Cat. # RR39LR

For Research Use

TaKaRa

Premix Ex Taq™ (Probe qPCR), ROX plus

Product Manual

v201903Da

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I. Description

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

The product includes ROX Reference Dye and is supplied as a 2X premix to facilitate easy preparation of reaction mixtures. The 2X premixed reagent also contains Tli RNaseH, a heat-resistant RNaseH, 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:

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

  etc.

*1 For Applied Biosystems 7500 Real-Time PCR System or 7500 Fast Real-Time PCR System (Thermo Fisher Scientific) requiring correction with ROX Reference Dye II, we recommend using *Premix Ex Taq* (Probe qPCR), Bulk (Cat. #RR390L).

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

II. Principle

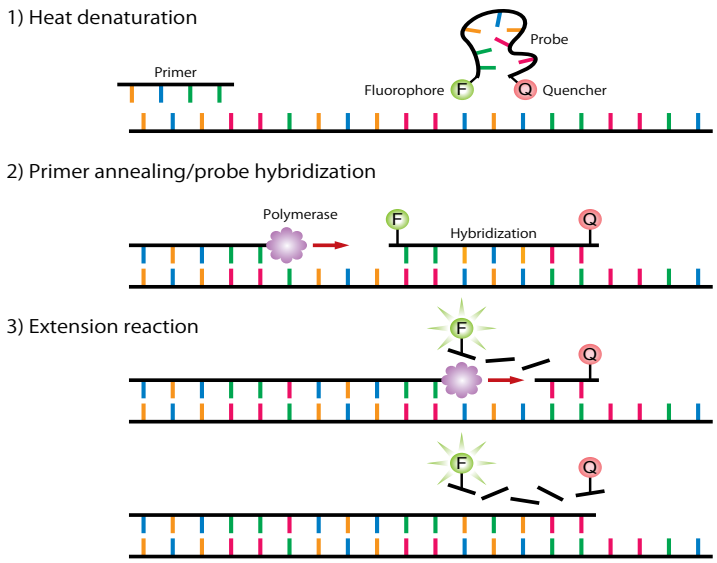
This product uses *TaKaRa Ex Taq* HS, a hot-start PCR enzyme, for PCR amplification. PCR amplification products can be monitored in real time using a probe.

1. PCR

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.

2. 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.



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

Premix Ex Taq (2X) (Probe qPCR), ROX plus*	5 ml

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

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℃ (stable for up to 6 months).

* Protect this kit from light and 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℃ 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* (2X) (Probe qPCR), ROX plus may precipitate. To dissolve the precipitate completely, let stand briefly at room temperature (below approximately 30℃), followed by inverting the bottle 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. Prepare the probe and primers separately.

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℃) required with 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

【 Protocol for the StepOnePlus Real-Time PCR System 】

1. Prepare the PCR mixture shown below.

<Per reaction>

Reagent	Volume	Final conc.
Premix Ex Taq (2X) (Probe qPCR), ROX plus	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 (< 100 ng)*3	2 μl	
Sterile purified water	6.4 μl	
Total	20 μl	

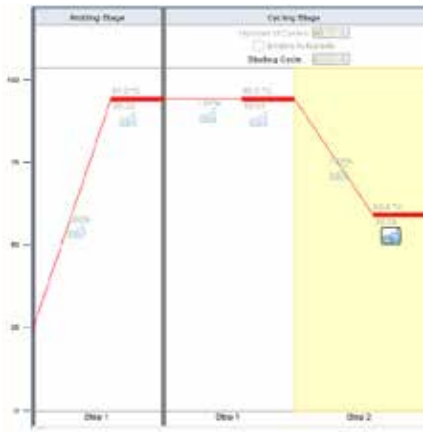
*1 Final primer concentration of 0.2 μM is most likely to yield good results. However, should further optimization be required, try adjusting primer concentrations in the range of 0.1 - 1.0 μM.

