

Cat. # RR820Q

For Research Use

TAKARA

**SYBR[®] *Premix Ex Taq*[™] II
(Tli RNaseH Plus)**

Product Manual

v201402Da

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I. Description

SYBR *Premix Ex Taq* II (Tli RNaseH Plus) is a reagent specifically designed for intercalator-based real-time PCR using SYBR Green I for detection. The premix is supplied at a 2X concentration and contains SYBR Green I 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, which minimizes PCR inhibition due to residual mRNA when using cDNA as template.

This product contains a modified buffer that offers higher reaction specificity than SYBR *Premix Ex Taq* (Tli RNaseH Plus) (Cat. #RR420A). By inhibiting non-specific amplification, which can interfere with quantitative determination, accurate assay over a wide range is possible. The combination of this buffer and *TAKARA Ex Taq*® HS, a hot start PCR enzyme that uses an anti-*Taq* antibody, allows highly reproducible and reliable real-time PCR analyses.

Benefits :

- (1) Allows rapid and accurate detection and gene expression analysis by real-time PCR.
- (2) Convenient ready-to-use 2X premix. Simply add primers, template, and sterile distilled water to perform intercalator-based real-time PCR.
- (3) *TAKARA Ex Taq* HS is an efficient, hot start PCR enzyme. The buffer system has been optimized for real-time PCR, enabling good amplification efficiency and high-sensitivity detection.
- (4) The 2X reagent is premixed with Tli RNase H, a heat-resistant RNase that minimizes inhibition of PCR due to residual mRNA when using cDNA as template.

II. Principle

This product includes *TAKARA Ex Taq* HS DNA polymerase for PCR amplification. PCR amplification products may be monitored in real time by SYBR Green I.

1. PCR

PCR is a technique used to amplify a target sequence from a minute amount of DNA. By repeating 3 cycles of DNA heat denaturation, primer annealing, and elongation, the target gene fragment may be quickly amplified using DNA polymerase.

This product uses *TAKARA Ex Taq* HS, a hot start PCR enzyme that prevents non-specific amplification 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 involves the addition of an intercalating reagent (e.g., SYBR Green I) that emits fluorescence when bound to double-strand DNA in the reaction mixture.

This enables the detection of amplified DNA by monitoring fluorescence. Fluorescence measurement not only allows for quantitative determination of target DNA but also for DNA composition by melting curve analysis.

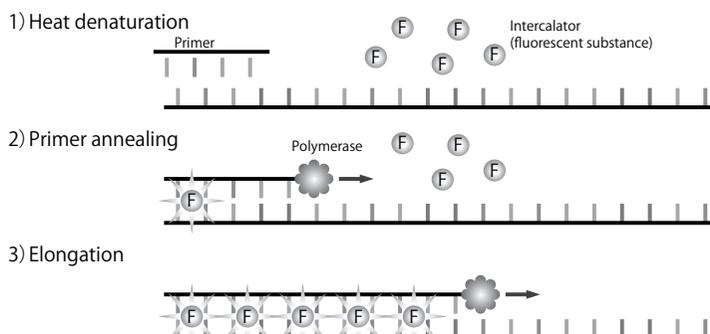


Figure 1. Fluorescent intercalator detection method.

III. Components [for 40 x 50 µl reactions]

SYBR <i>Premix Ex Taq</i> II (Tli RNaseH Plus) (2X conc.)* ¹	1 ml
ROX Reference Dye (50X conc.)* ²	40 µl
ROX Reference Dye II (50X conc.)* ²	40 µl

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

Note: For this product's quality specifications, refer to Section X.

* 2: ROX Reference Dye and ROX Reference Dye II are intended to be used with instruments that correct for between-well fluorescent signal, such as the real-time PCR devices by Life Technologies.

- ◆ Use the ROX Reference Dye
 - Applied Biosystems 7300 Real-Time PCR System
 - Applied Biosystems StepOnePlus Real-Time PCR System (Life Technologies)
 - ◆ Use the ROX Reference Dye II
 - Applied Biosystems 7500/7500 Fast Real-Time PCR System (Life Technologies)
 - ◆ Do not use this component
 - Thermal Cycler Dice™ Real Time System II (Cat. #TP900/TP960)*
 - LightCycler/LightCycler 480 System (Roche Diagnostics)
 - CFX96 Real-Time PCR Detection System (Bio-Rad)
- *: Not available in all geographic locations. Check for availability in your region.

