Cat. # RR830L

For Research Use

TaKaRa

TB Green® *Premix Ex Taq*™ II FAST qPCR, Bulk

Product Manual

v202604w

Table of Contents

I. Description

TB Green *Premix Ex Taq* II Fast qPCR, Bulk is a reagent specifically designed for intercalator-based real-time PCR. This product has an optimal reaction solution composition that combines high-speed variant *Taq* DNA polymerase with a buffer system that suppresses primer dimers. The addition of heat-resistant Tli RNaseH and Uracil-*N*-Glycosylase (UNG) minimizes PCR inhibition due to residual mRNA when cDNA is used as the template, and reduces amplification from carry-over contamination, respectively. Due to these attributes, this reagent allows for highly reproducible qPCR-based quantitation and detection over a wide dynamic range for a broad range of gene targets.

The 2X premixed reagent contains a universal reference and visualization dye to correct for variations in the fluorescence signal between wells, thereby minimizing operational error.

Models that are compatible with this product:

- Thermal Cycler Dice™ Real Time System IV (Cat. #TP1000/TP1010/TP1030)*
- Thermal Cycler Dice Real Time System III (Cat. #TP950/TP970/TP980/TP990)*
- CronoSTAR™ 96 Real-Time PCR System (Cat. #640231/640232)*
- Applied Biosystems QuantStudio 3/5 Real-Time PCR System (Thermo Fisher Scientific)
- Applied Biosystems StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)
- LightCycler 480 System (Roche Diagnostics)
- CFX96 Real-Time PCR Detection System (Bio-Rad)

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

II. Principle

This product contains a variant of *Taq* DNA Polymerase (refered to as variant *Taq* HS) that is used for PCR amplification. Amplification products are monitored in real time with TB Green dye.

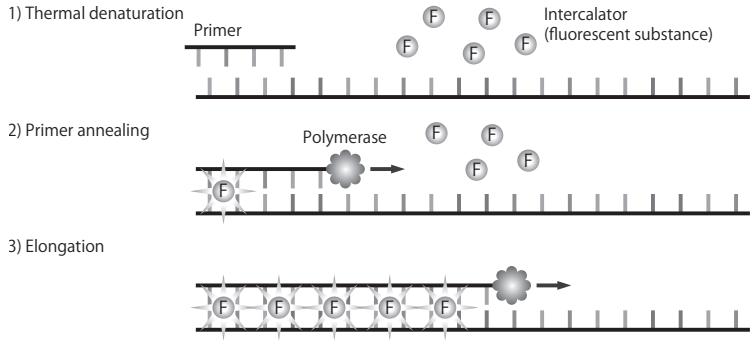
1. PCR

Polymerase chain reaction (PCR) uses primers to amplify a desired gene fragment from minute amounts of DNA. The reaction involves three steps: thermal denaturation, primer annealing, and elongation with DNA polymerase. By cycling through these three steps, it is possible to rapidly amplify the desired gene fragment by one-million fold.

The use of variant *Taq* HS for hot start PCR prevents nonspecific amplification caused by precycle mispriming during sample preparation and specifically-chosen reaction component ingredients suppress primer dimer formation. As a result, highly sensitive detection is possible.

2. Fluorometric detection (intercalator method)

The intercalator method of flourometric detection uses a reagent that emits fluorescence when it binds to double-stranded DNA (intercalator: TB Green dye, etc.). The intercalator is added to the reaction mixture and when double-stranded DNA is synthesized through PCR, fluorescence is emitted. By detecting the intensity of this fluorescence, it is possible to quantitate sample input and also measure the melting temperature of the amplified DNA.



III. Components [400 cycles, 25 µl volume per reaction]

TB Green *Premix Ex Taq* II FAST qPCR (2X), Bulk*1	5 ml
EASY Dilution II (for Real Time PCR)*2,3	1 ml x 3

*1 Contains variant *Taq* HS, dNTP Mixture, Mg^{2+}, Tli RNaseH, Uracil-*N*-Glycosylase (UNG), universal reference, visualization dye, and TB Green dye.

*2 EASY Dilution II (for Real Time PCR) is used as a diluent for serial dilution of total RNA and cDNA. Unlike water or TE buffer, EASY Dilution II (for Real Time PCR) allows for accurate dilution down to low concentrations and a wide range of calibration curves can be prepared. Moreover, this buffer never affects the reactivity of reverse transcription or PCR. The diluted template solution can be used as-is as the template for the reverse transcription or PCR reaction.

