Cat. # RR392A/S

For Research Use

TaKaRa

Probe qPCR Mix, with UNG

Product Manual

v201905Da

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I. Description

Probe qPCR Mix, with UNG is designed for probe-based qPCR. A combination of a hot-start PCR enzyme and an optimized buffer results in excellent suppression of non-specific amplification, high amplification efficiency, and high detection sensitivity in real-time PCR analyses. This product is also suitable for high-speed PCR. It 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. Probe qPCR Mix, with UNG is supplied as a 2X premix containing Tli RNase H and Uracil N-Glycosylase (UNG). A heat-resistant Tli RNase H can minimize PCR inhibition by degrading residual mRNA in reactions using cDNA templates, while UNG can avoid false positives due to carryover contamination by degrading previously amplified PCR products with this kit.

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 Inc.)
- CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.)

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

II. Principle

This product uses a hot-start PCR enzyme for PCR amplification. PCR amplification products can be monitored in real time using a probe.

1. PCR

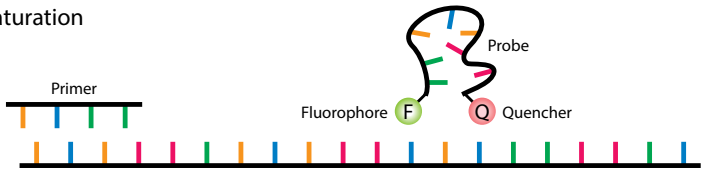
PCR is a technique used to amplify specific target sequences from minute amounts of DNA. By repeating three steps—heat denaturation, primer annealing, and primer extension—the target fragment is amplified up to a million times by DNA polymerase within a short time.

2. Fluorescence Detection

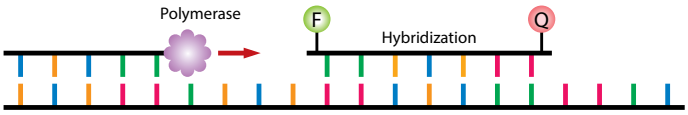
Probe oligonucleotides labeled with a 5′ fluorophore (e.g., FAM) and a 3′ quencher (e.g., TAMRA) are added to the reaction. Fluorescence of the fluorophore is suppressed by the quencher.

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

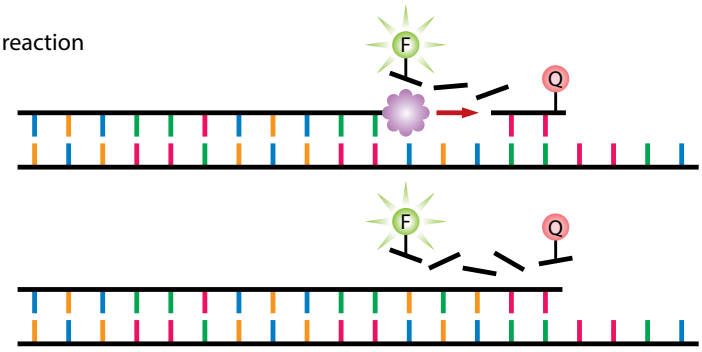
1) Heat denaturation



2) Primer annealing/probe hybridization



3) Extension reaction



III. Components (for 200 reactions)

	Cat. # RR392A	RR392S
Probe qPCR Mix, with UNG (2X)*1	1 ml x 5	1 ml
ROX Reference Dye (50X)*2	200 µl	40 µl
ROX Reference Dye II (50X)*2	200 µl	40 µl

*1 Contains PCR enzyme, dNTP Mixture, Mg^{2+}, Tli RNase H, and UNG.

*2 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 (Thermo Fisher Scientific Inc.)
  - StepOnePlus Real-Time PCR System (Thermo Fisher Scientific Inc.)
- ◆ 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 (Thermo Fisher Scientific Inc.)
  - 7500 Fast Real-Time PCR System (Thermo Fisher Scientific Inc.)
- ◆ 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)*
  - CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.)

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

IV. Materials Required but not Provided

1. Real-time PCR instrument
2. Reaction tubes or plates designed specifically for the qPCR instrument used
3. PCR primers
4. Probe for detection
5. Sterile purified water
6. Micropipette and tips (sterile, with filter)

V. Storage -20℃

VI. Features

1. This product allows rapid and accurate gene detection and quantification using real-time PCR.
2. The 2X premix enables easy pipetting.
3. High resistance to inhibitors allows for successful PCR reactions with templates purified using simpler methods.
4. The premix includes Tli RNaseH, a heat-resistant RNase H that minimizes PCR inhibition by degrading residual mRNA in input cDNA, and Uracil N-Glycosylase (UNG), which avoids false positives due to carryover contamination by degrading PCR products amplified previously with this kit.

