Cat. # RR82LR

For Research Use

TaKaRa

# TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus), ROX plus

Product Manual

v202112Da

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## I. Description

TB Green *Premix Ex Taq* II (Tli RNaseH Plus), ROX plus is designed for intercalator-based real-time PCR using TB Green. The premix is supplied as a 2X concentrate with TB Green and ROX Reference Dye to facilitate easy preparation of reaction mixtures for real-time monitoring.

The 2X premix also contains Tli RNaseH, a heat-resistant RNase H, to minimize PCR inhibition due to residual mRNA in reactions that use cDNA as the template.

The modified buffer composition of this product enables accurate measurement over a wide range of template concentrations. This, plus *TaKaRa Ex Taq*® HS DNA Polymerase (a hot-start PCR enzyme that uses an anti-*Taq* antibody), allows highly reproducible and reliable real-time PCR analysis.

**Compatible instrument systems include:**

- Devices that require signal correction using ROX Reference Dye ＊1
  StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)
- Devices that do not require signal correction using ROX Reference Dye
  Thermal Cycler Dice™ Real Time System III (Cat. #TP950/TP970/TP980/TP990) ＊2
  Thermal Cycler Dice Real Time System *II* (Cat. #TP900/TP960: discontinued)
  Thermal Cycler Dice Real Time System *Lite* (Cat. #TP700/TP760: discontinued))

**Note:** For the Smart Cycler System/Smart Cycler II System (Cepheid), the use of TB Green *Premix Ex Taq* (Tli RNaseH Plus) (Cat. #RR420A/B/L/W, RR42LR/WR) is recommended.

＊1 For the Applied Biosystems 7500/7500 Fast Real-Time PCR System (Thermo Fisher Scientific) requiring correction with ROX Reference Dye II, we recommend using TB Green *Premix Ex Taq* II (Tli RNaseH Plus), Bulk (Cat. #RR820L/W).

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

## II. Principle

This product is used for PCR amplification with *TaKaRa Ex Taq* HS DNA Polymerase. PCR amplification products can be monitored in real time using TB Green as an intercalator.

*TaKaRa Ex Taq* HS DNA Polymerase, a hot-start PCR enzyme, prevents non-specific amplifications due to mispriming or primer-dimer formation during reaction mixture preparation or other pre-cycling steps, allowing highly sensitive detection.

Fluorescence Detection - Intercalator Method

This method involves the addition of an intercalator (TB Green) that fluoresces when bound to double-stranded DNA in the reaction mixture, allowing the detection of fluorescence associated with amplification. Measuring the fluorescence intensity not only allows quantitative determination but also provides the melting temperature (Tm) of amplified DNA products.

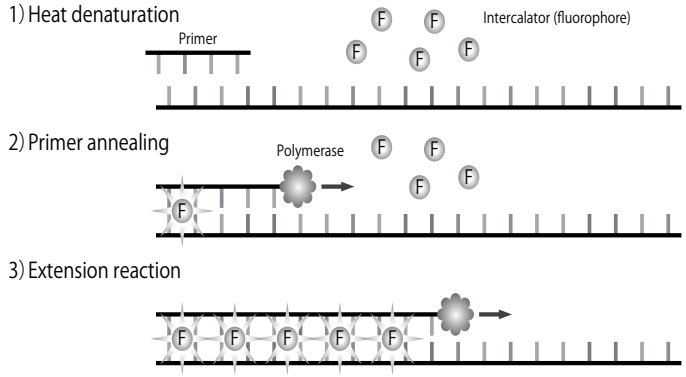



Figure 1. Fluorescent intercalator detection method.

## III. Components (200 reactions, 50 μl per reaction)

| | |
|---|---|
| TB Green *Premix Ex Taq* II (2X) (Tli RNaseH Plus), ROX plus | 5 ml |

＊ Contains *TaKaRa Ex Taq* HS, dNTP mixture, $Mg^{2+}$, Tli RNaseH, TB Green, and ROX Reference Dye.

## 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. Materials Required but not Provided

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

* For designing real-time PCR primers, please see section VIII.-(2) Primer design.

## VI. Precautions Before Use

Before using this product, read these precautions and follow them carefully.

1. Prior to use, make sure the reagent is evenly mixed by gently inverting the bottle several times without creating bubbles. Uneven reagent composition will result in inadequate reactivity.
   - Do not mix by vortexing.
   - When stored at -20℃, TB Green *Premix Ex Taq* II (2X) (Tli RNase H Plus), ROX plus may develop a white to pale yellow precipitate. To dissolve the precipitate completely, briefly let the reagent stand protected from light at room temperature (<30℃), then invert the bottle several times.
   - The presence of precipitate is indicative of uneven reagent composition; make sure the reagent is evenly mixed before use.
2. Place reagent on ice immediately after thawing.
3. This product contains ROX Reference Dye and TB Green. Take care to avoid exposure to bright light while preparing the reaction mixture.
4. While preparing or dispensing reaction mixtures, use sterile, disposable tips to avoid cross-contamination between samples.
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 to 15 min at 95℃) 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.

