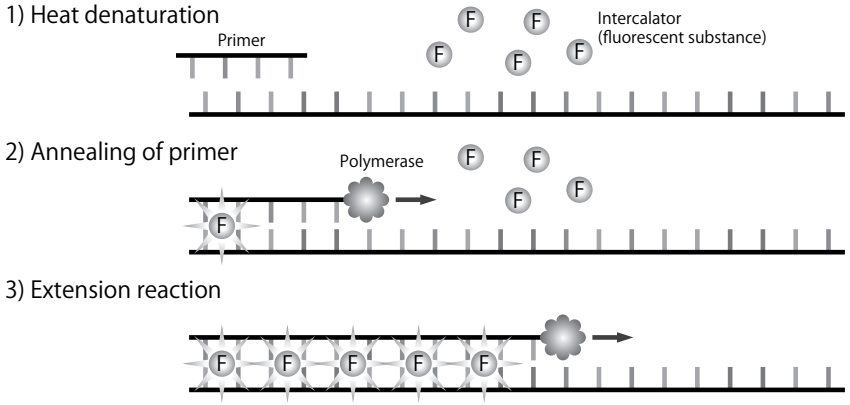
This product uses a mutant-type *Taq* hot-start polymerase for PCR amplification. PCR amplification products may be monitored in real time using TB Green as an intercalator.

### 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.
This product contains mutant-type *Taq* DNA polymerase for hot-start PCR reactions. This enzyme prevents non-specific amplification resulting from mispriming or primer-dimer formation during reaction mixture preparation or other pre-cycling steps, thereby allowing high-sensitivity detection.

### 2. Fluorescence detection - Intercalator method

This method uses a DNA intercalator (i.e., TB Green) that emits fluorescence when bound to double-stranded DNA. Monitoring fluorescence allows quantification of amplified products. Measuring the fluorescence intensity also provides the melting temperature of amplified DNA.



## III. Components [40 reactions (RR430S) / 200 reactions (RR430A), 50 μl volume]

| | Cat. #RR430S | RR430A |
|---|---|---|
| TB Green Fast qPCR Mix (2X conc.)*1 | 1 ml | 1 ml x 5 |
| ROX Reference Dye (50X conc.)*2 | 40 μl | 200 μl |
| ROX Reference Dye II (50X conc.)*2 | 40 μl | 200 μl |

*1 Contains a modified mutant-type *Taq* HS polymerase, dNTP mixture, $Mg^{2+}$, Tli RNaseH, and TB Green.

*2 ROX Reference Dyes are used for analyses with instruments that correct for cross-talk between wells, such as the real-time PCR instruments by Applied Biosystems.

◆ Add ROX Reference Dye (50X) when using the following instruments:
- Applied Biosystems 7300 Real-Time PCR System (Thermo Fisher Scientific)
- StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)

◆ Add ROX Reference Dye II (50X) when using the following instruments:
- Applied Biosystems 7500/7500 Fast Real-Time PCR System (Thermo Fisher Scientific)

◆ No ROX Reference Dye is required when using the following instruments:
- Thermal Cycler Dice Real Time System III (Cat. #TP950/TP970/TP980/TP990)*3
- Thermal Cycler Dice Real Time System *II* (Cat. #TP900/TP960)*3
- Thermal Cycler Dice Real Time System *Lite* (Cat. #TP700/TP760)*3
- LightCycler 96/LightCycler 480 system (Roche Diagnostics)
- CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories)
- Smart Cycler System/Smart Cycler II System (Cepheid)

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

**Materials Required but not Provided**

1. Real-time PCR machine (authorized instruments)
2. Reaction tubes and plates designed specifically for the real-time PCR instrument used
3. PCR Primers*
4. Sterile purified water
5. Micropipettes and tips (sterile, with filters)

* For designing real-time PCR primers, please refer to Section VIII-1.

## IV. Storage

Store at 4℃ (stable for up to 6 months.)
Always protect from light and avoid contamination.

For long-term storage, store at -20℃. Store thawed or opened product at 4℃ and use within 6 months.

