Cat. # RR390L

For Research Use

TaKaRa

Premix Ex Taq™ (Probe qPCR), Bulk

Product Manual

v202201Da

Table of Contents

I. Description

Premix Ex Taq (Probe qPCR) is designed for probe-based qPCR. This product is also suitable for high-speed PCR. *Premix Ex Taq* allows accurate target quantification and detection over a broad dynamic range and makes it possible to perform 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 RNaseH, a heat-resistant RNase H, to minimize PCR inhibition by residual mRNA in reactions with cDNA as a template. A combination of *TaKaRa Ex Taq*® HS (a hot-start PCR enzyme that uses an anti-*Taq* antibody) and a buffer optimized for real-time PCR results in excellent suppression of non-specific amplification, high amplification efficiency, and high detection sensitivity in real-time PCR analyses.

Compatible instrument systems include:

- Thermal Cycler Dice™ Real Time System III (Cat. #TP950/TP970/TP980/TP990)*
- Thermal Cycler Dice Real Time System *II* (Cat. #TP900/TP960: discontinued)
- Thermal Cycler Dice Real Time System *Lite* (Cat. #TP700/TP760: discontinued)
- Smart Cycler II System (Cepheid)
- Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific)
- Applied Biosystems 7500 Fast Real-Time PCR System (Thermo Fisher Scientific)
- StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)
- LightCycler/LightCycler 480 System (Roche Diagnostics)
- CFX96 Real-Time PCR Detection System (BIO-RAD)

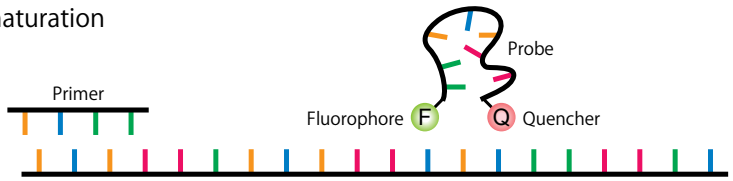
* Not available in all geographic locations. Check for availability in your area.

II. Principle

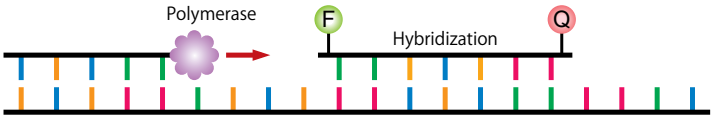
This product uses *TaKaRa Ex Taq* HS, a hot-start PCR enzyme, for PCR amplification. PCR Amplification products can be monitored in real time using a probe.
TaKaRa Ex Taq HS prevents non-specific amplifications from mispriming or formation of primer dimers during reaction mixture preparation or other pre-cycling steps and allows high-sensitivity detections.

Fluorescence Detection
Oligonucleotides modified by a 5′ fluorophore (e.g., FAM) and a 3′ quencher (e.g., TAMRA) are added to the reaction.
Under annealing conditions, the probe hybridizes specifically 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 detection.

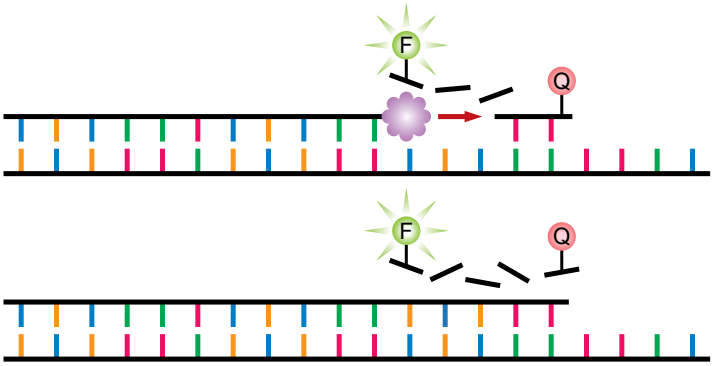
1) Heat denaturation



2) Primer annealing/probe hybridization



3) Extension



III. Components (for 200 reactions, 50 μl reaction volume)

(1) *Premix Ex Taq* (2X) (Probe qPCR), Bulk*1	5 ml
(2) ROX Reference Dye (50X)*2	200 μl

