

Cat. # RR390A

For Research Use

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**TAKARA**

***Premix Ex Taq*<sup>™</sup>**  
**(Probe qPCR)**

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Product Manual

v201903Da

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## I. Description

*Premix Ex Taq* (Probe qPCR) is designed for probe-based qPCR. This product is suitable for high-speed PCR. *Premix Ex Taq* allows accurate target quantification and detection over a broad dynamic range and makes it possible to conduct highly reproducible and reliable real-time PCR analyses.

The product is supplied as a 2X premix to facilitate easy preparation of reaction mixtures. The 2X premixed reagent also contains Tli RNase H, a heat-resistant RNase H, to minimize PCR inhibition by residual mRNA in reactions using cDNA templates. A combination of *TaKaRa Ex Taq*® HS polymerase, a hot-start PCR enzyme that uses an anti-*Taq* antibody, and a buffer optimized for real-time PCR suppresses non-specific amplification, and allows high amplification efficiency and high detection sensitivity in real-time PCR analyses.

### Advantages:

- (1) This product allows rapid and accurate detection and quantitative gene expression analysis by real-time PCR.
- (2) The premixed reagent simplifies reaction set up.
- (3) Includes *TaKaRa Ex Taq* HS polymerase, an enzyme designed for hot-start PCR. The buffer system has been optimized for real-time PCR, and provides excellent amplification efficiency and highly sensitive detection.
- (4) The 2X premixed reagent includes Tli RNase H, a heat-resistant RNase H, to minimize PCR inhibition by residual mRNA in reactions using cDNA templates.

### Compatible 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 7300/7500/7500 Fast Real-Time PCR System and StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)
- LightCycler/LightCycler 480 System (Roche Diagnostics)
- CFX96 Real-Time PCR Detection System (Bio-Rad)
- 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 *TaKaRa Ex Taq* HS for PCR amplification. PCR amplification products may be monitored in real time using a probe.

### 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.

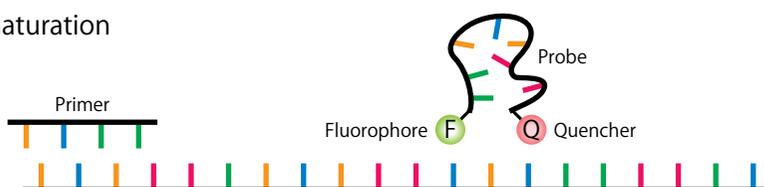
This product uses *TaKaRa Ex Taq* HS, a hot-start PCR enzyme that prevents non-specific amplification resulting from mispriming or primer dimer formation during reaction mixture preparation or other pre-cycling steps thereby allowing high-sensitivity detection.

### 2. Fluorescence Detection

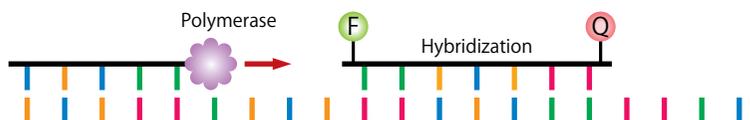
Oligonucleotides modified with a 5' fluorophore (e.g., FAM) and a 3' quencher (e.g., TAMRA) are added to the reaction.

Under annealing conditions, the probe hybridizes in a sequence-specific manner to the template DNA. Fluorescence of the fluorophore is suppressed by the quencher. During the extension reaction, the 5' → 3' exonuclease activity of *Taq* DNA polymerase degrades the hybridized probe, releasing quencher suppression and allowing fluorescence.