## V. Features

1. It is possible to quickly and accurately detect and quantify a gene using real-time PCR.
2. This product consists of a 2X premix containing TB Green. By adding primers, cDNA template, and sterile purified water, you can perform real-time PCR using the intercalation method.
3. Mutant-type *Taq* HS enables a high-speed PCR reaction. Since the buffer system is optimized for real-time PCR, high amplification efficiency and high-sensitivity detection are possible.
4. Tli RNaseH, a heat-resistant RNase H, is included in the 2X premix. It minimizes PCR inhibition by degrading residual mRNA when using cDNA as a template.
5. The improved enzyme and reaction mixture composition provides resistance to PCR inhibitors.

## VI. Precautions

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

1. When cDNA synthesis is performed with the real time RT-PCR assay, use PrimeScript™ RT reagent Kit (Perfect Real Time) (Cat. #RR037A/B) or PrimeScript RT Master Mix (Perfect Real Time) (Cat. #RR036A/B) to obtain highly reliable results. Never use PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time) (Cat. #RR047A/B) for cDNA synthesis because it may result in abnormal reaction.
2. Before use, make sure the reagent is evenly mixed by gently inverting several times without creating bubbles. Uneven reagent mixing will result in inadequate reactivity.
   Do not mix by vortexing.

   TB Green Fast qPCR Mix may develop a white or yellowish-white precipitate when stored at -20℃. Gently hand-warm or bring to room temperature protected from light, then invert gently several times to dissolve the precipitate completely.
   The precipitate is indicative of uneven reagent distribution; make sure that the reagent is evenly mixed before use.
3. Place reagents on ice when preparing the reaction mixture.
4. This product contains TB Green. Avoid exposure to strong light when preparing the reaction mixture.
5. Use fresh, disposable tips to avoid contamination between samples when preparing or dispensing reaction mixtures.

## VII. Protocol

**【 Protocol when using the Thermal Cycler Dice Real Time System III, *II*, and *Lite* 】**

* Please follow the procedures outlined in the manual of each respective instrument.

1. Prepare the PCR reaction mixture as indicated below.

< Per reaction >

| Reagent | Volume | Final conc. |
|---|---|---|
| TB Green Fast qPCR Mix (2X) | 12.5 μl | 1X |
| PCR Forward Primer (10 μM) | 1 μl | 0.4 μM*1 |
| PCR Reverse Primer (10 μM) | 1 μl | 0.4 μM*1 |
| Template (<100 ng)*2 | 2 μl | |
| Sterile purified water | 8.5 μl | |
| Total | 25 μl*3 | |

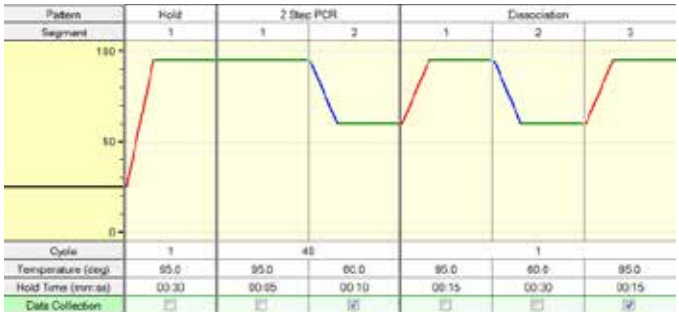
*1 A final primer concentration of 0.4 μM is likely to yield good results. However, if further optimization is required, try adjusting the primer concentration in the range of 0.2 to 1.0 μM.

*2 The optimal amount varies, depending on the number of target copies present in the template solution. Make serial dilutions to determine the appropriate amount, and use no more than 100 ng of DNA template per 25 μl. Furthermore, if cDNA (RT reaction mixture) is used as a template, the volume of the RT reaction mixture should be no more than 10% of the PCR mixture.

*3 The recommended total reaction volume is 25 μl.

2. Start the reaction.

The recommended shuttle PCR (i.e., 2-step PCR) protocol is described below. Use this protocol first and optimize PCR conditions when necessary. Perform 3-step PCR when using primers with low Tm values or when shuttle PCR is not feasible.