*1 Contains *TaKaRa Ex Taq* HS, dNTP Mixture, Mg^{2+}, and Tli RNaseH

*2 Use when performing analyses with real-time PCR instruments that normalize fluorescent signals between wells, such as Applied Biosystems instruments. The amount of ROX Reference Dye to use varies depending on the instrument. Follow the guidelines below:

- ◆ Add ROX Reference Dye (50X) in a volume equivalent to 1/50 of the PCR reaction mixture when using the following system:
  - StepOnePlus Real-Time PCR System (Thermo Fisher Scientific)
- ◆ Add ROX Reference Dye (50X) in a volume equivalent to 1/500 of the PCR reaction mixture when using the following system:
  - Applied Biosystems 7500/7500 Fast Real-Time PCR System (Thermo Fisher Scientific)
- ◆ No ROX Reference Dye (50X) is required when using any of the following systems:
  - Thermal Cycler Dice Real Time System III (Cat. #TP950/TP970/TP980/TP990)*3
  - Thermal Cycler Dice Real Time System *III*/*Lite* (Cat. #TP900/TP960/TP700/TP760: discontinued)
  - Smart Cycler II System (Cepheid)
  - LightCycler/LightCycler 480 System (Roche Diagnostics)
  - CFX96 Real-Time PCR Detection System (BIO-RAD)

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

IV. Materials Required but not Provided

- 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 (TaKaRa qPCR Probe, etc.)
- Sterile purified water
- Micropipette and tips (sterile, with filter)

V. Storage

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

It should be protected from light, and careful attention should be made not to cause 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℃ for long term storage. Once thawed, it should be stored at 4℃ and used within 6 months.

VI. Precautions before Use

This section describes precautions for using this product. Read before use.

(1) Before use, make sure the reagent is evenly mixed by gently inverting the tube several times without creating bubbles; otherwise the reagent may not provide sufficient reactivity. Do not mix by vortexing.
When stored frozen at -20℃, *Premix Ex Taq* (Probe qPCR) (2X), Bulk may precipitate. To dissolve the precipitate completely, let stand at room temperature (below approximately 30℃) briefly, followed by inverting the tube several times. The presence of precipitate is indicative of poorly mixed reagent. 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 and primers.

(4) Use fresh disposable tips to minimize potential cross-contamination between samples when preparing reaction mixtures or dispensing aliquots.

(5) *TaKaRa Ex Taq* HS included in this premix is a hot-start PCR enzyme with an anti-*Taq* antibody that inhibits polymerase activity. Do not perform the pre-PCR incubation (5 - 15 min at 95℃) that is required for other companies' chemically modified hot-start PCR enzymes. Prolonged denaturation may inactivate the enzyme, affecting amplification efficiency and quantification accuracy.
Even for the initial denaturation step, incubation at 95℃ for 30 sec is generally sufficient.

VII. Protocol

【For the Thermal Cycler Dice Real Time System III (*II* and *Lite*: discontinued)】

1. Prepare the PCR mixture shown below.

<Per reaction>

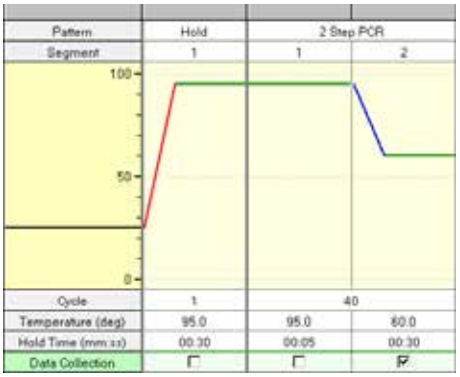
Reagent	Volume	Final conc.
Premix Ex Taq (2X) (Probe qPCR), Bulk	12.5 μl	1X
PCR Forward Primer (10 μM)	0.5 μl	0.2 μM*1
PCR Reverse Primer (10 μM)	0.5 μl	0.2 μM*1
Probe*2	1 μl	
Template*3	2 μl	
Sterile purified water	8.5 μl	
Total	25 μl	

*1 Final primer concentration of 0.2 μM 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 μM.