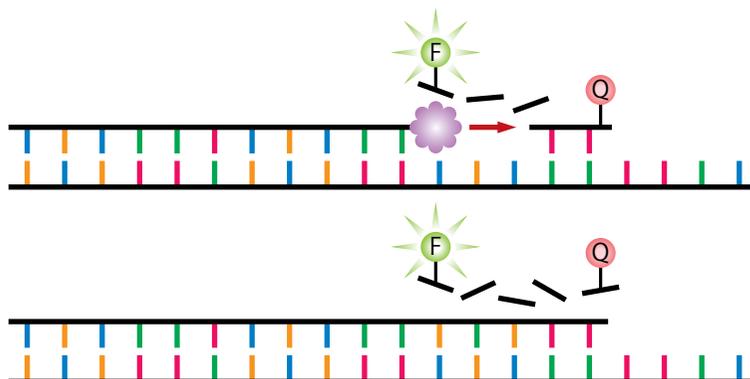
#### 1) Heat denaturation



#### 2) Primer annealing/probe hybridization



#### 3) Extension



### III. Components

(1) <i>Premix Ex Taq</i> (Probe qPCR) (2X conc.)* <sup>1</sup>	1 ml x 5
(2) ROX Reference Dye (50X conc.)* <sup>2</sup>	200 µl
(3) ROX Reference Dye II (50X conc.)* <sup>2</sup>	200 µl

\*<sup>1</sup> Contains *TAKARA Ex Taq* HS, dNTP Mixture, Mg<sup>2+</sup>, and Tli RNase H

\*<sup>2</sup> Use when performing analyses with real-time PCR instruments that normalize fluorescent signals between wells, such as Applied Biosystems instruments.

◆ Add ROX Reference Dye (50X) in a volume equivalent to 1/50 of the PCR reaction mixture when using the following Applied Biosystems systems:

- 7300 Real-Time PCR System
- StepOnePlus Real-Time PCR System

◆ Add ROX Reference Dye II (50X) in a volume equivalent to 1/100 of the PCR reaction mixture when using the following Applied Biosystems systems:

- 7500 Real-Time PCR System
- 7500 Fast Real-Time PCR System

◆ No ROX Reference Dye (50X) is required when using any of the following systems:

- Thermal Cycler Dice Real Time System series (Cat. #TP950/TP970/TP980/TP990, TP900/TP960, TP700/TP760)\*
- LightCycler/LightCycler 480 System (Roche Diagnostics)
- CFX96 Real-Time PCR Detection System (Bio-Rad)
- Smart Cycler System/Smart Cycler II System (Cepheid)

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

### IV. Materials Required but not Provided

- DNA amplification system for real-time PCR (authorized instruments)
- Reaction tubes or plates designed specifically for the qPCR instrument used
- PCR primers
- Probe for detection (Dual Labeled Probe, etc.)
- Sterile purified water
- Micropipette and tips (sterile, with filter)

### V. Storage

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

Every precaution should be taken to avoid contamination.

1. Before use, gently invert tube to make sure reagent is completely dissolved and evenly mixed.
2. This product may be frozen at -20°C for long term storage. Once thawed, it should be stored at 4°C and used within 6 months.

## VI. Precautions before Use

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 the tube several times without creating bubbles. Uneven reagent mixing will result in inadequate reactivity. Do not mix by vortexing.

When stored frozen at  $-20^{\circ}\text{C}$ , *Premix Ex Taq* (Probe qPCR) (2X conc.) may precipitate. To dissolve the precipitate completely, warm by hand or let stand at room temperature briefly, then invert the tube several times. Make sure reagent is evenly mixed before use.

2. Place reagent on ice immediately after it has thawed.
3. This product is not supplied with probe or primers.
4. Use fresh disposable tips to minimize potential cross-contamination between samples when preparing reaction mixtures or dispensing aliquots.

## VII. Protocol

### 1. Protocol using the Thermal Cycler Dice Real Time System III, II, and Lite

1. Prepare the PCR reaction mixture shown below.

<Per reaction>		
Reagent	Volume	Final Conc.
<i>Premix Ex Taq</i> (Probe qPCR) (2X conc.)	12.5 $\mu\text{l}$	1X
PCR Forward Primer (10 $\mu\text{M}$ )	0.5 $\mu\text{l}$	0.2 $\mu\text{M}^{*1}$
PCR Reverse Primer (10 $\mu\text{M}$ )	0.5 $\mu\text{l}$	0.2 $\mu\text{M}^{*1}$
Probe <sup>*2</sup>	1 $\mu\text{l}$	
Template <sup>*3</sup>	2 $\mu\text{l}$	
Sterile purified water	8.5 $\mu\text{l}$	
Total	25 $\mu\text{l}$	

