

For Research Use

Takara

TB Green[®] *Premix Ex Taq*[™] (Tli RNaseH Plus), Bulk

Product Manual

The long-term storage temperature of this product has been changed to -20°C since Lot. AK7801. See section V. Storage.

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.

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I. Description

TB Green *Premix Ex Taq* (Tli RNaseH Plus), Bulk is designed for intercalator-based real-time PCR using TB Green. 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 template.

The combination of *TaKaRa Ex Taq*® HS, a hot-start PCR enzyme that includes an anti-*Taq* antibody, and a buffer optimized for real-time PCR, allows high amplification efficiency and detection sensitivity. This product is suitable for high-speed PCR and enables accurate assay and detection of targets, making it possible to obtain highly reproducible and reliable real-time PCR results.

Benefits

- (1) Allows rapid and accurate detection and assay of targets by real-time PCR.
- (2) 2X concentration premixed with TB Green; simply add primers, template, and sterile purified water to perform intercalator-based real-time PCR.
- (3) *TaKaRa Ex Taq* HS, a hot-start PCR enzyme, is used for PCR. The buffer system has been optimized for real-time PCR, allowing good amplification efficiency and high-sensitivity detection.
- (4) The 2X reagent is premixed with Tli RNase H, a heat-resistant RNase H that minimizes PCR inhibition by residual mRNA when using cDNA as template.

II. Principle

This product uses *TaKaRa Ex Taq* HS 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 cycles of heat denaturation, primer annealing, and elongation, the target fragment is amplified up to a million times by DNA polymerase within a short time.

This product uses *TaKaRa Ex Taq* HS, a hot-start PCR enzyme that 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. Fluorescent detection - Intercalator method

This method uses a DNA intercalator (e.g., TB Green) that emits fluorescence when bound to double-strand DNA. Monitoring fluorescence allows for quantification of amplified products.

Measuring the fluorescence intensity also provides the melting temperature of amplified DNA.

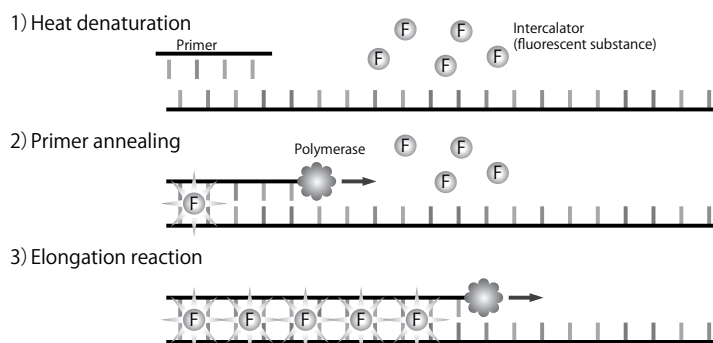


Figure 1. Fluorescent intercalator detection method.

III. Components (200 reactions, 50 µl volume)

| | |
|--|--------|
| TB Green <i>Premix Ex Taq</i> (2X conc.) (Tli RNaseH Plus), Bulk* ¹ | 5 ml |
| ROX Reference Dye (50X conc.)* ² | 200 µl |

*¹ Contains *TaKaRa Ex Taq* HS, dNTP Mixture, Mg²⁺, Tli RNase H, and TB Green.

*² ROX Reference Dye is used for analyses with instruments that correct for between-well fluorescence signal, such as the real-time PCR instruments by Applied Biosystems.

- ◆ Add ROX Reference Dye (50X) in a volume equivalent to 1/50 of the PCR reaction mixture when using the following instrument:
 - StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)
- ◆ Add ROX Reference Dye (50X) in a volume equivalent to 1/250 of the PCR reaction mixture when using the following instrument:
 - Applied Biosystems 7500/7500 Fast Real-Time PCR System (Thermo Fisher Scientific)
- ◆ No ROX Reference Dye (50X) is required when using any of the following instruments:
 - Thermal Cycler Dice™ Real Time System III (Cat. #TP950/TP970/TP980/TP990)*³
 - Thermal Cycler Dice Real Time System *Lite* (Cat. #TP700/TP760)*³
 - Smart Cycler II System (Cepheid)
 - LightCycler/LightCycler 480 System (Roche Diagnostics)
 - CFX96 Real-Time PCR Detection System (Bio-Rad)

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

IV. Materials Required but not Provided

- Gene amplification system for real-time PCR (authorized instruments)
- Reaction tubes and plates designed specifically for the real-time PCR instrument used
- PCR primers*
- Sterile purified water
- Micropipettes and tips (sterile, with filter)

* For designing real-time PCR primers, please see Section IX-1. Primer design.