*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. When using the Thermal Cycler Dice Real Time System, use a final concentration in the range of 0.1 to 0.5 μM.

*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 μl cDNA template solution for a 25 μl PCR reaction).

2. Start the reaction.
   The recommended protocol for PCR reactions is the shuttle PCR standard protocol below. You may set the annealing/extension time between 20 and 30 sec, but first try 30 sec, which generally yields stable results. (See "PCR Conditions" on page 14.)



Shuttle PCR standard protocol

Hold (Initial denaturation)
Cycle: 1
95℃ 30 sec

2 Step PCR
Cycles: 40
95℃ 5 sec
60℃ 30 sec

3. After the reaction is complete, assess the amplification curve and create a standard curve if a quantitative determination will be performed.
   For the analytical method, refer to the manual for the Thermal Cycler Dice Real Time System.

【For the Applied Biosystems 7500/7500 Fast Real-Time PCR System or StepOnePlus Real-Time PCR System】

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

1. Prepare the PCR mixture shown below.

● For the StepOnePlus Real-Time PCR System, add ROX Reference Dye (50X) in a volume equivalent to 1/50 of the PCR reaction mixture.

<Per reaction>

Reagent	Volume	Final conc.
Premix Ex Taq (2X) (Probe qPCR), Bulk	10 μl	1X
PCR Forward Primer (10 μM)	0.4 μl	0.2 μM*1
PCR Reverse Primer (10 μM)	0.4 μl	0.2 μM*1
Probe*2	0.8 μl	
ROX Reference Dye (50X)*3	0.4 μl	1X
Template*4	2 μl	
Sterile purified water	6 μl	
Total	20 μl*5	

● For the Applied Biosystems 7500/7500 Fast Real-Time PCR System, add ROX Reference Dye (50X) in a volume equivalent to 1/500 of the PCR reaction mixture.

<Per reaction>

Reagent	Volume	Volume	Final conc.
Premix Ex Taq (2X) (Probe qPCR), Bulk	10 μl	25 μl	1X
PCR Forward Primer (10 μM)	0.4 μl	1 μl	0.2 μM*1
PCR Reverse Primer (10 μM)	0.4 μl	1 μl	0.2 μM*1
Probe*2	0.8 μl	2 μl	
ROX Reference Dye (50X)*3	0.04 μl	0.1 μl	0.1X
Template*4	2 μl	4 μl	
Sterile purified water	6.36 μl	16.9 μl	
Total	20 μl*5	50 μl*5	

*1 Final primer concentration of 0.2 μM 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 μM.

*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 manual and the probe data sheet to determine the appropriate concentration.

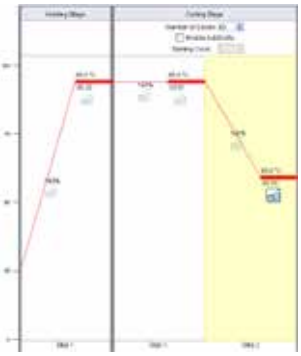
*3 Add ROX Reference Dye (50X) to a final concentration of 1X when using the StepOnePlus Real-Time PCR System and a final concentration of 0.1X when using the Applied Biosystems 7500/7500 Fast Real-Time PCR System.

*4 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 for a 20 μl PCR reaction. 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 μl cDNA template solution for a 20 μl PCR reaction).

*5 Adjust according to the recommended volume for each instrument system.

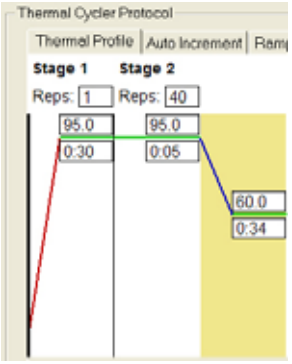
2. Start the reaction.
   The recommended protocol for PCR reactions is the shuttle PCR standard protocol below. Try this protocol first and optimize PCR conditions as necessary. (See "PCR Conditions" on page 14.)