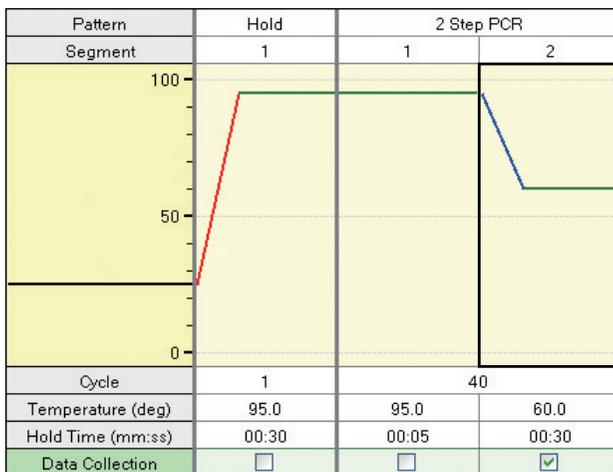
\*1 Final primer concentration of 0.2  $\mu\text{M}$  is likely to yield good results. However, if further optimization is required, adjust the primer concentration in the range of 0.1 - 1.0  $\mu\text{M}$ .

\*2 The probe concentration varies depending on the real-time PCR instrument being used and the type of fluorescent label. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration. When using the Thermal Cycler Dice Real Time System, use a final concentration in the range of 0.1 - 0.5  $\mu\text{M}$ .

\*3 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. 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 (e.g., no more than 2.5  $\mu\text{l}$  cDNA template solution for 25  $\mu\text{l}$  PCR reaction).

2. Start the reaction.

The recommended shuttle PCR (i.e., 2-step PCR) protocol is described below. Try this protocol first and optimize PCR conditions as necessary (refer to VII-7. "PCR Reaction Conditions").



Shuttle PCR Standard Protocol

Hold (initial denaturation)

Number of cycle: 1

95°C 30 sec

2-Step PCR

Number of cycles: 40

95°C 5 sec

60°C 30 sec

**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.

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

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

**2. Protocol using the Applied Biosystems 7300 Real-Time PCR System or StepOnePlus Real-Time PCR System**

\* Follow the procedures provided in the manual of the respective apparatus.

## 1. Prepare the PCR reaction mixture shown below.

<Per reaction>

Reagent	Volume	Volume	Final Conc.
<i>Premix Ex Taq</i> (Probe qPCR) (2X conc.)	10 $\mu$ l	25 $\mu$ l	1X
PCR Forward Primer (10 $\mu$ M)	0.4 $\mu$ l	1 $\mu$ l	0.2 $\mu$ M* <sup>1</sup>
PCR Reverse Primer (10 $\mu$ M)	0.4 $\mu$ l	1 $\mu$ l	0.2 $\mu$ M* <sup>1</sup>
Probe* <sup>2</sup>	0.8 $\mu$ l	2 $\mu$ l	
ROX Reference Dye (50X)* <sup>3</sup>	0.4 $\mu$ l	1 $\mu$ l	1X
Template* <sup>4</sup>	2 $\mu$ l	4 $\mu$ l	
Sterile purified water	6 $\mu$ l	16 $\mu$ l	
Total	20 $\mu$ l* <sup>5</sup>	50 $\mu$ l* <sup>5</sup>	

\*<sup>1</sup> Final primer concentration of 0.2  $\mu$ M is likely to yield good results. However, if further optimization is required, adjust the primer concentration in the range of 0.1 - 1.0  $\mu$ M.

\*<sup>2</sup> The probe concentration varies depending on the real-time PCR instrument being used and the type of fluorescent label. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration.

\*<sup>3</sup> Use the ROX Reference Dye at a final concentration of 1X.

\*<sup>4</sup> 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  $\mu$ 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 (e.g., no more than 2  $\mu$ l cDNA template solution for 20  $\mu$ l PCR reaction).

\*<sup>5</sup> Adjust according to the recommended volume for the instrument.