IV. Materials Required but not Provided

1. Reagents

- PCR primers
For guidelines for real-time PCR primer design, refer to Section IX.1.
- Sterile distilled water

2. Materials

- Real-time PCR reaction tubes or plates
- Micropipettes and tips (autoclave treated)
- Gene amplification system for real-time PCR (authorized instruments)

Compatible instruments include:

- Thermal Cycler Dice Real Time System // (Cat. #TP900/TP960)*
Thermal Cycler Dice Real Time System Single (Cat. #TP850/TP870)*
Thermal Cycler Dice Real Time System *Lite* (Cat. #TP700/TP760)*
*: Not available in all geographic locations. Check for availability in your area.
- Applied Biosystems 7300/7500, 7500 Fast Real-Time PCR System, StepOnePlus Real-Time PCR System (Life Technologies)
- LightCycler/LightCycler 480 System (Roche Diagnostics)
- CFX96 Real-Time PCR Detection System (Bio-Rad)

Note: SYBR *Premix Ex Taq* (Tli RNaseH Plus) (Cat. #RR420S/A/B) is recommended when using the Smart Cycler System/Smart Cycler II System (Cepheid).

V. Storage

- Store at 4°C (stable for up to 6 months).
- Protect this kit from light and avoid contamination.
- This kit is shipped in a package containing dry ice. Store the kit protected from light at 4°C after its receipt.
- For long-term storage, keep at -80°C. Do not store at -20°C. Store thawed product at 4°C, and use within 6 months.

VI. Precautions

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

1. Prior to use, make sure the reagent is evenly mixed by gently inverting several times without creating bubbles. Uneven reagent mixture will result in inadequate reactivity.
 - Do not mix by vortexing.
 - When stored at -80°C SYBR *Premix Ex Taq* II (Tli RNaseH Plus) (2X conc.) may develop a white to pale yellow precipitate. Gently hand-warm and allow to stand protected from light at room temperature briefly, then invert several times to dissolve the precipitate completely.
 - The presence of precipitate is indicative of uneven reagent composition; make sure the reagent is evenly mixed before use.
2. Place reagents on ice when preparing the reaction mixture.
3. This product contains SYBR Green I. 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.

VII. Protocol

Note: Please follow the procedures provided in the manual provided with each respective instrument.

[For the Applied Biosystems 7300/7500/7500 Fast Real-Time PCR System and StepOnePlus Real-Time PCR System]

A. Prepare the PCR mixture shown below.

<Per reaction>

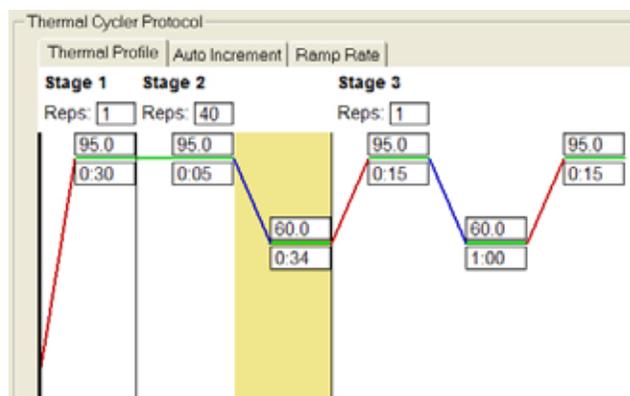
Reagent	Volume	Volume	Final conc.
SYBR <i>Premix Ex Taq</i> II (Tli RNaseH Plus) (2X)	10 μ l	25 μ l	1X
PCR Forward Primer (10 μ M)	0.8 μ l	2 μ l	0.4 μ M *1
PCR Reverse Primer (10 μ M)	0.8 μ l	2 μ l	0.4 μ M *1
ROX Reference Dye (50X) or Dye II (50X) *2	0.4 μ l	1 μ l	1X
Template *3	2 μ l	4 μ l	
dH ₂ O (sterile distilled water)	6 μ l	16 μ l	
Total	20 μ l *4	50 μ l *4	

- *1: A final primer concentration of 0.4 μ M is likely to yield good results. However, if there is insufficient reactivity, use a primer concentration between 0.2 and 1.0 μ M.
- *2: The concentration for ROX Reference Dye II (50X) is lower than that for ROX Reference Dye (50X).
- Use ROX Reference Dye II (50X) when performing analyses with Applied Biosystems 7500/7500 Fast Real-Time PCR System.
 - Use ROX Reference Dye (50X) when using StepOnePlus or Applied Biosystems 7300 Real-Time PCR System.
- *3: 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 μ l. Furthermore, if cDNA (RT reaction mixture) is used as the template, the volume of the RT reaction mixture should not exceed 10% of the PCR mixture.
- *4: Prepare in accordance with the recommended volume for each instrument.