< StepOnePlus Real-Time PCR System >



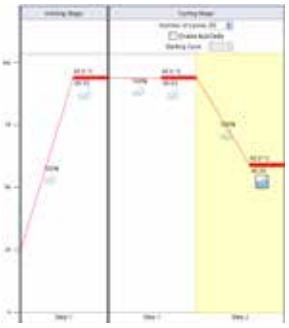
Shuttle PCR standard protocol
- Fast mode
- Holding stage:
  - Number of cycle: 1
  - 95℃ 20 sec
- Cycling stage:
  - Number of cycles: 40
  - 95℃ 1 sec
  - 60℃ 20 sec

< 7500 Real-Time PCR System >



Shuttle PCR standard protocol
- Stage 1: Initial denaturation
  - Number of cycle: 1
  - 95℃ 30 sec
- Stage 2: PCR
  - Number of cycles: 40
  - 95℃ 5 sec
  - 60℃ 34 sec

< 7500 Fast Real-Time PCR System >



Shuttle PCR standard protocol
- Fast mode
- Holding stage:
  - Number of cycle: 1
  - 95℃ 20 sec
- Cycling stage:
  - Number of cycles: 40
  - 95℃ 3 sec
  - 60℃ 30 sec

3. After the reaction is complete, assess the amplification curve and create a standard curve if a quantitative determination will be performed.
   For analytical methods, refer to the manual for the real-time PCR instrument used.

【 For the LightCycler/LightCycler 480 System 】

Note: Please follow the procedures provided in the manual for the LightCycler/LightCycler 480 System (Roche Diagnostics).

1. Prepare the PCR mixture shown below.

<Per reaction>

Reagent	Volume	Final conc.
Premix Ex Taq (2X) (Probe qPCR), Bulk	10 μl	1X
PCR Forward Primer (10 μM)	0.4 μl	0.2 μM*1
PCR Reverse Primer (10 μM)	0.4 μl	0.2 μM*1
Probe*2	0.8 μl	
Template*3	2 μl	
Sterile purified water	6.4 μl	
Total	20 μl	

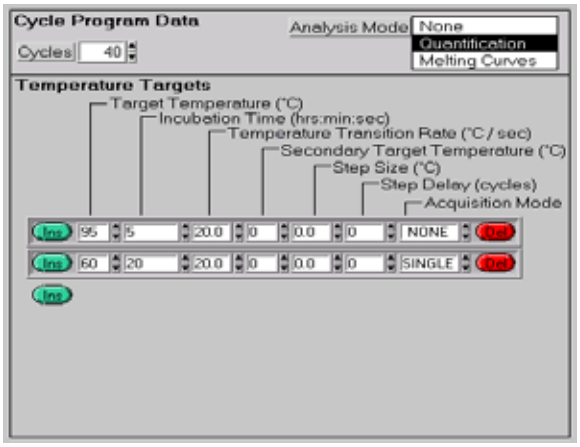
*1 Final primer concentration of 0.2 μM is most likely to yield good results. However, should further optimization be required, try adjusting primer concentrations in the range of 0.1 to 1.0 μM.

*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 μl cDNA template solution for a 20 μl PCR reaction).

2. Start the reaction.
   The recommended protocol for PCR reactions is the shuttle PCR standard protocol below. Try this protocol first and optimize PCR conditions as necessary. (See "PCR Reaction Conditions" on page 14.)