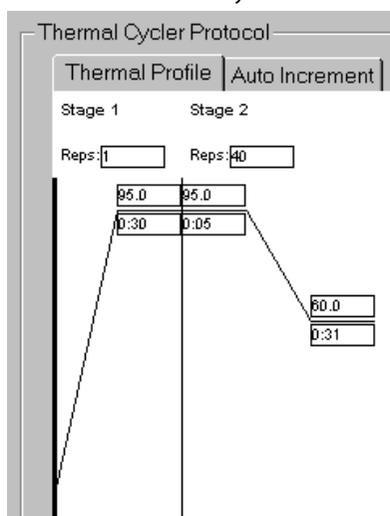
## 2. Start the reaction.

The recommended shuttle PCR (i.e., 2-step PCR) protocol is described below. Try this protocol first and optimize PCR conditions as necessary (refer to VII-7. "PCR Reaction Conditions").

**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.

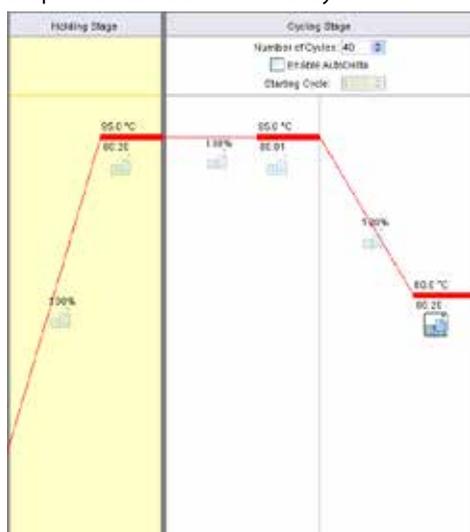
< 7300 Real-Time PCR System >



Shuttle PCR Standard Protocol

Stage 1: initial denaturation  
 Number of cycle: 1  
 95°C 30 sec  
 Stage 2: PCR  
 Number of cycles: 40  
 95°C 5 sec  
 60°C 31 sec

< StepOnePlus Real-Time PCR System >



Shuttle PCR Standard Protocol

Fast Mode  
 Holding Stage  
 Number of cycle: 1  
 95°C 20 sec  
 Cycling Stage  
 Number of cycles: 40  
 95°C 1 sec  
 60°C 20 sec

3. After the reaction is complete, check the amplification curves and plot a standard curve if absolute quantification will be performed. Refer to the instrument's instruction manual for specific analysis methods.

**3. Protocol using the Applied Biosystems 7500/7500 Fast Real-Time PCR System**

\* Follow the procedures provided in the manual of the respective apparatus.

## 1. Prepare the PCR reaction mixture shown.

<Per reaction>

Reagent	Volume	Volume	Final Conc.
<i>Premix Ex Taq</i> (Probe qPCR) (2X conc.)	10 $\mu$ l	25 $\mu$ l	1X
PCR Forward Primer (10 $\mu$ M)	0.4 $\mu$ l	1 $\mu$ l	0.2 $\mu$ M* <sup>1</sup>
PCR Reverse Primer (10 $\mu$ M)	0.4 $\mu$ l	1 $\mu$ l	0.2 $\mu$ M* <sup>1</sup>
Probe* <sup>2</sup>	0.8 $\mu$ l	2 $\mu$ l	
ROX Reference Dye II (50X)* <sup>3</sup>	0.2 $\mu$ l	0.5 $\mu$ l	0.5X
Template* <sup>4</sup>	2 $\mu$ l	4 $\mu$ l	
Sterile purified water	6.2 $\mu$ l	16.5 $\mu$ l	
Total	20 $\mu$ l* <sup>5</sup>	50 $\mu$ l* <sup>5</sup>	

\*<sup>1</sup> Final primer concentration of 0.2  $\mu$ M is likely to yield good results. However, if further optimization is required, adjust the primer concentration in the range of 0.1 - 1.0  $\mu$ M.

\*<sup>2</sup> The probe concentration varies depending on the real-time PCR instrument being used and the type of fluorescent label. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration.

\*<sup>3</sup> Use the ROX Reference Dye II at a final concentration of 0.5X.