*3 EASY Dilution II (for Real Time PCR) can be purchased separately (Cat. #9451).

Materials required but not provided

1. Real-time PCR equipment
2. Dedicated reaction PCR tubes or plates
3. PCR primer*4
4. Sterile purified water
5. Micropipettes and tips

*4 For guidelines on how to design primers for real-time PCR, see "VIII. Appendix: Primer Design".

IV. Storage

Store at 4℃: stable for 6 months.
Shield from light and exercise care to avoid contamination.

Notes:

- EASY Dilution II (for Real Time PCR) should be stored at –20℃ after use.
- TB Green *Premix Ex Taq* II FAST qPCR (2X), Bulk should be stored at 4℃ and used within 6 months once the tube has been opened. For long-term storage, store at –20℃.

V. Features

1. Gene detection and quantitation can be performed rapidly and accurately by real-time PCR using this product.
2. This is a premixed reagent with a 2X concentration that includes TB Green dye. Real-time PCR using the intercalator method can be performed simply by adding primer, template, and sterile purified water.
3. High-speed reactions are performed with the variant *Taq* HS. The buffer system, which has been optimized to suppress primer dimer formation, allows for a high signal/noise ratio and sensitivity despite a short elongation time.
4. This product contains heat-resistant Tli RNaseH and UNG. Tli RNaseH minimizes PCR inhibition by residual RNA in cases where cDNA is used as template. UNG makes it possible to avoid carry-over contamination.
5. Universal reference and visualization dye come already added to the 2X premixed reagent. There is no need to add additional reference reagent to the premix to correct for well-to-well differences in the fluorescence signal.

VI. Precautions for Use

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

1. The following reagents are recommended for use in reverse transcription reactions when performing real-time RT-PCR. Highly reliable results can be obtained by using these reagents together with this product.
   - 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)
2. Before use, mix by gently inverting the tube, taking care not to create bubbles, to ensure that the reagent is evenly mixed. Sufficient reactivity may not be obtained if the reagent composition is uneven. DO NOT mix with a vortex mixer.

   When TB Green *Premix Ex Taq* II FAST qPCR (2X), Bulk has been stored at –20℃, a white to yellowish-white precipitate may form. To dissolve the precipitate, tap the tube for crushing the precipitate, then warm the product with your hands and mix by inversion. After dissolving, store at 4℃ in the dark. It is important to make sure the precipitate is completely dissolved and mixed before use to ensure that the reagent composition is even.
3. When preparing samples for PCR, keep the reagent on ice.
4. This product includes intercalator TB Green dye, which is light-sensitive. Be careful not to expose the reagent to strong light during sample preparation. Used TB Green *Premix Ex Taq* II Fast qPCR (2X), Bulk should be stored immediately away from light.
5. Be sure to use a new disposable pipette tip when preparing and dispensing the reaction solution, and take every precaution to prevent sample-to-sample contamination.

VII. Protocol

Note: **Please operate all equipment in accordance with the instruction manual.**

1. Preparation of PCR reaction solution

<Per reaction>

Reagents	Volume	Final conc.
TB Green *Premix Ex Taq* II Fast qPCR (2X), Bulk	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]	≤ 2.5 μl	
Sterile purified water	x μl	
Total	25 μl	

*1 In many cases, good results can be obtained with a final primer concentration of 0.4 μM. If you are having problems with amplification, it may be useful to try primer concentrations within the range of 0.2 - 1.0 μM.

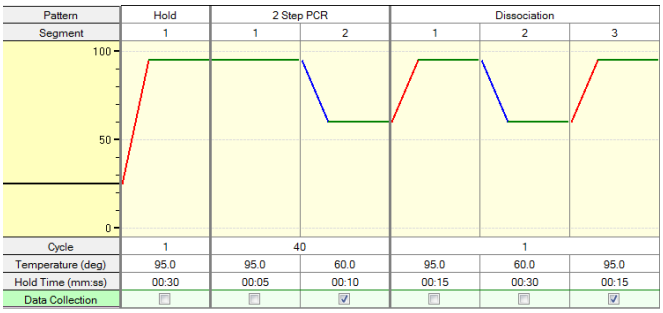
*2 For DNA templates, a concentration of no more than 100 ng is recommended. For cDNA templates (using RT reaction solution), the added amount should not exceed 10% of the total volume of the PCR reaction solution.