<LightCycler>

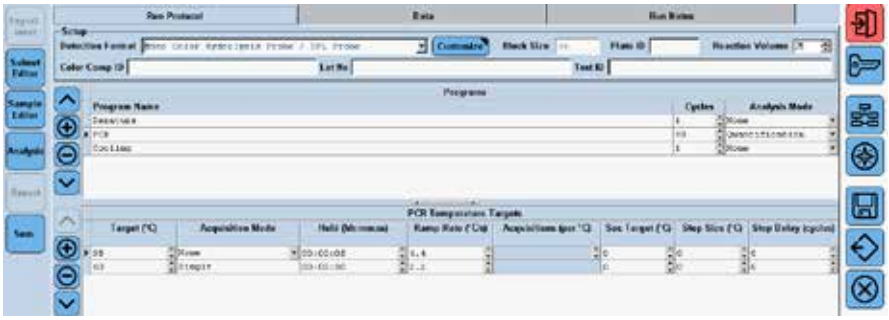


Shuttle PCR standard protocol

Stage 1: Initial denaturation
95℃ 30 sec 20℃/sec
1 cycle

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

<LightCycler 480 System>



Shuttle PCR standard protocol

Denature

95℃ 30 sec (Ramp Rate 4.4℃/sec)
1 cycle

PCR

Analysis Mode: Quantification
95℃ 5 sec (Ramp Rate 4.4℃/sec)
60℃ 30 sec (Ramp Rate 2.2℃/sec, Acquisition Mode: Single)
40 cycles

Cooling

50℃ 30 sec (Ramp Rate 2.2℃/sec)
1 cycle

3. After the reaction is complete, assess the amplification curve and create a standard curve if a quantitative determination will be performed.
   For analytical methods, refer to the manual for the real-time PCR instrument used.

【 For the CFX96 Real-Time PCR Detection System 】

Note: Please 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>

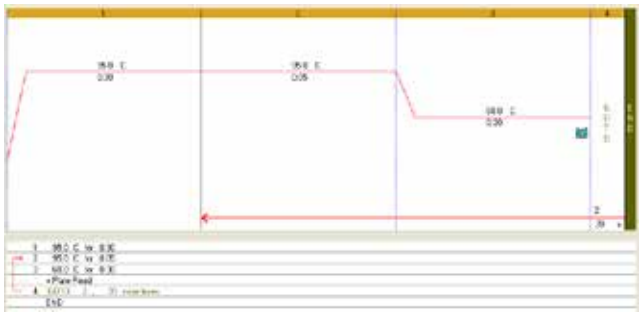
Reagent	Volume	Final conc.
Premix Ex Taq (2X) (Probe qPCR), Bulk	12.5 μl	1X
PCR Forward Primer (10 μM)	0.5 μl	0.2 μM*1
PCR Reverse Primer (10 μM)	0.5 μl	0.2 μM*1
Probe*2	1 μl	
Template*3	2 μl	
Sterile purified water	8.5 μl	
Total	25 μl	

*1 Final primer concentration of 0.2 μM 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 μM.

*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 μl cDNA template solution for a 25 μl 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. (See "PCR Conditions" on page 14.)



Shuttle PCR standard protocol
Sample volume: 25 μl
Step 1: 95℃ 30 sec
Step 2: PCR
GOTO: 39 (40 cycles)
95℃ 5 sec
60℃ 30 sec

3. After the reaction is complete, assess the amplification curve and create a standard curve if a quantitative determination will be performed.
   For analytical methods, refer to the CFX96 Real-Time PCR Detection System manual.

【 For the Smart Cycler II System 】

1. Prepare the PCR mixture shown below.

<Per reaction>

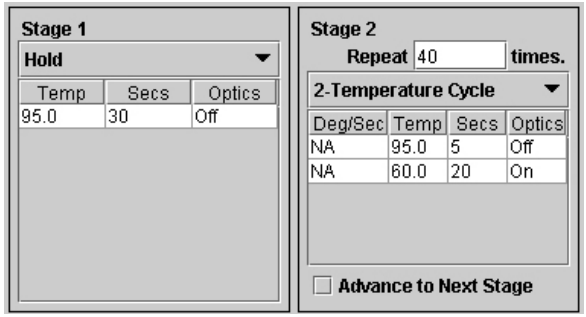
Reagent	Volume	Final conc.
Premix Ex Taq (2X) (Probe qPCR), Bulk	12.5 μl	1X
PCR Forward Primer (10 μM)	0.5 μl	0.2 μM*1
PCR Reverse Primer (10 μM)	0.5 μl	0.2 μM*1
Probe*2	1 μl	
Template*3	2 μl	
Sterile purified water	8.5 μl	
Total	25 μl	

*1 Final primer concentration of 0.2 μM 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 μM.