\*<sup>4</sup> 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  $\mu$ 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 (e.g., no more than 2  $\mu$ l cDNA template solution for 20  $\mu$ l PCR reaction).

\*<sup>5</sup> Adjust according to the recommended volume for the instrument.

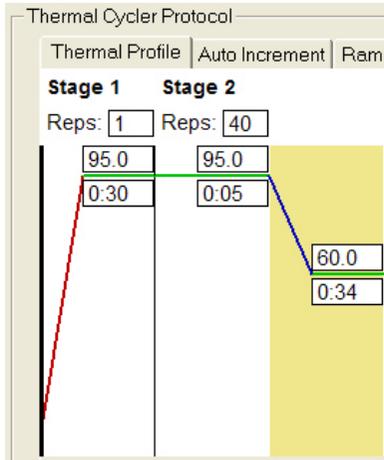
## 2. Start the reaction.

The recommended shuttle PCR (i.e., 2-step PCR) protocol is described below. Try this protocol first and optimize PCR conditions as necessary (refer to VII-7. "PCR Reaction Conditions").

**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.

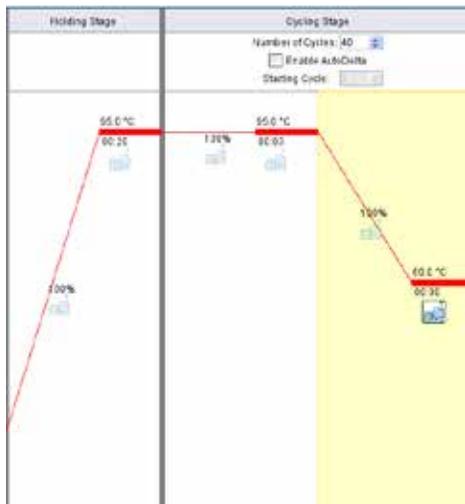
< 7500 Real-Time PCR System >



Shuttle PCR Standard Protocol

- Stage 1: initial denaturation
  - Number of cycle: 1
  - 95°C 30 sec
- Stage 2: PCR reaction
  - Number of cycles: 40
  - 95°C 5 sec
  - 60°C 34 sec

< 7500 Fast Real-Time PCR System >



Shuttle PCR Standard Protocol

- Fast Mode
- Holding Stage
  - Number of cycle: 1
  - 95°C 20 sec
- Cycling Stage
  - Number of cycles: 40
  - 95°C 3 sec
  - 60°C 30 sec

3. After the reaction is complete, check the amplification curves and plot a standard curve if absolute quantification will be performed. Refer to the instrument's instruction manual for specific analysis methods.

**4. Protocol using the LightCycler/LightCycler 480 System**

- \* Follow the procedures provided in the manual for the LightCycler System (Roche Diagnostics).

## 1. Prepare the PCR reaction mixture shown below.

&lt;Per reaction&gt;

Reagent	Volume	Final Conc.
<i>Premix Ex Taq</i> (Probe qPCR) (2X conc.)	10 $\mu$ l	1X
PCR Forward Primer (10 $\mu$ M)	0.4 $\mu$ l	0.2 $\mu$ M*1
PCR Reverse Primer (10 $\mu$ M)	0.4 $\mu$ l	0.2 $\mu$ M*1
Probe*2	0.8 $\mu$ l	
Template*3	2 $\mu$ l	
Sterile purified water	6.4 $\mu$ l	
Total	20 $\mu$ l	

- \*1 Final primer concentration of 0.2  $\mu$ M is likely to yield good results. However, if further optimization is required, adjust the primer concentration in the range of 0.1 - 1.0  $\mu$ M.
- \*2 The probe concentration varies depending on the real-time PCR instrument being used and the type of fluorescent label. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration.
- \*3 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. 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 (e.g., no more than 2  $\mu$ l cDNA template solution for 20  $\mu$ l PCR reaction).