   For the initial denaturation step, incubation at 95℃ for 30 sec is generally sufficient.

## VII. Protocol

**【 For the StepOnePlus Real-Time PCR System 】**

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

1. Prepare the PCR mixture shown below. To account for pipetting error, make a master mix with at least 10% more than the total volume needed for the total number of reactions.

<Per reaction>

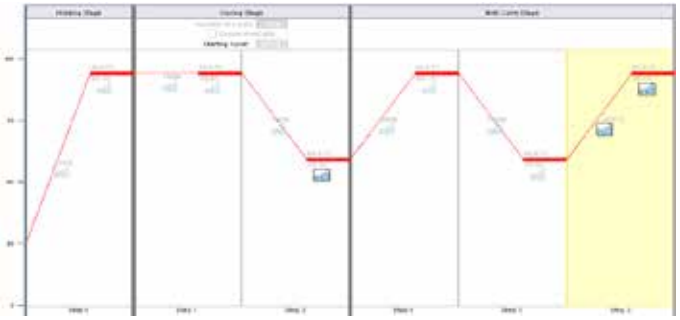
| Reagent | Volume | Final conc. |
|---|---|---|
| TB Green *Premix Ex Taq* II (2X) (Tli RNaseH Plus), ROX plus | 10 μl | 1X |
| PCR Forward Primer (10 μM) | 0.8 μl | 0.4 μM＊1 |
| PCR Reverse Primer (10 μM) | 0.8 μl | 0.4 μM＊1 |
| Template (<100 ng)＊2 | 2 μl | |
| Sterile purified water | 6.4 μl | |
| Total | 20 μl | |

＊1 A final primer concentration of 0.4 μM is likely to yield good results. If there is insufficient reactivity, use a concentration between 0.2 and 1.0 μM.

＊2 The optimal 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 μl reaction. Furthermore, if cDNA (RT reaction mixure) is used as the template, the template volume should not exceed 10% of the PCR mixture.

2. Start the reaction.

The shuttle PCR standard protocol is recommended. Try this protocol first and optimize PCR conditions as necessary. Perform 3-step PCR when using a primer with a low Tm value or when shuttle PCR is not feasible. To further optimize PCR conditions, please see section VIII.-(1) Optimization.



Shuttle PCR standard protocol

Holding Stage
Step 1: 95℃ 30 sec

Cycling Stage
Cycles: 40
Step 1: 95℃ 5 sec
Step 2: 60℃ 30 sec

Melt Curve Stage

3. After the reaction is complete, check the amplification and melting curves and plot a standard curve if absolute quantification will be performed. For specific analysis methods, refer to the manual for the StepOnePlus Real-Time PCR System.

**【For the Thermal Cycler Dice Real Time System series】**

1. Prepare the PCR mixture shown below. To account for pipetting error, make a master mix with at least 10% more than the total volume needed for the total number of reactions.

<Per reaction>

| Reagent | Volume | Final conc. |
|---|---|---|
| TB Green *Premix Ex Taq* II (2X) (Tli RNaseH Plus), ROX plus | 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 | |

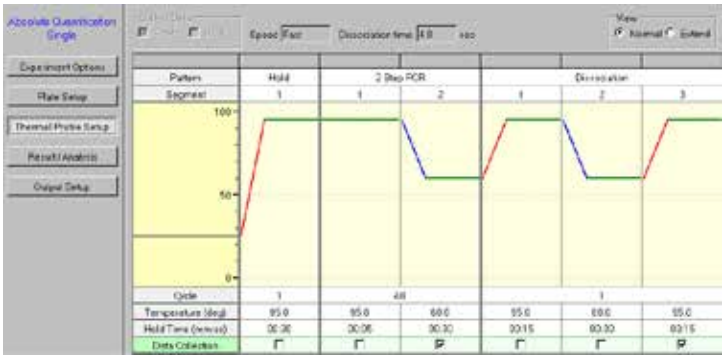
＊1 A final primer concentration of 0.4 μM is likely to yield good results. If there is insufficient reactivity, use a concentration between 0.2 and 1.0 μM.

＊2 The optimal 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 25 μl reaction. Furthermore, if cDNA (RT reaction mixure) is used as the template, the template volume should not exceed 10% of the PCR mixture.

＊3 The recommended reaction volume is 25 μl.