Shuttle PCR standard protocol

Hold (Initial denaturation)
Cycle: 1
95℃ 30 sec
2 Step PCR*4
Cycles: 40
95℃ 5 sec
60℃ 10 sec
Dissociation stage

*4 For information on optimizing PCR conditions, please refer to the section on "How to select experimental conditions."

**Note:**
The mutant-type *Taq* HS polymerase 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 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 absolute quantification will be performed.

Refer to the instrument's instruction manual for specific analysis methods.

**【 Protocol when using the Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System and StepOnePlus Real-Time PCR System 】**

* Please follow the procedures outlined in the manual of each respective instrument.

1. Prepare the PCR reaction mixture as indicated below.

< Per reaction >

| Reagent | Volume | Volume | Final conc. |
|---|---|---|---|
| TB Green Fast qPCR Mix (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 (50X) or Dye II (50X)[*2] | 0.4 μl | 1 μl | 1X |
| Template[*3] | 2 μl | 4 μl | |
| Sterile purified water | 6 μl | 16 μl | |
| Total | 20 μl[*4] | 50 μl[*4] | |

*1 A final primer concentration of 0.4 μM is likely to yield good results. However, if further optimization is required, try adjusting the primer concentration in the range of 0.2 to 1.0 μM.

*2 The concentration of ROX Reference Dye II (50X) is lower than that of ROX Reference Dye (50X). When you perform an analysis using the Applied Biosystems 7500 Real-Time PCR System or 7500 Fast Real-Time PCR System, use ROX Reference Dye II (50X).
For the StepOnePlus or 7300 Real-Time PCR System, use ROX Reference Dye (50X).

*3 The optimal amount varies, depending on the number of target copies present in the template solution. Make serial dilutions to determine the appropriate amount, and use no more than 100 ng of template per 20 μl total volume. Furthermore, if cDNA (RT reaction mixture) is added as the template, the template volume should be no more than 10% of the volume of the PCR mixture.

*4 Prepare this according to the recommended amount for each instrument.

2. Start the reaction.

   The recommended shuttle PCR (i.e., 2-step PCR) protocol is described below. Use this protocol first and optimize PCR conditions when necessary. Perform 3-step PCR when using primers with low Tm values or when shuttle PCR is not feasible. For optimizing PCR conditions, please refer to the section on "How to select experimental conditions."

   <Applied Biosystems 7300/7500 Real-Time PCR System and StepOnePlus>

   Shuttle PCR standard protocol

   Stage 1: Initial denaturation
   Cycle: 1
   95℃ 30 sec
   Stage 2: PCR reaction
   Cycles: 40
   95℃ 5 sec
   60℃ 10 - 15 sec *
   Stage 3: Melt Curve Analysis

   * Select the Filter (FAM or ROX) you use, and set the shortest detection time that can be performed with the filter. (Ten sec can be set for the StepOnePlus instrument.)

   <Applied Biosystems 7500 Fast Real-Time PCR System>

   Shuttle PCR standard protocol

   Holding Stage
   Cycle: 1
   95℃ 30 sec
   Cycling Stage
   Cycles: 40
   95℃ 3 sec
   60℃ 12 - 15 sec *
   Melt Curve Stage

   * Select the Filter (FAM or ROX) you use, and set the shortest detection time that can be performed with the filter.

   **Note:**
   The mutant-type *Taq* HS polymerase contained 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 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 absolute quantification will be performed.

   Refer to the instrument's instruction manual for specific analysis methods.

**【 Protocol when using the LightCycler 96/LightCycler 480 system 】**

* Please follow the procedures outlined in the manual of each respective instrument.

1. Prepare the PCR reaction mixture as indicated below.

< Per reaction >

| Reagent | Volume | Final conc. |
|---|---|---|
| TB Green Fast qPCR Mix (2X) | 10 μl | 1X |
| PCR Forward Primer (10 μM) | 0.8 μl | 0.4 μM[*1] |
| PCR Reverse Primer (10 μM) | 0.8 μl | 0.4 μM[*1] |
| Template (<100 ng)[*2] | 2 μl | |
| Sterile purified water | 6.4 μl | |
| Total | 20 μl | |

*1 A final primer concentration of 0.4 μM is most likely to yield good results. However, if further optimization is required, try adjusting the primer concentration in the range of 0.2 to 1.0 μM.