B. Start the reaction.

The recommended shuttle PCR standard protocol is described below. Try this protocol first and optimize PCR conditions as necessary. Perform 3-step PCR when using primers with low T_m values or when shuttle PCR is not feasible. To optimize PCR conditions further, please see section VIII. Optimization.

1) Applied Biosystems 7300/7500 and, StepOnePlus Real-Time PCR System



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

*: With StepOnePlus, set to 30 sec.;
with 7300, set to 31 sec.; and with
7500, set to 34 sec.

Figure 2. Shuttle PCR standard protocol.

2) Applied Biosystems 7500 Fast Real-Time PCR System

Shuttle PCR standard protocol

Holding Stage

Reps: 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 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 quantification will be performed.

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

[For the LightCycler/LightCycler 480 System]

A. Prepare the PCR mixture shown below.

<Per reaction>

Reagent	Volume	Final conc.
SYBR <i>Premix Ex Taq</i> II (Tli RNaseH Plus) (2X)	10 μ l	1X
PCR Forward Primer (10 μ M)	0.8 μ l	0.4 μ M *1
PCR Reverse Primer (10 μ M)	0.8 μ l	0.4 μ M *1
Template (< 100 ng)*2	2 μ l	
dH ₂ O (sterile distilled water)	6.4 μ l	
Total	20 μ l	

- *1: A final primer concentration of 0.4 μ M is likely to yield good results. However, if there is insufficient reactivity, use a primer concentration between 0.1 and 1.0 μ M.
- *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 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.

B. Start the reaction.

The shuttle PCR standard protocol is recommended for PCR. Try this protocol first and optimize PCR conditions as necessary. Perform 3-step PCR when using primers with low T_m values or when shuttle PCR is not feasible. To optimize PCR conditions further, please see section VIII. Optimization.

<LightCycler>

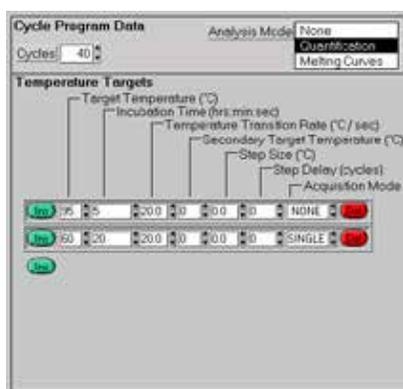


Figure 3. Shuttle PCR standard protocol

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.

<LightCycler480 System>

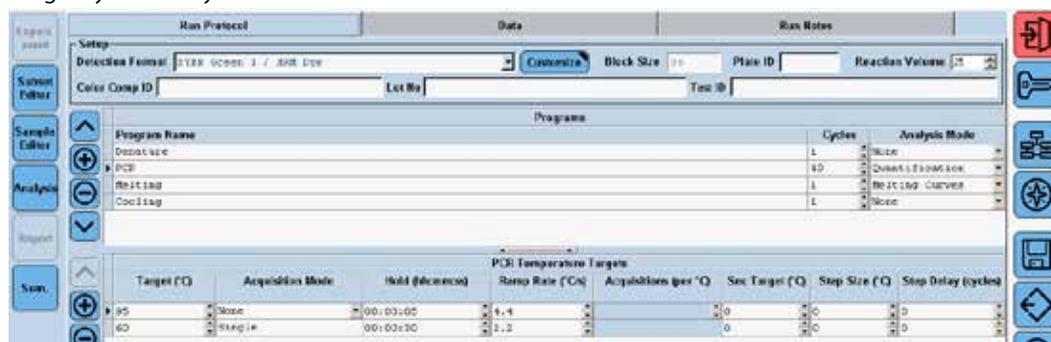


Figure 4. Shuttle PCR standard protocol

Initial denaturation

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 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 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 quantification will be performed.
Refer to the instrument's instruction manual for specific analysis methods.