V. Storage

Store at 4°C (stable for up to 6 months.)

Always protect from light and avoid contamination.

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

VI. Precautions

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 several times without creating bubbles. Uneven reagent mixing will result in inadequate reactivity.
 - Do not mix by vortexing.
 - When stored at -20°C, TB Green *Premix Ex Taq* (2X conc.) may develop a white to pale yellow precipitant. Gently hand-warm and let stand protected from light at room temperature. Invert gently several times to dissolve the precipitate completely.
 - The presence of precipitant is indicative of uneven reagent distribution; make sure that the reagent is evenly mixed before use.
2. Place reagents on ice when preparing the reaction mixture.
3. This product contains TB Green. Avoid exposure to strong light when preparing the reaction mixture.
4. Use fresh disposable tips to avoid contamination between samples when preparing or dispensing reaction mixtures.
5. *TaKaRa Ex Taq* HS is a hot-start PCR enzyme with an anti-*Taq* antibody that inhibits polymerase activity. Do not perform the pre-PCR incubation (5 - 15 min at 95°C) that is required with other companies' chemically modified hot-start PCR enzymes. The activity of *TaKaRa Ex Taq* HS decreases with longer heat treatment and the amplification efficiency and quantification accuracy can be affected.
Even for the initial denaturation step, 95°C for 30 sec is generally sufficient.

VII. Protocol

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

[Applied Biosystems 7500/7500 Fast Real-Time PCR System and StepOnePlus Real-Time PCR System]

A. Prepare the PCR mixture shown below.

<Per reaction>

StepOnePlus Real-Time PCR System

| Reagent | Volume | Final conc. |
|---|--------------------------|---------------------------|
| TB Green <i>Premix Ex Taq</i> (2X) (Tli RNaseH Plus), Bulk | 10 μ l | 1X |
| PCR Forward Primer (10 μ M) | 0.4 μ l | 0.2 μ M ^{*1} |
| PCR Reverse Primer (10 μ M) | 0.4 μ l | 0.2 μ M ^{*1} |
| ROX Reference Dye (50X) | 0.4 μ l | 1X |
| Template ^{*2} | 2 μ l | |
| Sterile purified water | 6.8 μ l | |
| Total | 20 μ l ^{*3} | |

Applied Biosystems 7500/7500 Fast Real-Time PCR System

| Reagent | Volume | Volume | Final conc. |
|---|--------------------------|--------------------------|---------------------------|
| TB Green <i>Premix Ex Taq</i> (2X) (Tli RNaseH Plus), Bulk | 10 μ l | 25 μ l | 1X |
| PCR Forward Primer (10 μ M) | 0.4 μ l | 1 μ l | 0.2 μ M ^{*1} |
| PCR Reverse Primer (10 μ M) | 0.4 μ l | 1 μ l | 0.2 μ M ^{*1} |
| ROX Reference Dye (50X) | 0.08 μ l | 0.2 μ l | 0.2X |
| Template ^{*2} | 2 μ l | 4 μ l | |
| Sterile purified water | 7.12 μ l | 18.8 μ l | |
| Total | 20 μ l ^{*3} | 50 μ l ^{*3} | |

^{*1} A final primer concentration of 0.2 μ M likely will provide good results. However, if there is an issue with reactivity, use a primer concentration between 0.1 and 1.0 μ M.

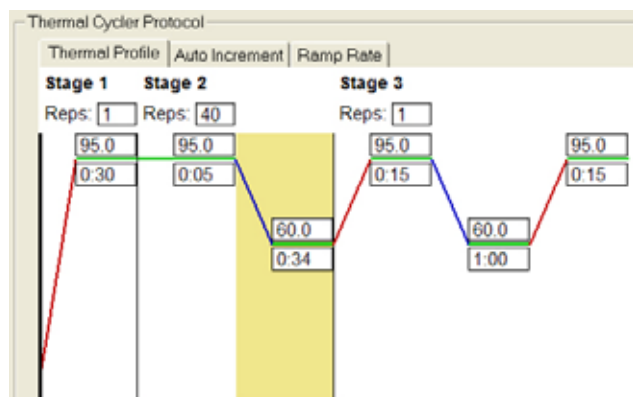
^{*2} The quantity varies depending on the number of target copies present in the template solution. Make serial dilutions to determine the appropriate template amount and use no more than 100 ng of DNA template per 20 μ l. Furthermore, if cDNA (RT reaction mixture) is used as template, the volume of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

^{*3} Prepare in accordance with the recommended volume for each instrument.