*2 The optimal amount varies, depending on the number of target copies present in the template solution. Make serial dilutions to determine the appropriate amount, and use no more than 100 ng of template per 20 μl total volume. Furthermore, if cDNA (RT reaction mixture) is added as the template, template volume should be no more than 10% of the volume of the PCR mixture.

2. Start the reaction.

The recommended shuttle PCR (i.e., 2-step PCR) protocol is described below. Use this protocol first and optimize PCR conditions as necessary. Perform 3-step PCR when using primers with low Tm values or when shuttle PCR is not feasible. For optimizing PCR conditions, please refer to the section on "How to select experimental conditions."

<LightCycler 96>

Shuttle PCR standard protocol

Stage 1: Initial denaturation
94℃ 30 sec 20℃/sec
1 Cycle
Stage 2: PCR reaction
94℃ 5 sec 20℃/sec
60℃ 10 sec 20℃/sec
40 Cycles
Stage 3: Melt Curve Analysis
95℃ 0 sec 20℃/sec
60℃ 15 sec 20℃/sec
95℃ 0 sec 0.1℃/sec

< LightCycler 480 system>

Shuttle PCR standard protocol

Denature
94℃ 30 sec (Ramp Rate 4.8℃/sec)
1 Cycle

PCR
Analysis Mode: Quantification
94℃ 5 sec (Ramp Rate 4.8℃/sec)
60℃ 10 sec (Ramp Rate 2.5℃/sec, Acquisition Mode: Single)
40 Cycles

Melting
Analysis Mode: Melting Curves
95℃ 5 sec (Ramp Rate 4.8℃/sec)
60℃ 1 min (Ramp Rate 2.5℃/sec)
95℃ (Ramp Rate 0.11℃/sec,
Acquisition Mode: Continuous,
Acquisitions: 5 per℃)
1 Cycle

Cooling
50℃ 30 sec (Ramp Rate 2.5℃/sec)
1 Cycle

**Note:**
The mutant-type *Taq* HS polymerase contained 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 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 absolute quantification will be performed.

   Refer to the instrument' s instruction manual for specific analysis methods.

**【 Protocol when using the CFX96 Real-Time PCR Detection System 】**

* Please follow the procedures outlined in the manual of this instrument.

1. Prepare the PCR reaction mixture as indicated below.

< Per reaction >

| Reagent | Volume | Final conc. |
|---|---|---|
| TB Green Fast qPCR Mix (2X) | 10 μl | 1X |
| PCR Forward Primer (10 μM) | 0.8 μl | 0.4 μM[*1] |
| PCR Reverse Primer (10 μM) | 0.8 μl | 0.4 μM[*1] |
| Template (<100 ng)[*2] | 2 μl | |
| Sterile purified water | 6.4 μl | |
| Total | 20 μl | |

*1 A final primer concentration of 0.4 μM is likely to yield good results. However, if further optimization is required, try adjusting the primer concentration in the range of 0.2 to 1.0 μM.

*2 The optimal amount varies, depending on the number of target copies present in the template solution. Make serial dilutions to determine the appropriate amount, and use no more than 100 ng of template per 20 μl total volume. Furthermore, if cDNA (RT reaction mixture) is added as the template, the template volume should be no more than 10% of the volume of the PCR mixture.

2. Start the reaction.

The recommended shuttle PCR (i.e., 2-step PCR) protocol is described below. Use this protocol first and optimize PCR conditions when necessary. Perform 3-step PCR when using primers with low Tm values or when shuttle PCR is not feasible. For optimizing PCR conditions, please refer to the section on "How to select experimental conditions."

Shuttle PCR standard protocol

Sample volume: 20 μl
Step 1: 95℃ 30 sec
Stage 2: PCR reaction
GOTO: 39 (40 Cycles)
95℃ 5 sec
60℃ 10 sec
Stage 3: Melt Curve

**Note:**
The mutant-type *Taq* HS polymerase contained 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 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 absolute quantification will be performed.

Refer to the instrument's instruction manual for specific analysis methods.