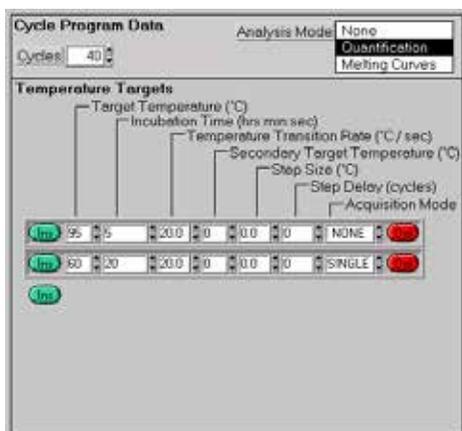
## 2. Briefly centrifuge PCR capillaries. Place them in the LightCycler instrument and start the reaction.

The recommended shuttle PCR (i.e., 2-step PCR) protocol is described below. Try this protocol first and optimize PCR conditions as necessary (refer to VII-7. "PCR Reaction Conditions").

**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.

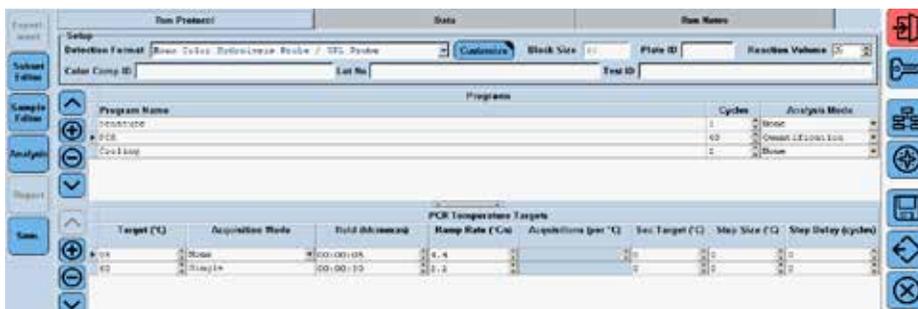
&lt;LightCycler&gt;

Shuttle PCR Standard Protocol

Stage 1: initial denaturation  
95°C 30 sec 20°C/sec  
1 Cycle

Stage 2: PCR reaction  
95°C 5 sec 20°C/sec  
60°C 20 sec 20°C/sec  
40 Cycles

&lt;LightCycler 480 System&gt;

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

## Cooling

50°C 30 sec (Ramp rate: 2.2°C/sec)  
1 cycle

- After the reaction is complete, check the amplification curves and plot a standard curve if absolute quantification will be performed. Refer to the instrument's instruction manual for specific analysis methods.

## 5. Protocol using the CFX96 Real-Time PCR Detection System

- \* Follow the procedures provided in the instruction manual for the CFX96 Real-Time PCR Detection System (BIO-RAD).

### 1. Prepare the PCR mixture shown below.

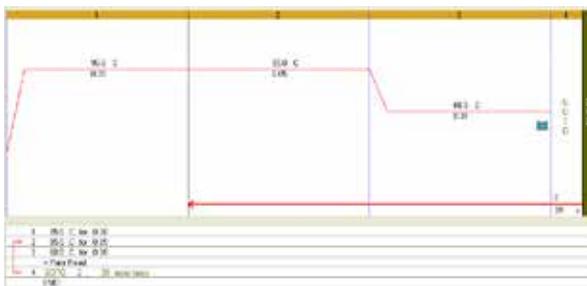
<Per reaction>

Reagent	Volume	Final conc.
<i>Premix Ex Taq</i> (2X) (Probe qPCR), Bulk	12.5 $\mu$ l	1X
PCR Forward Primer (10 $\mu$ M)	0.5 $\mu$ l	0.2 $\mu$ M*1
PCR Reverse Primer (10 $\mu$ M)	0.5 $\mu$ l	0.2 $\mu$ M*1
Probe*2	1 $\mu$ l	
Template*3	2 $\mu$ l	
Sterile purified water	8.5 $\mu$ l	
Total	25 $\mu$ l	

- \*1 Final primer concentration of 0.2  $\mu$ M is most likely to yield good results. However, should further optimization be required, try adjusting primer concentrations in the range of 0.1 to 1.0  $\mu$ M.
- \*2 The probe concentration varies depending on the model of real-time PCR instrument used and the fluorescent labeling dye of the probe. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration.
- \*3 The optimal quantity depends on the copy number of the target in the template solution. Test serial dilutions to select the appropriate quantity. It is preferable to use no more than 100 ng of DNA template. When using cDNA (RT reaction mixture) as a template, the template volume should not exceed 10% of the PCR reaction mixture (e.g., no more than 2.5  $\mu$ l cDNA template solution for a 25  $\mu$ l PCR reaction).