B. 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 T_m values or when a shuttle PCR is not feasible. (For optimizing PCR conditions, please refer to Section VIII.)

1) Applied Biosystems 7500 Real-Time PCR System and StepOnePlus



Stage 1: Initial denaturation

Reps: 1
95°C 30 sec

Stage 2: PCR

Reps: 40
95°C 5 sec
60°C 30 - 34 sec*

Dissociation stage

* StepOnePlus, set to 30 sec;
7500, set to 34 sec.

Figure 2. Shuttle PCR standard protocol.

2) Applied Biosystems 7500 Fast Real-Time PCR System

Shuttle PCR standard protocol

Hold Stage

Number of Cycle: 1
95°C 30 sec

Cycling Stage

Number of Cycles: 40
95°C 3 sec
60°C 30 sec

Melt Curve Stage

Note:

- *TaKaRa Ex Taq HS* 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.
- For initial template denaturation before PCR, 95°C for 30 sec is sufficient.

C. 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.

【 LightCycler/LightCycler 480 System 】

A. Prepare the PCR mixture shown below.

<Per reaction>

| Reagent | Volume | Final conc. |
|--|-------------|---------------------------|
| TB Green <i>Premix Ex Taq</i> (2X) (Tli RNaseH Plus), Bulk | 10 μ l | 1X |
| PCR Forward Primer (10 μ M) | 0.4 μ l | 0.2 μ M ^{*1} |
| PCR Reverse Primer (10 μ M) | 0.4 μ l | 0.2 μ M ^{*1} |
| Template (< 100 ng) ^{*2} | 2 μ l | |
| Sterile purified water | 7.2 μ l | |
| Total | 20 μ l | |

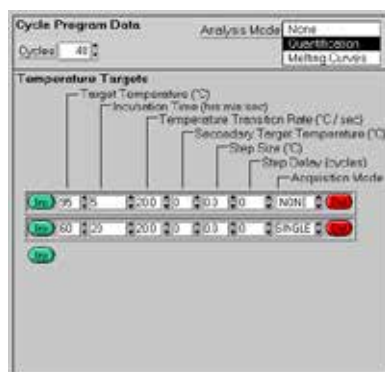
*1 A final primer concentration of 0.2 μ M likely will provide good results. However, if there is an issue with reactivity, use a primer concentration between 0.1 and 1.0 μ M.

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

B. 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 T_m values or when a shuttle PCR is not feasible. (For optimizing PCR conditions, please refer to Section VIII.)

<LightCycler>



Stage 1: Initial denaturation
95°C 30 sec 20°C/sec
1 cycle

Stage 2: PCR (See figure on the left)
95°C 5 sec 20°C/sec
60°C 20 sec 20°C/sec
40 cycles

Stage 3: Melt Curve Analysis
95°C 0 sec 20°C/sec
65°C 15 sec 20°C/sec
95°C 0 sec 0.1°C/sec

Figure 3. Shuttle PCR standard protocol.

<LightCycler 480 System>

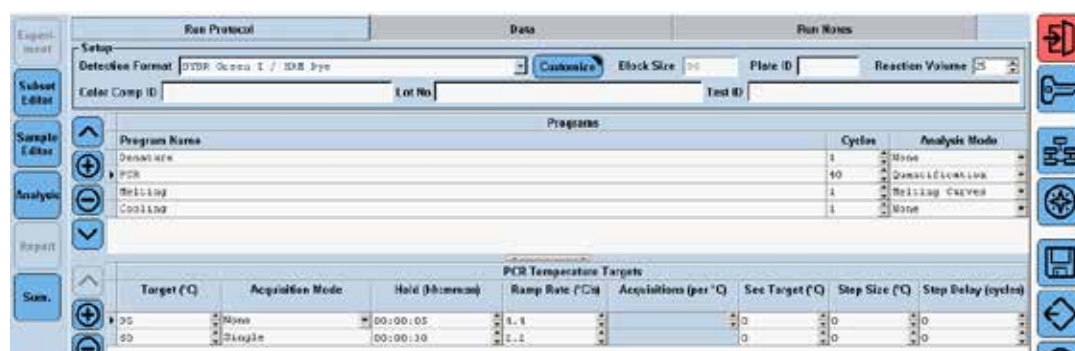


Figure 4. Shuttle PCR standard protocol

Denature

95°C 30 sec (Ramp rate: 4.4°C/sec)