**【 Protocol when using the Smart Cycler II System 】**

* Please follow the procedures outlined in the manual of this instrument.

1. Prepare the PCR reaction mixture as indicated below.

< Per reaction >

| Reagent | Volume | Final conc. |
|---|---|---|
| TB Green Fast qPCR Mix (2X) | 12.5 μl | 1X |
| PCR Forward Primer (10 μM) | 1 μl | 0.4 μM*1 |
| PCR Reverse Primer (10 μM) | 1 μl | 0.4 μM*1 |
| Template (<100 ng)*2 | 2 μl | |
| Sterile purified water | 8.5 μl | |
| Total | 25 μl | |

*1 A final primer concentration of 0.4 μM is likely to yield good results. However, if further optimization is required, try adjusting the primer concentration in the range of 0.2 to 1.0 μM.

*2 The optimal amount varies, depending on the number of target copies present in the template solution. Make serial dilutions to determine the appropriate amount, and use no more than 100 ng of template per 25 μl total volume. Furthermore, if cDNA (RT reaction mixture) is added as the template, the template volume should be no more than 10% of the volume of the PCR mixture.

2. Briefly centrifuge reaction tubes with the Smart Cycler centrifuge and then set them in the Smart Cycler instrument to initiate the reaction. The recommended shuttle PCR (i.e., 2-step PCR) protocol is described below. Use this protocol first and optimize PCR conditions when necessary. Perform 3-step PCR when using primers with low Tm values or when a shuttle PCR is not feasible. For optimizing PCR conditions, please refer to the section on "How to select experimental conditions."

Shuttle PCR standard protocol

Stage 1: Initial denaturation
Hold
95℃ 30 sec
Stage 2: PCR
Repeat: 40 times
95℃ 5 sec
60℃ 10 sec
Stage 3: Melt curve

**Note:**
The mutant-type *Taq* HS polymerase contained 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 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 absolute quantification will be performed.

Refer to the instruction manual for the Smart Cycler System for specific analysis methods.

**How to select experimental conditions**

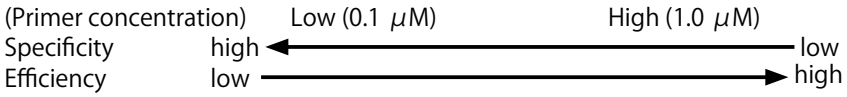
If unsatisfactory results are obtained using the recommended shuttle PCR standard protocol, follow the procedures below to optimize the primer concentration and PCR conditions. Select PCR conditions based on comprehensive analysis of both reaction specificity and amplification efficiency. Using conditions that balance these two aspects will allow accurate measurement over a wide range of concentrations.

○ System with a high reaction specificity
- With a "no template" control, non-specific amplification (e.g., primer dimers) does not occur
- Non-specific amplification products, those other than the target product, are not generated.

○ System with a high amplification efficiency
- Amplification product is detected early (low Ct value).
- PCR amplification efficiency is high (near the theoretical value of 100%).

[Evaluation of primer concentration]

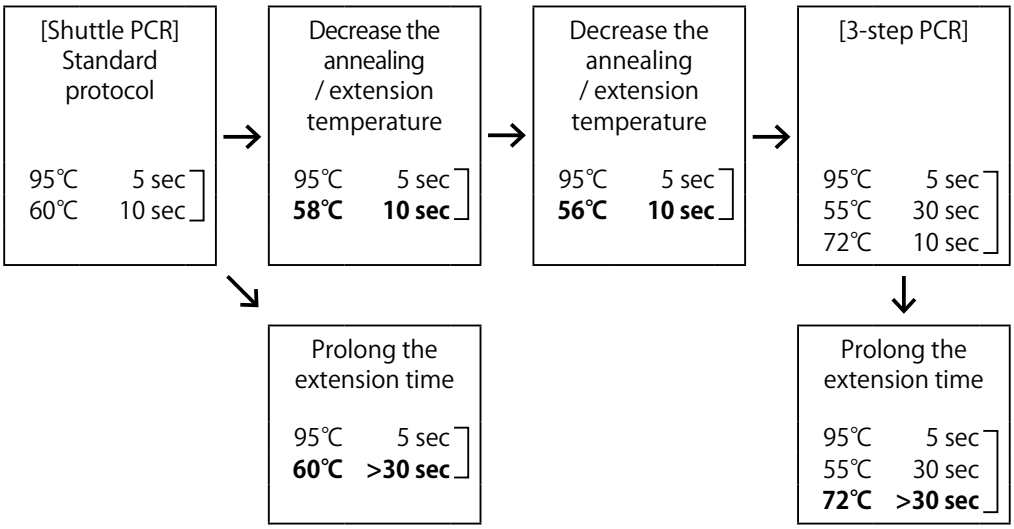
The relationship between primer concentration and the reaction's specificity and amplification efficiency is illustrated below. Reducing primer concentration raises reaction specificity. In contrast, increasing primer concentration raises amplification efficiency.