[For theCFX96 Real-Time PCR Detection System]

A. Prepare the PCR mixture shown below.

<Per reaction>

Reagent	Volume	Final conc.
SYBR <i>Premix Ex Taq</i> II (Tli RNaseH Plus) (2X)	12.5 μ l	1X
PCR Forward Primer (10 μ M)	1 μ l	0.4 μ M *1
PCR Reverse Primer (10 μ M)	1 μ l	0.4 μ M *1
Template (<100 ng)*2	2 μ l	
dH ₂ O (sterile distilled water)	8.5 μ l	
Total	25 μ l	

- * 1: A final primer concentration of 0.4 μ M is likely to yield good results. However, if there is insufficient reactivity, use a primer concentration between 0.2 and 1.0 μ M.
- * 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 μ l. Furthermore, if cDNA (RT reaction mixture) is used as the template, the volume of the RT reaction mixture should not exceed 10% of the PCR mixture.

[For the Thermal Cycler Dice Real Time System II]

A. Prepare the PCR mixture shown below.

<Per reaction>

Reagent	Volume	Final conc.
SYBR <i>Premix Ex Taq</i> II (Tli RNaseH Plus) (2X)	12.5 μ l	1X
PCR Forward Primer (10 μ M)	1.0 μ l	0.4 μ M *1
PCR Reverse Primer (10 μ M)	1.0 μ l	0.4 μ M *1
Template (<100 ng)*2	2.0 μ l	
dH ₂ O (sterile distilled water)	8.5 μ l	
Total	25 μ l *3	

- *1: A final primer concentration of 0.4 μ M is likely to yield good results. However, if there is insufficient reactivity, use a primer concentration between 0.1 and 1.0 μ M.
- *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 μ l. Furthermore, if cDNA (RT reaction mixture) is used as the template, the volume of the RT reaction mixture should not exceed 10% of the PCR mixture.
- *3: The recommended reaction volume is 25 μ l.

- B. Start the reaction.
The shuttle PCR standard protocol is recommended for PCR. Try this protocol first and optimize PCR conditions as necessary. Perform a 3-step PCR when using primers with low T_m values or when shuttle PCR is not feasible.



Hold (initial denaturation)

Cycle: 1
95°C 30 sec.

2 Step PCR*4

Cycle: 40
95°C 5 sec.
60°C 30 sec.

Dissociation

*4: For optimizing PCR conditions, please refer to Section VIII.

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 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 quantification will be performed.
Refer to the instrument's instruction manual for specific analysis methods.

VIII. Optimization

If the recommended conditions (shuttle PCR standard protocol) do not provide sufficient reactivity, follow the procedures below to optimize primer concentration and PCR conditions. In addition, depending on the reaction system, switching to a different real-time PCR reagent from the Perfect Real Time series (Cat. #RR420S/A/B, RR091A/B*) may greatly improve the results.

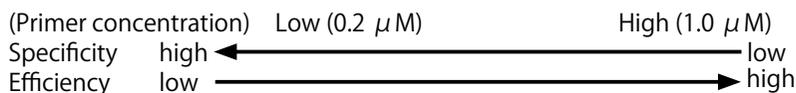
Select PCR conditions based on comprehensive analysis of both reaction specificity and amplification efficiency. Using conditions that balance these two aspects will allow accurate measurement over a wide range of concentrations.

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

- 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 at earlier cycles (small Ct value).
 - PCR amplification efficiency is high (near the theoretical value of 100%).

1. Evaluation of primer concentration

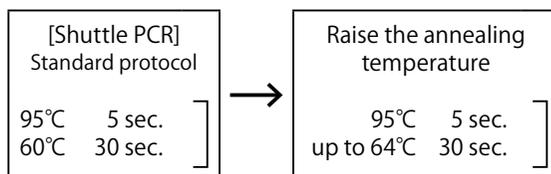
The relationship between primer concentration and reaction specificity and amplification efficiency is illustrated below. Reducing primer concentration raises reaction specificity. In contrast, increasing the primer concentration raises amplification efficiency.