2. Start of reaction

It is recommended that you perform the PCR reaction according to the following standard two-step PCR protocol. Try this protocol first, and then optimize your cycling conditions as necessary.

If your assay is designed in such a way in which two-step PCR would be difficult, such as when using a primer with a low Tm value, perform three-step PCR instead.

Two-step PCR Standard Protocol



Hold (Initial Denaturation)[*3]
Cycle：1
95℃ 30 sec

Two-Step PCR[*4]
Cycles：40
95℃ 5 sec
60℃ 10 sec

Dissociation

*3 If contamination by PCR products (including dUTP) is suspected, perform a step at 25℃ for 2 - 10 min before the initial denaturation. PCR products carried over from the previous experiment will be degraded by the action of UNG.

*4 When optimizing PCR conditions, refer to "Optimizing PCR conditions" on page 7.

Note: The variant *Taq* HS used in this product is a hot start DNA polymerase with anti-*Taq* antibodies that inhibit polymerase activity at low temperatures. DO NOT perform a 5 - 15 min activation step at 95℃ that is usually necessary with other chemically modified hot start PCR enzymes. Excessive heat treatment tends to cause a decrease in enzyme activity, adversely affecting amplification efficiency and quantitation accuracy.
Even if initial denaturation of the template is performed before the PCR reaction, the usual 95℃ for 30 seconds will be sufficient.

3. End of reaction

Check the amplification curve and melting curve, and prepare a calibration curve if quantitation is to be performed.

Optimizing PCR conditions

If good amplification cannot be obtained with the recommended conditions (Two-step PCR Standard Protocol), consider the primer concentration and PCR conditions described below.

When optimizing PCR conditions, take into account both reaction specificity and amplification efficiency. In an experimental system with a good balance of the two, it is possible to perform accurate quantitation over a wide range of concentrations.

○ PCR optimized for high specificity

- Nonspecific amplification of primer dimers, etc., does not occur in the no-template control
- Nonspecific amplification of items other than the desired product does not occur.

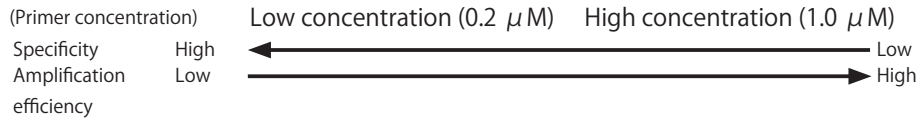
○ PCR optimized for high amplification efficiency

- Amplification products are detected in an earlier cycle (Ct value is small).
- PCR amplification efficiency is high (close to the theoretical value of 100%).

[Primer concentration considerations]

The following relationship exists between the primer concentration, PCR specificity, and amplification efficiency:

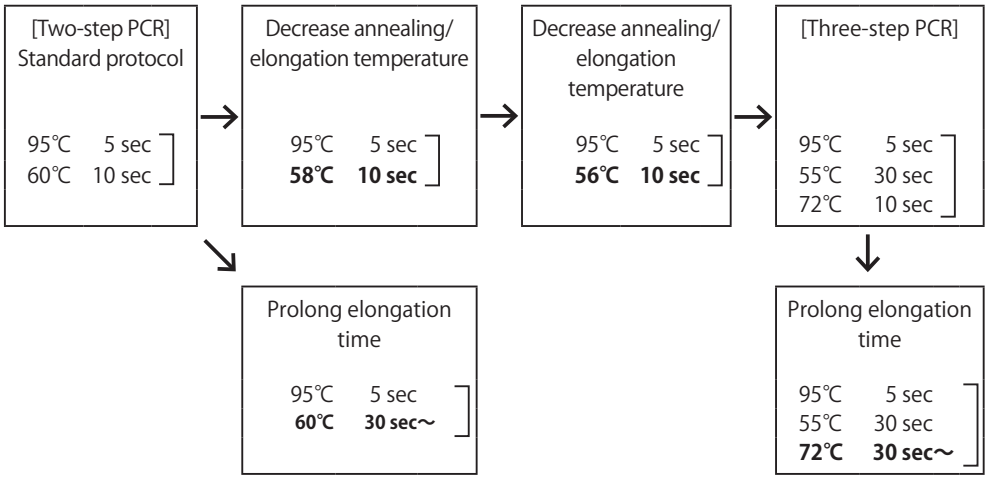
- To increase the assay specificity, decrease the primer concentration.
- To increase the amplification efficiency, increase the primer concentration.