1 cycle

PCR

Analysis Mode: Quantification

95°C 5 sec (Ramp rate: 4.4°C/sec)

60°C 30 sec (Ramp rate: 2.2°C/sec, Acquisition Mode: Single)

40 cycles

Melting

Analysis Mode: Melting Curves

95°C 5 sec (Ramp rate: 4.4°C/sec)

60°C 1 min (Ramp rate: 2.2°C/sec)

95°C (Ramp rate: 0.11°C/sec, Acquisition Mode: Continuous, Acquisitions: 5 per °C)

1 cycle

Cooling

50°C 30 sec (Ramp rate: 2.2°C/sec)

1 cycle

Note:

- *Takara Ex Taq HS* is a hot-start PCR enzyme 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.
- For initial template denaturation before PCR, 95°C for 30 sec is sufficient.

- C. 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 manual for specific analysis methods.

[Smart Cycler II System]

- A. Prepare the PCR mixture shown below.

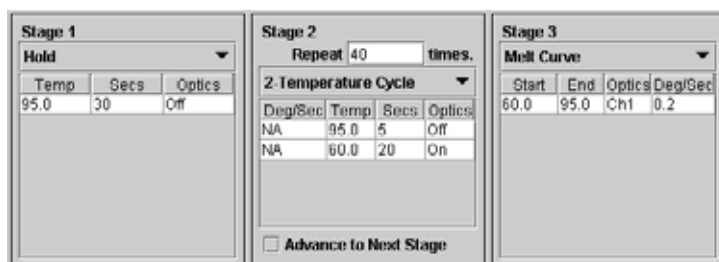
<Per reaction>

| Reagent | Volume | Final conc. |
|--|-----------------------------|---------------------------|
| TB Green <i>Premix Ex Taq</i> (2X) (Tli RNaseH Plus), Bulk | 12.5 μ l | 1X |
| PCR Forward Primer (10 μ M) | 0.5 μ l | 0.2 μ M* ¹ |
| PCR Reverse Primer (10 μ M) | 0.5 μ l | 0.2 μ M* ¹ |
| Template (< 100 ng)* ² | 2.0 μ l | |
| Sterile purified water | 9.5 μ l | |
| Total | 25 μl | |

- *¹ A final primer concentration of 0.2 μ M likely will provide good results. However, if there is an issue with reactivity, use a primer concentration between 0.1 and 1.0 μ M.
- *² The quantity 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. If cDNA (RT reaction mixture) is used as template, the volume of the RT reaction mixture should be no more than 10% of the PCR mixture.

- B. 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 T_m values or when a shuttle PCR is not feasible. (For optimizing PCR conditions, please refer to Section VIII.)



Stage 1: Initial denaturation
Hold
95°C 30 sec

Stage 2: PCR
Repeat: 40 times
95°C 5 sec
60°C 20 sec

Stage 3: Melt curve

Figure 5. Shuttle PCR standard protocol.

Note:

- *TAKARA Ex Taq* HS 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.
- For initial template denaturation before PCR, 95°C for 30 sec is sufficient.

- C. After the reaction is complete, check the amplification and melting curves and plot a standard curve if absolute quantification will be performed.

For the analysis methods when using the Smart Cycler System, please refer to the instruction manual for Smart Cycler System.

【 CFX96 Real-Time PCR Detection System 】

- A. Prepare the PCR mixture shown below.