*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 Optimal template quantity depends on the copy number of the target in the template solution. Test serial dilutions to select an appropriate quantity. Use no more than 100 ng of DNA template. When using cDNA (RT reaction mixture) as a template in RT-PCR, template volume should not exceed 10% of the PCR reaction mixture (e.g., no more than 2.0 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 shown below. Try this protocol first and optimize PCR conditions as needed. (See "PCR Conditions" on page 9.)



Shuttle PCR standard protocol

Fast Mode
Holding Stage
Number of cycle: 1
Step 1: 95℃ 20 sec
Cycling Stage
Number of cycles: 40
Step 1: 95℃ 1 sec
Step 2: 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 StepOnePlus Real-Time PCR System.

【 Protocol For the Thermal Cycler Dice Real Time System III, *II*, and *Lite*】

1. Prepare the PCR mixture shown below.

<Per reaction>

Reagent	Volume	Final conc.
Premix Ex Taq (2X) (Probe qPCR), ROX plus	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 (< 100 ng)*3	2 μl	
Sterile purified water	8.5 μl	
Total	25 μl	

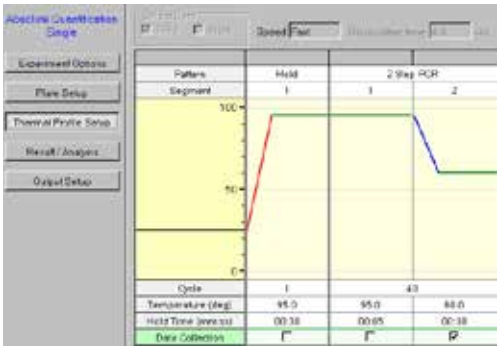
*1 Final primer concentration of 0.2 μM is most likely to yield good results. However, should further optimization be required, try adjusting primer concentrations in the range of 0.1 - 1.0 μM.

*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 III, *II,* and *Lite*, use a final concentration in the range of 0.1 - 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 9.)



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 analytical methods, refer to the manual for the Thermal Cycler Real Time System.

【 Protocol for the Smart Cycler II System 】

1. Prepare the PCR mixture shown below.

<Per reaction>

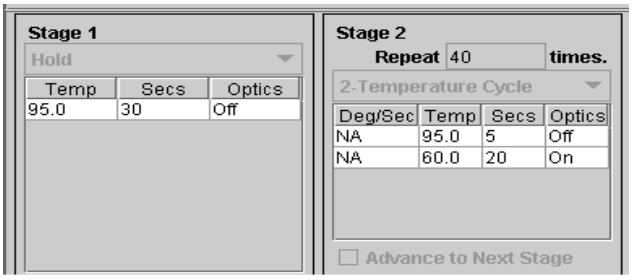
Reagent	Volume	Final conc.
Premix Ex Taq (2X) (Probe qPCR), ROX plus	12.5 μl	1X
PCR Forward Primer (10 μM)	0.5 μl	0.2 μM*1
PCR Reverse Primer (10 μM)	0.5 μl	0.2 μM*1
Probe*2	1 μl	
Template (<100 ng)*3	2 μl	
Sterile purified water	8.5 μl	
Total	25 μl	

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

*3 Optimal template quantity depends on the copy number of the target in the template. Test serial dilutions to select an appropriate quantity. Use no more than 100 ng of DNA template. When using cDNA (RT reaction mixture) as a template in RT-PCR, 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 9.)



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 - 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 - 5 sec is usually sufficient.
Annealing/ extension	56 - 64℃	20 - 30 sec	On	When optimizing reaction conditions, evaluate results using annealing/ extension temperature in the range of 56 - 64℃. If poor reactivity occurs, increasing incubation time for this step may improve results.

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), ROX plus 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 volume 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)*1
Premix Ex Taq™ (Probe qPCR) (Cat. #RR390A/B)
Premix Ex Taq™ (Probe qPCR), Bulk (Cat. #RR390L)
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

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.