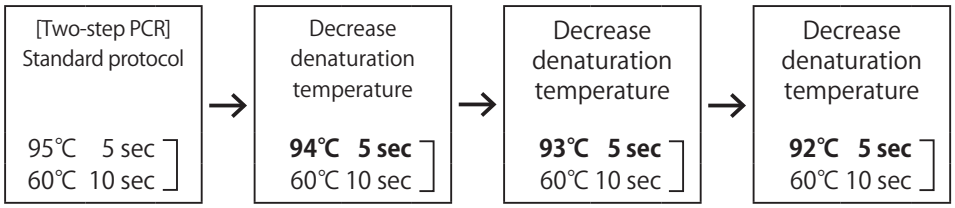
[PCR condition considerations]

○ To increase the amplification efficiency:

(1) Decreasing the annealing/elongation temperature, switching to three-step PCR, or prolonging the elongation time may improve the amplification efficiency.

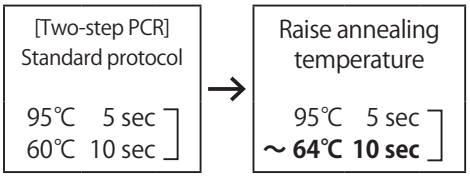


(2) Decreasing the denaturation temperature from 95℃ to 92℃ in increments of 1℃ may improve amplification efficiency.



○ To increase specificity:

Raising the annealing temperature may improve specificity, however, this may affect amplification efficiency.



○ Initial denaturation

For initial denaturation, 30 sec at 95℃ is usually sufficient, even with a template that is difficult to denature such as circular plasmid or genomic DNA. Depending on the condition of the template, it may be possible to extend the initial denaturation to it 1 - 2 min at 95℃. Please note that if the initial denaturation is extended for too long it may cause deactivation of the enzyme. Therefore, an initial denaturation cycle of >2 min is not recommended.

VIII. Appendix: Primer Design

In order to perform real-time PCR efficiently, it is important to design good quality primers. Please design primers in accordance with the following guidelines for optimal assay specificity and amplification efficiency. Primers designed and synthesized using these guidelines are compatibile with the Two-step PCR Standard Protocol (Section VII).

■ Amplification products

Amplification size	80 - 150 bp is optimal. (Amplification up to 300 bp is possible.)

■ Primer

Length	17 - 25 mers
GC content	40 - 60% (preferably, 45 - 55%)
Tm	The Tm values of the forward primer and reverse primer should not differ greatly. Calculation of Tm value is performed with specialized software. OLIGO* : 63 - 68℃ Primer3 : 60 - 65℃
Sequence	Use a sequence without overall base biases. Avoid sequences with GC-rich or AT-rich regions (particularly at the 3′ end). Avoid consecutive T/C pairings (polypyrimidine). Avoid consecutive A/G pairings (polypurine).
3′ end sequence	Avoid sequences with GC-rich or AT-rich regions at the 3′ end. The base at the 3′ end should preferably be G or C. Primers with T at the 3′ end should be avoided.
Complementation	Avoid complementary sequences of 3 or more bases within or between primers. Avoid complementary sequences of 2 or more bases at 3′ ends of primers.
Specificity	Confirm primer specificity by BLAST search.

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

IX. Related Products

TB Green® *Premix Ex Taq*™ II FAST qPCR (Cat. #RR830S/A/B)
TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820S/A/B)
TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus), Bulk (Cat. #RR820L/W)*
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)
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)*
EASY Dilution II (for Real Time PCR) (Cat. #9451)
Thermal Cycler Dice™ Real Time System IV (Cat. #TP1000/TP1010/TP1030)*
Thermal Cycler Dice™ Real Time System III (Cat. #TP950/TP970/TP980/TP990)*
CronoSTAR™ 96 Real-Time PCR System (Cat. #640231/640232)*

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