*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. When using the Smart Cycler II System, generally, try a final concentration in the range of 0.1 to 0.5 μM.

*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 μl cDNA template solution for a 25 μl PCR reaction).

2. Start the reaction.
   The recommended protocol for PCR reactions is the shuttle PCR standard protocol below. Try this protocol first and optimize PCR conditions as necessary. (See "PCR Conditions" on page 14.)



Shuttle PCR standard protocol

Stage 1: Initial denaturation
Hold
95℃ 30 sec

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

3. After the reaction is complete, assess the amplification curve and create a standard curve if a quantitative determination will be performed.
   For analytical methods, refer to the manual for the Smart Cycler II System.

<PCR Conditions>

Initial denaturation

Step	Temperature	Time	Detection	Comment
Initial denaturation	95℃	30 sec	Off	Generally, 95℃ for 30 sec is sufficient for initial denaturation in most cases, even with difficult to denature templates such as circular plasmids and genomic DNAs. This procedure may be extended to 1 to 2 min at 95℃ 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 to 45 cycles.**

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

* Some Applied Biosystems systems do not allow a setting of 30 sec or less for the detection step. For the 7500 Real-Time PCR System, set the time to 34 sec or longer.

VIII. Procedures for Performing Real-Time RT-PCR

For highly reliable results in real-time RT-PCR, we recommend using *Premix Ex Taq* (Probe qPCR) in combination with the following kits for reverse transcription.

- 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)

[Example Protocol]

The following is a protocol for probe assays when using the PrimeScript RT reagent Kit (Perfect Real Time) (Cat. #RR037A/B).

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

<Per reaction>

Reagent	Volume	Final conc.
5X PrimeScript Buffer (for Real Time)	2 μl	1X
PrimeScript RT Enzyme Mix I	0.5 μl	
Oligo dT Primer (50 μM)*1	0.5 μl	25 pmol
Random 6 mers (100 μM)*1	2 μl	200 pmol
total RNA		
RNase Free dH_2O		
Total	10 μl*2	

*1 Using both the Oligo dT Primer and the Random 6 mers allows efficient cDNA synthesis for the entire length of mRNA. The primer amount when using each primer alone or when using Gene Specific Primer is shown below.

Primer	Volume	Amount
Oligo dT Primer (50 μM)	0.5 μl	25 pmol
Random 6 mers (100 μM)	2 μl	200 pmol
Gene Specific Primer (2 μM)	0.5 μl	1 pmol

*2 Scale up the reverse transcription reaction as necessary. A 10 μl of reaction mixture amount can reverse-transcribe up to approximately 1 μg of total RNA.

2. Perform a reverse transcription reaction.

37℃ 15 min*3 (reverse transcription reaction)
85℃ 5 sec (heat inactivation of reverse transcriptase)
4℃

*3 When using the Gene Specific Primer:
Perform reverse transcription at 42℃ for 15 min. If non-specific PCR amplification occurs, performing the reverse transcription step at 50℃ may improve results.

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

IX. Related Products

Probe qPCR Mix (Cat. #RR391A/B)
Premix Ex Taq™ (Probe qPCR) (Cat. #RR390A/B)
Premix Ex Taq™ (Probe qPCR), ROX plus (Cat. #RR39LR)
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)
TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Cat. #RR820A/B)
TB Green® *Premix Ex Taq*™ II (Tli RNaseH Plus), Bulk (Cat. #RR820L)
TB Green® *Premix Ex Taq*™ (Tli RNaseH Plus) (Cat. #RR420A/B)
TB Green® *Premix Ex Taq*™ (Tli RNaseH Plus), Bulk (Cat. #RR420L)
TB Green® Fast qPCR Mix (Cat. #RR430A/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.