2. Start the reaction.

   The shuttle PCR standard protocol is recommended. Try this protocol first and optimize PCR conditions as necessary. Perform 3-step PCR when using a primer with a low Tm value or when shuttle PCR is not feasible. To further optimize PCR conditions, please see section VIII.-(1) Optimization.




Shuttle PCR standard protocol

Hold (Initial Denaturation)
Cycles: 1
95℃ 30 sec

2-step PCR
Cycles: 40
95℃ 5 sec
60℃ 30 sec

Dissociation

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

## VIII. Appendix

### (1) Optimization

If the recommended conditions (shuttle PCR standard protocol) do not provide sufficient reactivity, follow the procedures below to optimize the primer concentration and PCR conditions. In addition, using another real-time PCR reagent in the Perfect Real Time series (Cat. #RR420A/B/L/W, RR42LR/WR, or RR091A/B*) may greatly improve the results.

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

PCR conditions should be selected by considering both reaction specificity and amplification efficiency. A PCR system that balances these two aspects allows accurate measurement over a wide range of concentrations.

○ System with a high reaction specificity

- Using a negative, 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 at earlier cycles (lower $C_t$ value).
- PCR amplification efficiency is high (near the theoretical maximum of 100%).

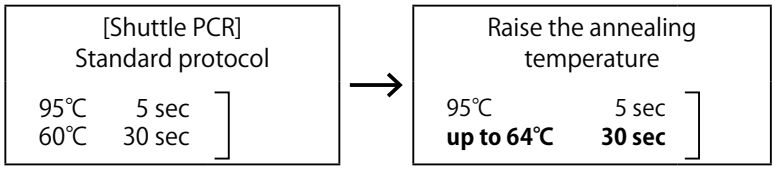
[Optimization of primer concentration]

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

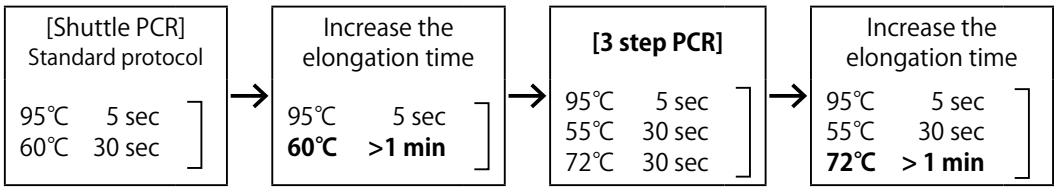
| (Primer concentration) | Low (0.1 μM) | | High (1.0 μM) |
|---|---|---|---|
| Specificity | High | ← | Low |
| Efficiency | Low | → | High |

[Optimization of PCR conditions]

○ To increase reaction specificity
Raising the annealing temperature may improve reaction specificity. Perform optimization while checking amplification efficiency.




○ To increase amplification efficiency
Increasing the elongation time or switching to 3-step PCR may improve amplification efficiency. Optimize using the steps below.




○ 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. Denaturation may be extended to 1 to 2 min at 95℃ 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.

[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* II (Tli RNaseH Plus) (Cat. #RR820A/B/L/W, RR82LR/WR) is a versatile reagent that balances specificity and amplification efficiency. To further increase specificity, TB Green Premix DimerEraser™ (Perfect Real Time) (Cat. #RR091A/B)＊ may be used. For products that are difficult to amplify, TB Green *Premix Ex Taq* (Tli RNaseH Plus) (Cat. #RR420A/B/L/W, RR42LR/WR) provides high amplification efficiency and is suitable for high-speed reactions.

(Reagent)

| | TB Green *Premix Ex Taq* (Tli RNaseH Plus) (Cat. #RR420A/L, RR42LR) | TB Green *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820A/L, RR82LR) | TB Green Premix DimerEraser (Perfect Real Time) (Cat. #RR091A/B)＊ |
|---|---|---|---|
| Specificity | Lower | ← | Higher |
| Efficiency | Higher | → | Lower |

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

**(2) Primer design**

Designing primers with good reactivity is critical to successful real-time PCR. 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 shuttle PCR standard protocol (Section VII.).

■ 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 the forward primer and the reverse primer do not differ greatly in Tm values.<br>Use software to determine Tm values.<br>OLIGO＊1 : 63 - 68℃<br>Primer3 : 60 - 65℃ |
| Sequence | Make sure there are no sequence biases overall.<br>Avoid having 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 GC-rich or AT-rich regions at the 3′ end.<br>It is preferable to have a G or C as the terminal base at the 3′ end.<br>Avoid a primer design with T as the terminal base at the 3′ end. |
| Complementation | Avoid having any complementary sequences of three bases or more within a primer and between primers.<br>Avoid having any complementary sequences of two bases or more at a primer's 3′ end. |
| Specificity | Verify primer specificity with a BLAST search.＊2 |

＊1 OLIGO Primer Analysis Software (Molecular Biology Insights, Inc.)