### 2. Start the reaction.

The recommended protocol for PCR reactions is the shuttle PCR standard protocol described below. Try this protocol first and optimize PCR conditions as necessary. (refer to VII-7. "PCR Reaction Conditions")



#### Shuttle PCR standard protocol

Sample volume: 25  $\mu$ l

Step 1: 95°C 30 sec

Step 2: PCR

GOTO: 39 (40 cycles)

95°C 5 sec

60°C 30 sec

#### 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.

- 3. After the reaction is complete, assess the amplification curve and create a standard curve if a quantitative determination will be performed. Refer to the instrument's instruction manual for specific analysis methods.

**6. Protocol using the Smart Cycler II System**

1. Prepare the PCR reaction mixture shown below.

<Per reaction>

Reagent	Volume	Final Conc.
<i>Premix Ex Taq</i> (Probe qPCR) (2X conc.)	12.5 $\mu$ l	1X
PCR Forward Primer (10 $\mu$ M)	0.5 $\mu$ l	0.2 $\mu$ M*1
PCR Reverse Primer (10 $\mu$ M)	0.5 $\mu$ l	0.2 $\mu$ M*1
Probe*2	1 $\mu$ l	
Template*3	2 $\mu$ l	
Sterile purified water	8.5 $\mu$ l	
Total	25 $\mu$ l	

- \*1 Final primer concentration of 0.2  $\mu$ M is likely to yield good results. However, if further optimization is required, adjust the primer concentration in the range of 0.1 - 1.0  $\mu$ M.
- \*2 The probe concentration varies depending on the real-time PCR instrument being used and the type of fluorescent label. Refer to the instrument manual and the probe data sheet to determine the appropriate concentration. When using the Smart Cycler System/Smart Cycler II System, use a final concentration in the range of 0.1 - 0.5  $\mu$ M.
- \*3 Optimal template quantity depends on the target copy concentration of the template solution. Prepare and test serial dilutions to select an appropriate quantity. Use no more than 100 ng of DNA template. When using cDNA as a template in RT-PCR (RT reaction mixture), template solution volume should not exceed 10% of the PCR reaction mixture (e.g., no more than 2.5  $\mu$ l cDNA template solution for a 25  $\mu$ l PCR reaction).

- Briefly centrifuge the reaction tubes with a Smart Cycler centrifuge. Place them in Smart Cycler and start the reaction.

The recommended protocol for PCR reactions is the shuttle PCR standard protocol described below. Try this protocol first and optimize PCR conditions as necessary. (Refer to VII-7. "PCR Reaction Conditions").

**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.

Stage 1				Stage 2			
Hold				Repeat 40 times.			
Temp	Secs	Optics		2-Temperature Cycle			
95.0	30	Off		Deg/Sec	Temp	Secs	Optics
				NA	95.0	5	Off
				NA	60.0	20	On
				<input type="checkbox"/> Advance to Next Stage			

Shuttle PCR Standard Protocol

Stage 1: initial denaturation  
 Hold  
 95°C 30 sec  
 Stage 2: PCR reaction  
 Repeat 40 times  
 95°C 5 sec  
 60°C 20 sec

- After the reaction is complete, check the amplification curves and plot a standard curve if absolute quantification will be performed. Refer to the instrument's instruction manual for specific analysis methods.

## 7. PCR Reaction Conditions

## Initial denaturation

Step	Temperature	Time	Detection	Comment
Initial denaturation	95°C	30 sec	Off	In general, 95°C for 30 sec is sufficient for initial denaturation in most cases, even for difficult to denature templates such as circular plasmids and genomic DNA. This procedure may be extended to 1 - 2 min at 95°C depending on template condition. Prolonged denaturation may inactivate the enzyme. Therefore, do not perform denaturation for more than 2 min.