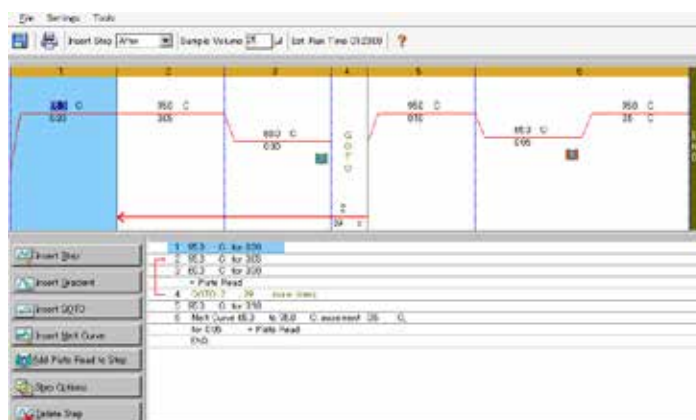
<Per reaction>

| Reagent | Volume | Final conc. |
|--|--------------|---------------|
| TB Green <i>Premix Ex Taq</i> (2X) (Tli RNaseH Plus), Bulk | 12.5 μ l | 1X |
| PCR Forward Primer (10 μ M) | 0.5 μ l | 0.2 μ M*1 |
| PCR Reverse Primer (10 μ M) | 0.5 μ l | 0.2 μ M*1 |
| Template (<100 ng)*2 | 2 μ l | |
| Sterile purified water | 9.5 μ l | |
| Total | 25 μ l | |

- *1 A final primer concentration of 0.2 μ M likely will provide good results. However, if there is an issue with reactivity, use a primer concentration between 0.1 and 1.0 μ M.
- *2 The optimal amount depends on the number of target copies in the template solution. Make serial dilutions to determine the appropriate amount and use no more than 100 ng of DNA template. If cDNA (RT reaction mixture) is added as template, the template volume should be no more than 10% of the PCR mixture.

- B. 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 T_m values or when a shuttle PCR is not feasible. (For optimizing PCR conditions, please refer to Section VIII.)

Sample volume: 25 μ lStep 1:
95°C 30 secStep 2: PCR
GOTO: 39 (40 cycles)
95°C 5 sec
60°C 30 sec

Step 3: Melt Curve

Figure 6. Shuttle PCR standard protocol.

Note:

- *TAKARA Ex Taq* HS 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.
- For initial template denaturation before PCR, 95°C for 30 sec is sufficient.

- C. After the reaction is complete, check the amplification and melting curves and plot a standard curve if absolute quantification will be performed.

For analysis methods, refer to the manual for CFX96 Real-Time PCR Detection System.

【 Thermal Cycler Dice Real Time System III and Lite 】

A. Prepare the PCR mixture shown below.

<Per reaction>

| Reagent | Volume | Final conc. |
|--|--------------------------|---------------------------|
| TB Green <i>Premix Ex Taq</i> (2X) (Tli RNaseH Plus), Bulk | 12.5 μ l | 1X |
| PCR Forward Primer (10 μ M) | 0.5 μ l | 0.2 μ M* ¹ |
| PCR Reverse Primer (10 μ M) | 0.5 μ l | 0.2 μ M* ¹ |
| Template (<100 ng)* ² | 2.0 μ l | |
| Sterile purified water | 9.5 μ l | |
| Total | 25 μ l* ³ | |

*1 A final primer concentration of 0.2 μ M likely will provide good results. However, if there is an issue with reactivity, use a primer concentration between 0.1 and 1.0 μ M.

*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. If cDNA (RT reaction mixture) is used as template, the volume of the RT reaction mixture should be no more than 10% of PCR mixture.

*3 The recommended volume is 25 μ l for reaction mixtures.

B. 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 T_m values or when a shuttle PCR is not feasible. (For optimizing PCR conditions, please refer to Section VIII.)

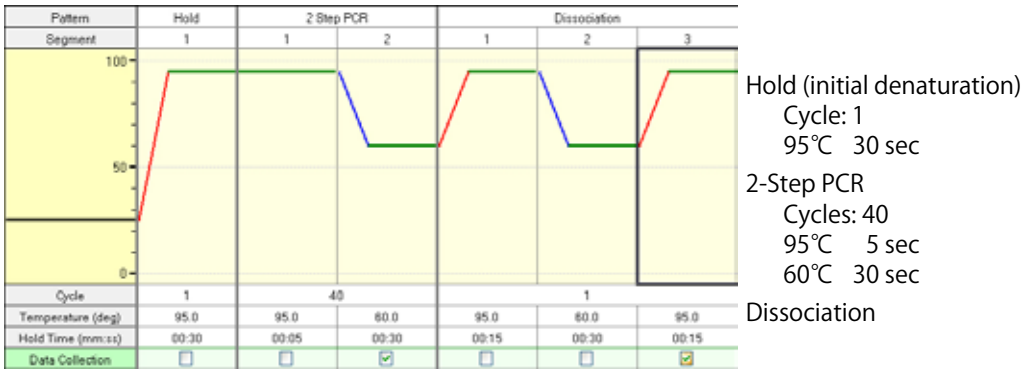


Figure 7. Shuttle PCR standard protocol.