＊2 https://blast.ncbi.nlm.nih.gov/Blast.cgi

**(3) Preparing templates for real-time RT-PCR**

When preparing cDNA templates for real-time RT-PCR, the following products are recommended:

- 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 provide reliable results. Refer to the product's user manual for RT reaction conditions.

Use the following protocol when using PrimeScript RT Master Mix (Perfect Real Time) (Cat. #RR036A/B).

1. Prepare the reverse transcription mixture shown below and keep on ice.

<Per reaction>

| Reagent | Amount | Final conc. |
|---|---|---|
| 5X PrimeScript RT Master Mix (Perfect Real Time) | 2 μl | 1X |
| Total RNA＊ | x μl | |
| RNase-free $dH_2O$ | to 10 μl | |

＊ Scale up the reverse transcription reaction as necessary. A 10 μl reaction mixture can reverse-transcribe up to 500 ng of total RNA.

2. Perform a reverse transcription reaction.

| | | |
|---|---|---|
| 37℃ | 15 min | (reverse transcription reaction) |
| 85℃ | 5 sec | (heat inactivation of reverse transcriptase) |
| 4℃ | | |

3. Perform PCR according to the method described in VII. Protocol.

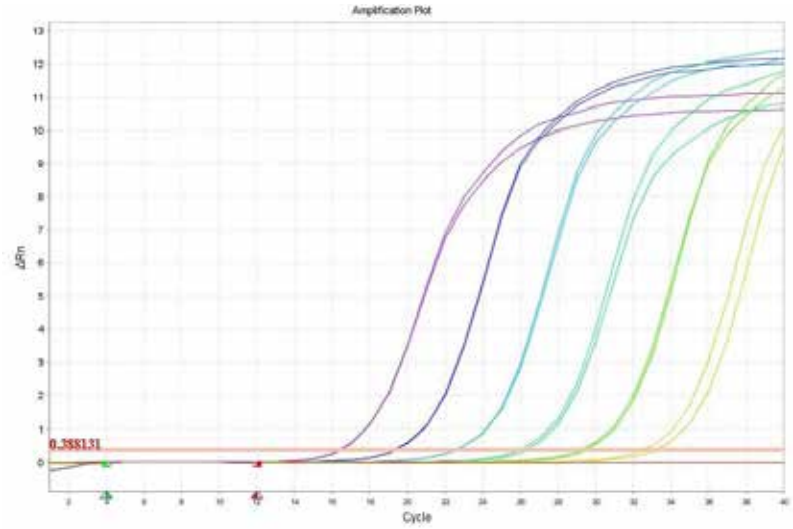
■ Experimental example (StepOnePlus Real-Time PCR System)

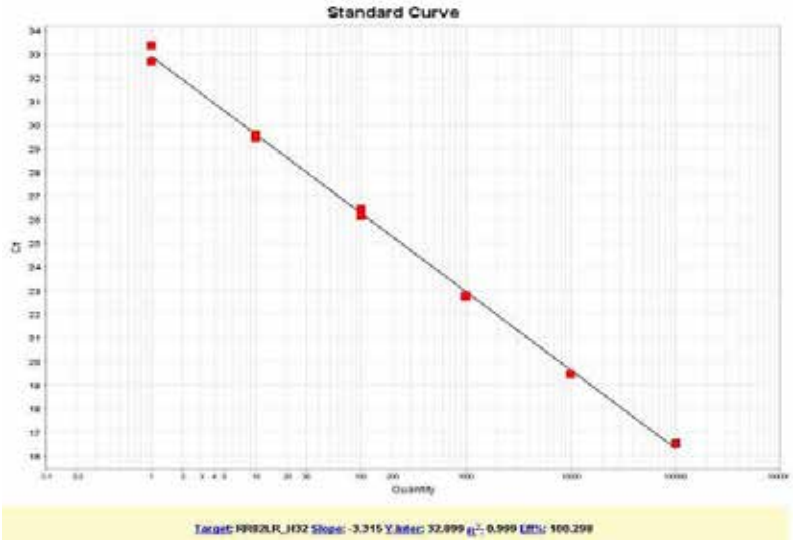
Reverse Transcription Reaction

Template: HL60 total RNA (1 pg to 100 ng)

Real-time PCR

Target: Human *TFRC*



## IX. Related Products

TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B)
TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus), Bulk (Cat. #RR820L/W)
TB Green® *Premix Ex Taq*™ (Tli RNaseH Plus) (Cat. #RR420A/B)
TB Green® *Premix Ex Taq*™ (Tli RNaseH Plus), Bulk (Cat. #RR420L/W)
TB Green® *Premix Ex Taq*™ (Tli RNaseH Plus), ROX plus (Cat. #RR42LR/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)*

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