2. Evaluation of PCR conditions

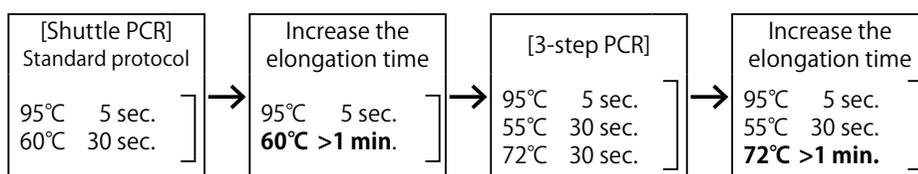
- To improve reaction specificity

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



- To improve amplification efficiency

Increasing the elongation time or switching to a 3-step PCR 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 procedure may be extended to 1 to 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 > 2 min.

3. Relationship between reagent and reactivity

Takara Bio supplies three different reagents for SYBR Green real-time PCR analysis. The relationship between reaction specificity and amplification efficiency for these reagents is described below.

SYBR *Premix Ex Taq* (Tli RNaseH Plus) (Cat. #RR420S/A/B) provides high amplification efficiency. SYBR *Premix Ex Taq* II (Tli RNaseH Plus) (Cat. #RR820S/A/B) and SYBR *Premix DimerEraser*™ (Perfect Real Time) (Cat. #RR091A/B)* have greater specificity.

(Reagent)	SYBR <i>Premix Ex Taq</i>	SYBR <i>Premix Ex Taq</i> II	SYBR <i>Premix DimerEraser</i>
Specificity	lower	←————→ higher	
Efficiency	higher	————→ lower	

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

IX. Appendix**1. Primer design**

Designing primers with a 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 shuttle PCR standard protocol (Section VII.).

■ Amplification product

Amplification size	The optimal size is 80 - 150 bp (amplification up to 300 bp is possible).
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■ 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. Use software to determine Tm values. OLIGO*1 : 63 - 68°C Primer3*2: 60 - 65°C
Sequence	Make sure that there are no overall 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. It is better to avoid a primer design 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 a BLAST search*3.

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

* 2: Primer3 (http://biotools.umassmed.edu/bioapps/primer3_www.cgi)

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

2. When performing real-time RT-PCR

For preparing cDNA templates for real-time RT-PCR, PrimeScript™ RT 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 highly reliable results.

- A. Prepare PCR mixtures according to 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.

<Per reaction>

Reagent	Volume	Final conc.
SYBR <i>Premix Ex Taq</i> II (2X)	12.5 μ l	1X
PCR Forward Primer (10 μ M)	1.0 μ l	0.4 μ M
PCR Reverse Primer (10 μ M)	1.0 μ l	0.4 μ M
dH ₂ O (sterile distilled water)	x μ l	
Total	22.5 - 24 μl	

- 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.

[Reaction example]

Human TBP mRNA was detected by real-time RT-PCR. cDNA equivalent to 1 pg - 100 ng of total RNA was used as the template, with dH₂O as the negative control.

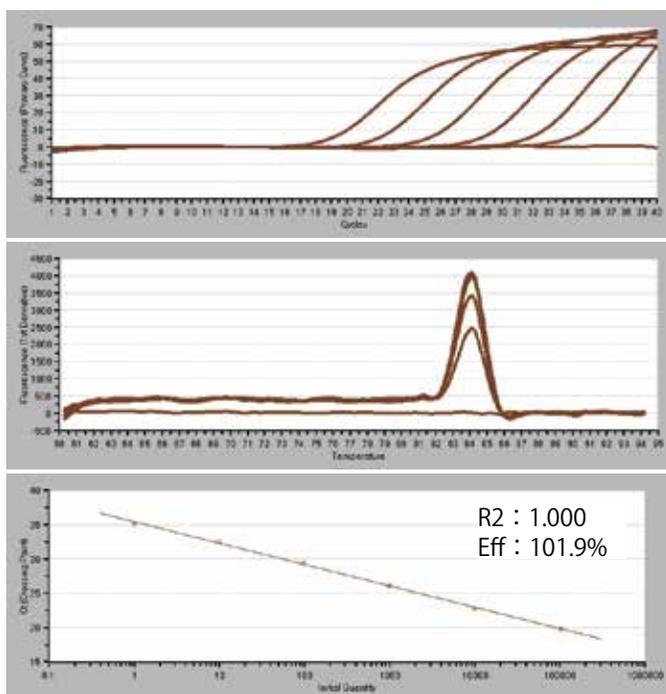


Figure 7. Detection of human TBP mRNA by real-time RT-PCR.