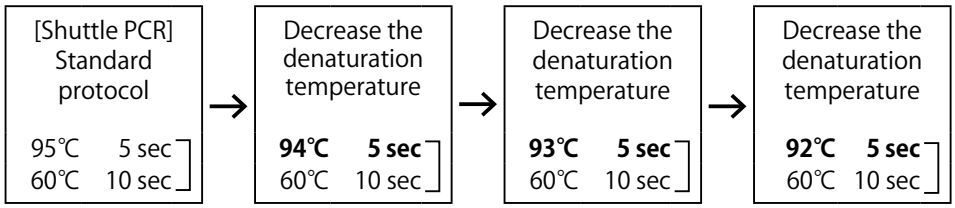
【 Evaluation of PCR conditions 】

○ To improve amplification efficiency

(1) Decreasing the annealing / extension temperature, switching to a 3-step PCR protocol, or prolonging the extension time may improve amplification effficiency. Perform optimization using the steps below.

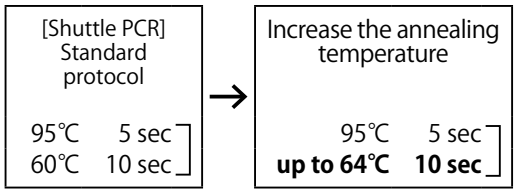



(2) Amplification efficiency can be improved by decreasing the denaturation temperature from 95℃ to 92℃ in 1℃ increments.




○ To improve reaction specificity

Raising the annealing temperature may improve reaction specificity. Perform optimization while checking for effects on amplification efficiency.




○ Initial denaturation

Generally, 95℃ for 30 sec is sufficient for initial denaturation, even for difficult-to-denature templates such as circular plasmids and genomic DNA. This step may be extended for 1 - 2 min at 95℃ depending on the condition of the template. However, prolonging this step may inactivate the enzyme. Therefore, it is recommended to avoid initial denaturation steps longer than 2 min.

## VIII. Appendix

### 1. Primer design

Designing primers with good reactivity is critical for efficient real-time PCR. Please follow the guidelines below to design primers that yield high amplification efficiency without non-specific amplification. RT-PCR primers designed and synthesized using these guidelines are compatible with the standard shuttle PCR protocol.

■ Amplification product

| | |
|---|---|
| Amplification size | The optimal size is 80 - 150 bp (amplification up to 300 bp is possible) |

■ Primer

| | |
|---|---|
| Length | 17 - 25 mer |
| GC content | 40 - 60% (preferably 45 - 55%) |
| Tm | Make sure that the Tm values for the forward primer and the reverse primer do not differ greatly.<br>Use primer design software to determine Tm values.<br>OLIGO*1 : 63 - 68℃<br>Primer3 : 60 - 65℃ |
| Sequence | Make sure that overall there are no base sequence biases.<br>Avoid having any GC-rich or AT-rich regions in the sequence (particularly at the 3′ end).<br>Avoid having consecutive T/C pairings (polypyrimidine).<br>Avoid having consecutive A/G pairings (polypurine). |
| 3′ end sequence | Avoid having any GC-rich or AT-rich sequence at the 3′ end.<br>It is preferable to have a G or C as the 3′ terminal base.<br>Avoid primers with T as the 3′ terminal base. |
| Complementation | Avoid having any complementary sequences of 3 bases or more within a primer and between primers.<br>Avoid having any complementary sequences of 2 bases or more at the primer's 3′ end. |
| Specificity | Verify the primer′ s specificity by performing a BLAST search.*2 |