## Shuttle PCR (2-step PCR)

number of cycles: 30 - 45 cycles

Step	Temperature	Time	Detection	Comment
Denaturation	95°C	3 - 5 sec	Off	Generally the amplification product size for real-time PCR does not exceed 300 bp. Therefore, 95°C for ~3 - 5 sec is usually sufficient.
Annealing/ extension	56 - 64°C	20 - 30 sec (31, 34 sec)*	On	When optimizing reaction conditions, evaluate results using annealing/ extension temperature in the range of 56 - 64°C. If poor reactivity occurs, increasing incubation time for this step may improve results.

- \* Some apparatuses do not allow a detection-step setting of 30 sec or shorter.  
Applied Biosystems 7300 allows a setting of 31 sec or longer.  
Applied Biosystems 7500 allows a setting of 34 sec or longer.

**8. Real-Time RT-PCR**

To synthesize cDNA templates for real-time RT-PCR, we recommend the PrimeScript™ RT Reagent Kit (Perfect Real Time) (Cat. #RR037A). Perform the reverse transcription reaction using the protocol below.

1. Prepare the reverse transcription reaction mixture shown below. Assemble the reaction mixture on ice.

<Per reaction>

Reagent	Volume	Final Conc.
5X PrimeScript Buffer (for Real Time)	2 $\mu$ l	1X
PrimeScript RT Enzyme Mix I	0.5 $\mu$ l	
Oligo dT Primer (50 $\mu$ M)* <sup>1</sup>	0.5 $\mu$ l	25 pmol
Random 6 mers (100 $\mu$ M)* <sup>1</sup>	2 $\mu$ l	200 pmol
total RNA		
RNase Free dH <sub>2</sub> O		
<b>Total</b>	<b>10 <math>\mu</math>l*<sup>2</sup></b>	

- \*<sup>1</sup> Using both the Oligo dT Primer and the Random 6 mers allows efficient cDNA synthesis for the entire region of mRNA. The amount to use when using each primer alone or when using a gene specific primer is shown below.

Primer	Volume	Amount
Oligo dT Primer (50 $\mu$ M)	0.5 $\mu$ l	25 pmol
Random 6 mers (100 $\mu$ M)	2 $\mu$ l	200 pmol
Gene Specific Primer (2 $\mu$ M)	0.5 $\mu$ l	1 pmol

- \*<sup>2</sup> Scale up the reverse transcription reaction as necessary. A 10  $\mu$ l reaction can reverse-transcribe ~1  $\mu$ g of total RNA.

2. Perform the reverse transcription reaction.

37°C 15 min\* (reverse transcription)

85°C 5 sec (heat inactivation of reverse transcriptase)

4°C

- \* When using a gene specific primer:

Perform reverse transcription at 42°C for 15 min. If non-specific PCR amplification occurs, conducting the reverse transcription step at 50°C may improve results.

3. Perform PCR.

Perform a PCR according to the method described in "VII. Protocol" on page 6.

**VIII. Related Products**

- Probe qPCR Mix (Cat. #RR391A/B)\*1
- 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 PrimeScript™ RT-PCR Kit (Perfect Real Time) (Cat. #RR064A)\*1
- TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B)\*2
- TB Green® Fast qPCR Mix (Cat.#RR430A/B)\*1, 2
- TB Green® *Premix Ex Taq*™ (Tli RNaseH Plus) (Cat. #RR420A/B)\*2
- One Step TB Green® PrimeScript™ PLUS RT-PCR Kit II (Perfect Real Time) (Cat. #RR096A/B)\*1, 2

- Thermal Cycler Dice™ Real Time System III (Cat. #TP950/TP970/TP980/TP990)\*1
- Thermal Cycler Dice™ Real Time System II (Cat. #TP900/TP960)\*1
- Thermal Cycler Dice™ Real Time System *Lite* (Cat. #TP700/TP760)\*1

\*1 Not available in all geographic locations. Check for availability in your area.

\*2 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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**NOTE:** This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.

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