X. Quality Specifications

1. Quality test

Consistent amplification results and SYBR Green I detection were observed for real-time PCR with HL60 cDNA as template using Thermal Cycler Dice Real Time System (amplified fragment: 185 bp).

2. *TAKARA Ex Taq* HS

A. Definition of activity

1 U is defined as the activity required to incorporate 10 nmol of total nucleotides into acid-insoluble precipitate over 30 min. at 74°C using activated salmon sperm DNA as template/primer.

Composition of the reaction mixture for activity assay:

25 mM	TAPS buffer (pH9.3, 25°C)
50 mM	KCl
2 mM	MgCl ₂
0.1 mM	DTT
200 μM each	dATP, dGTP and dCTP
100 μM	[³ H]-dTTP
0.25 mg/ml	Activated salmon sperm DNA

B. Purity

- 1) No change in DNA electrophoresis pattern is observed after 10 U of this enzyme and 0.6 μg of *λ-Hind* III digest are allowed to react at 74°C for 1 hour.
- 2) No change in DNA electrophoresis pattern is observed after 10 U of this enzyme and 0.6 μg of supercoiled pBR322 DNA are allowed to react at 74°C for 1 hour.
- 3) No change in DNA electrophoresis pattern is observed after 10 U of this enzyme and 0.6 μg of *λ* DNA are allowed to react at 74°C for 1 hour.

XI. Related Products

SYBR® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B)

SYBR® *Premix Ex Taq*™ (Tli RNaseH Plus) (Cat. #RR420S/A/B/L/W/LR/WR)

SYBR® *Premix DimerEraser*™ (Perfect Real Time) (Cat. #RR091A/B)*

SYBR® *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 SYBR® PrimeScript™ RT-PCR Kit (Perfect Real Time) (Cat. #RR066A/B)*

One Step SYBR® PrimeScript™ RT-PCR Kit II (Perfect Real Time) (Cat. #RR086A/B)

One Step SYBR® PrimeScript™ PLUS RT-PCR Kit (Perfect Real Time) (Cat. #RR096A/B)*

Thermal Cycler Dice™ Real Time System // (Cat. #TP900/TP960)*

Thermal Cycler Dice™ Real Time System Single (Cat. #TP850/TP870)*

Thermal Cycler Dice™ Real Time System *Lite* (Cat. #TP700/TP760)*

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

NOTICE TO PURCHASER: LIMITED LICENSE**[P5] PCR Notice**

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,994,056 and 6,171,785. The purchase of this product includes a limited, nontransferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

[L11] SYBR® Green I

This product is provided under an intellectual property license from Life Technologies Corporation. The transfer of this product is contingent on the buyer using the purchased product and components of the product solely in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The sale of this product is expressly conditioned on the buyer not using the product or its components for any Commercial Purposes. Commercial Purposes means any activity by a party to generate revenue, which may include, but is not limited to use of the product or its components: (i) in manufacturing; (ii) to provide a service, information, or data in return for payment; (iii) for therapeutic, diagnostic or prophylactic purposes; or (iv) for resale, regardless of whether they are resold for use in research. For information on purchasing a license to this product for purposes other than research, contact Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, CA 92008 USA or outlicensing@lifetech.com.

[L46] SYBR®/Melting Curve Analysis

The purchase of this product includes a limited, non-transferable license for all fields other than human or veterinary in vitro diagnostics under specific claims of U.S. Patent Nos. 6,174,670, 6,569,627 and 5,871,908, owned by the University of Utah Research Foundation or Evotec Biosystems GmbH and licensed to Idaho Technology, Inc. and Roche Diagnostics GmbH, to use only the enclosed amount of product according to the specified protocols. No right is conveyed, expressly, by implication, or by estoppel, to use any instrument or system under any claim of U.S. Patent Nos. 6,174,670, 6,569,627 and 5,871,908, other than for the amount of product contained herein.

[L52] Rox Reference Dye (Research Field)

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,928,907. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

[M40] Thermostable RNase H

This product is covered by the claims of U.S. Patent No. 7,422,888 and its foreign counterpart patent claims.

[M57] LA Technology

This product is covered by the claims 6-16 outside the U.S. corresponding to the expired U.S. Patent No. 5,436,149.

[M82] Tli RNaseH Plus

This product is the subject of the pending JP patent application.

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