*1 OLIGO Primer Analysis Software (Molecular Biology Insights, Inc.)
*2 http://www.ncbi.nlm.nih.gov/BLAST/

## 2. Preparing templates for real-time RT-PCR

The following products are recommended for the reverse transcription reaction in real-time RT-PCR:

- PrimeScript RT Reagent Kit (Perfect Real Time) (Cat. #RR037A/B)
- PrimeScript RT Master Mix (Perfect Real Time) (Cat. #RR036A/B)

When used in combination with this kit, these products provide reliable results. Refer to the product's user manual for RT reaction conditions.
Never use PrimeScript RT reagent Kit with gDNA Eraser (Perfect Real Time) (Cat. #RR047A/B) for cDNA synthesis because it may result in an abnormal reaction.

(1) Prepare a PCR reaction mixture as indicated below.
(When using the Thermal Cycler Dice Real Time System)

Prepare the following components in volumes slightly more than that needed for the required number of reactions and dispense 22.5 - 24 μl into each tube.

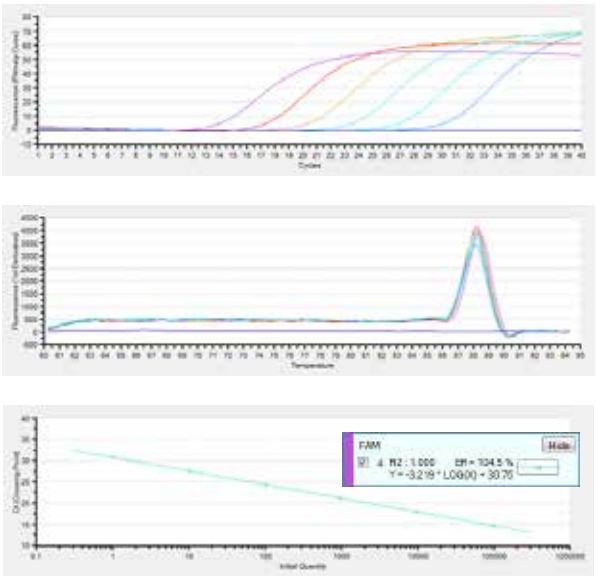
< Per reaction >

| Reagent | Volume | Final conc. |
|---|---|---|
| TB Green Fast qPCR Mix (2X) | 12.5 μl | 1X |
| PCR Forward Primer (10 μM) | 1 μl | 0.4 μM |
| PCR Reverse Primer (10 μM) | 1 μl | 0.4 μM |
| Sterile purified water | x μl | |
| Total | 22.5 - 24 μl | |

(2) Add 1 - 2.5 μl of the reverse transcription reaction mixture into each of the microtubes containing the dispensed reaction mixture.

Note: Add no more than 2.5 μl of the reverse transcription reaction mixture to the PCR mixture.

[Experimental example]




Human ACTB mRNA was detected by real-time RT-PCR. cDNA equivalent to 1 pg - 100 ng of total RNA was used as the template, with sterile purified water as the negative control.

## IX. Related products

TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B/L/W/LR/WR)
TB Green® Premix DimerEraser™ (Perfect Real Time) (Cat. #RR091A/B)*
TB Green® *Premix Ex Taq*™ GC (Perfect Real Time) (Cat. #RR071A/B)*
PrimeScript™ RT Reagent Kit (Perfect Real Time) (Cat. #RR037A/B)
PrimeScript™ RT Master Mix (Perfect Real Time) (Cat. #RR036A/B)
One Step TB Green® PrimeScript™ RT-PCR Kit (Perfect Real Time) (Cat. #RR066A/B)*
One Step TB Green® PrimeScript™ RT-PCR Kit II (Perfect Real Time) (Cat. #RR086A/B)
One Step TB Green® PrimeScript™ PLUS RT-PCR Kit (Perfect Real Time) (Cat. #RR096A/B)*

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)*

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