Note:

- *TaKaRa Ex Taq* HS 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.
- For initial template denaturation before PCR, 95°C for 30 sec is sufficient.

C. After the reaction is complete, check the amplification and melting curves and plot a standard curve if absolute quantification will be performed. When using Thermal Cycler Dice Real Time System, please refer to the instruction manual for analysis methods.

VIII. Optimization

If unsatisfactory results are obtained using the recommended conditions (shuttle PCR standard protocol), follow the procedures below to optimize the primer concentration and PCR conditions. In addition, depending on the reaction system, switching to another TB Green Premix (Cat. #RR820A/B, RR091A/B*) may greatly improve the results.

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.

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

☐ System with a high reaction specificity

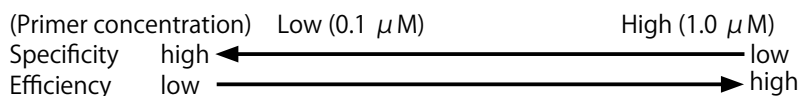
- With no template control, non-specific amplification (e.g., primer-dimers) does not occur.
- Non-specific amplification products, those other than the target product, are not generated.

☐ System with a high amplification efficiency

- Amplification product is detected early (small Ct value).
- PCR amplification efficiency is high (near the theoretical value of 100%).

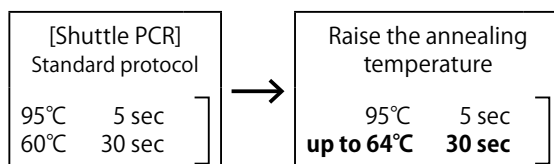
1. Evaluation of primer concentration

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

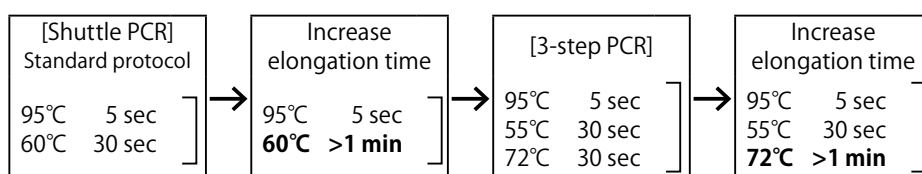


2. Evaluation of PCR conditions

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



- To improve amplification efficiency
Increasing elongation time or switching to a 3-step PCR protocol may improve amplification efficiency. Perform optimization using the steps below.



- Initial denaturation
Generally, 95°C 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 to 1 - 2 min at 95°C depending on the template. However, prolonging this step may inactivate the enzyme. Therefore, it is recommended to avoid initial denaturation steps longer than 2 min.

3. Relationship between reagent and reactivity

Takara Bio supplies several different reagents for intercalator-based real-time PCR analysis using TB Green. The relationship between reaction specificity and amplification efficiency for these reagents is described below.

TB Green *Premix Ex Taq* (Tli RNaseH Plus) (Cat. #RR420A/B) provides high amplification efficiency. TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820A/B) and TB Green *Premix DimerEraser*™ (Perfect Real Time) (Cat. #RR091A/B)* have greater specificity.

| (Reagent) | TB Green <i>Premix Ex Taq</i> (Cat. #RR420A) | TB Green <i>Premix Ex Taq</i> II (Cat. #RR820A) | TB Green <i>Premix DimerEraser</i> * (Cat. #RR091A) |
|-------------|---|--|--|
| Specificity | lower | ←————→ | higher |
| Efficiency | higher | ————→ | lower |

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

IX. Appendix

1. Primer design

Designing primers with good reactivity is critical to 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 (Section VII.).

■ Amplification product

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

■ Primer

| | |
|-----------------|--|
| Length | 17 - 25mer |
| 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. Use primer design software to determine Tm values. OLIGO*1 : 63 - 68°C Primer3 : 60 - 65°C |
| Sequence | Make sure that overall there are no base sequence biases. Avoid having any GC-rich or AT-rich regions in the sequence (particularly at the 3' end). Avoid having consecutive T/C pairings (polypyrimidine). Avoid having consecutive A/G pairings (polypurine). |
| 3' end sequence | Avoid having any GC-rich or AT-rich sequence at the 3' end. It is preferable to have a G or C as the 3' end-base. Avoid primers with T as the 3' end-base. |
| Complementation | Avoid having any complementary sequences of 3 bases or more within a primer and between primers. Avoid having any complementary sequences of 2 bases or more at the primer's 3' ends. |
| Specificity | Verify primer specificity by BLAST search.*2 |

*1 OLIGO Primer Analysis Software (Molecular Biology Insights)

*2 <http://www.ncbi.nlm.nih.gov/BLAST/>

2. When performing real-time RT-PCR

To synthesize cDNA templates for real-time RT-PCR, we recommend using PrimeScript™ reverse transcriptase products.

- PrimeScript RT reagent Kit (Perfect Real Time) (Cat. #RR037A/B)
- PrimeScript RT Master Mix (Perfect Real Time) (Cat. #RR036A/B)
- PrimeScript RT Reagent Kit with gDNA Eraser (Perfect Real Time) (Cat. #RR047A/B)

When used in combination with this kit, these products can provide highly reliable results.

- A. Prepare a PCR mixture by the following procedure.
(When using Thermal Cycler Dice Real Time System)

Prepare the following components in volumes slightly more than that needed for the required number of tubes and dispense 22.5 - 24 μ l into tubes.

<Per reaction>

| Reagent | Volume | Final conc. |
|--|-------------------|-------------|
| TB Green <i>Premix Ex Taq</i> (2X) (Tli RNaseH Plus), Bulk | 12.5 μ l | 1X |
| PCR Forward Primer (10 μ M) | 0.5 μ l | 0.2 μ M |
| PCR Reverse Primer (10 μ M) | 0.5 μ l | 0.2 μ M |
| Sterile purified water | x μ l | |
| Total | 22.5 - 24 μ l | |

- B. Add 1 - 2.5 μ l of the reverse transcription reaction mixture to each of the microtubes containing aliquots of the reaction mixture.

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

[Experimental example]

Human ATP5F1 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.

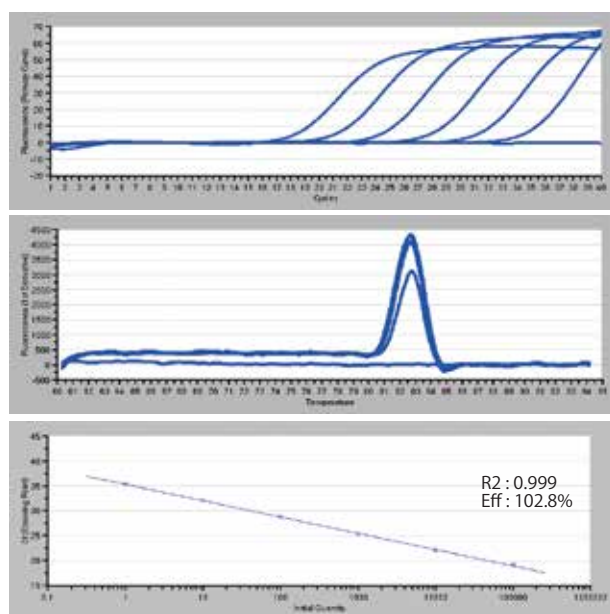


Figure 8. Detection of human ATP5F1 mRNA using real-time RT-PCR

X. Related Products

TB Green® *Premix Ex Taq*™ (Tli RNaseH Plus) (Cat. #RR420A/B/W, RR42LR/WR)
TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B/L/W, RR82LR/WR)
TB Green® Fast qPCR Mix (Cat. #RR430A/B)
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)
PrimeScript™ RT Reagent Kit with gDNA Eraser (Perfect Real Time) (Cat. #RR047A/B)
Thermal Cycler Dice™ Real Time System III (Cat. #TP950/TP970/TP980/TP990)*
Thermal Cycler Dice™ Real Time System *Lite* (Cat. #TP700/TP760)*

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

TB Green and *Takara Ex Taq* are registered trademarks of Takara Bio Inc.

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