VII. 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℃, Probe qPCR Mix (2X) may precipitate. To dissolve the precipitate completely, warm by hand or let stand at room temperature for a while, 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 and primers.

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

5. The probe and primers are susceptible to degradation by nuclease. Take care to avoid nuclease contamination during sample handling.

6. We recommend designating and physically segregating three separate areas, as described below, for preparing reaction mixtures, preparing samples, and performing the detection reaction. Please avoid opening or closing a tube containing amplified products in any areas.

   - Area 1: Reaction mixture preparation and dispensing
   - Area 2: Sample preparation
   - Area 3: Addition of samples to reaction mixtures

   This kit allows amplification and detection to take place simultaneously in real time. Thus, no electrophoresis or other analytical methods are required after the reaction is complete. Never remove amplification products from tubes as doing so may introduce contamination.

7. Operate real-time PCR instruments in accordance with the manufacturer's instructions.

VIII. Precautions during Use

When contamination from the previous reaction using this kit is suspected, perform a step that incubates the reaction mixture at 25℃ for 10 minutes. PCR products that were carried over from the previous reaction will be degraded by the UNG activity.

The DNA polymerase 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 heat treatment may inactivate the enzyme, affecting amplification efficiency and quantification accuracy. Even for the initial denaturation, incubation at 95℃ for 30 sec is sufficient.

IX. Protocol

[Protocol when using the Thermal Cycler Dice Real Time System III, *II*, and *Lite*]

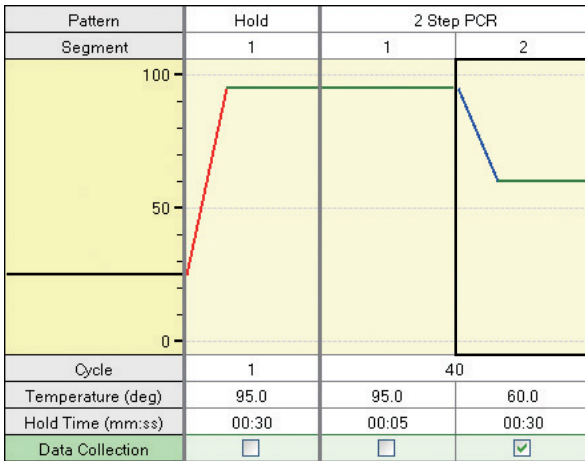
* Follow the instructions in the user manual for each instrument.

1. Prepare a PCR reaction mixture as indicated below.

<For 1 reaction>

Reagent	Volume	Final conc.
Probe qPCR Mix, with UNG (2X)	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	

2. Start the reaction.
   The recommended protocol for PCR reactions is the shuttle PCR standard protocol shown below. Although the annealing/extension time can be set between 20 and 30 sec, try 30 sec first for more consistent results.
   (See <PCR reaction conditions> on page 11.)



Shuttle PCR standard protocol
Hold (UNG)
Number of cycles: 1
25℃ 10 min*4
Hold (Initial denaturation)
Number of cycles: 1
95℃ 30 sec
2 Step PCR
Number of cycles: 40
95℃ 5 sec
60℃ 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.

*1 - 4: Refer to <PCR reaction conditions> on page 11.

[Protocol when using the Applied Biosystems 7300 Real-Time PCR System, StepOnePlus Real-Time PCR System]

* Follow the instructions in the user manual for each instrument.

1. Prepare a PCR reaction mixture as indicated below.

<For 1 reaction>

Reagent	Volume	Volume	Final conc.
Probe qPCR Mix1, with UNG (2X)	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)*5	0.4 µl	1 µl	1X
Template*3	2 µl	4 µl	
Sterile purified water	6 µl	16 µl	
Total	20 µl *7	50 µl *7	

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 11.)

<7300 Real-Time PCR System>

Shuttle PCR standard protocol

<Hold>
25℃ 10 min*4

Stage 1: Initial denaturation
Number of cycles: 1
95℃ 30 sec

Stage 2: PCR reaction
Number of cycles: 40
95℃ 5 sec
60℃ 31 sec*8

<StepOnePlus Real-Time PCR System>

Fast Protocol

<Hold>
25℃ 10 min*4

Holding stage
Number of cycles: 1
95℃ 20 sec

Cycling stage
Number of cycles: 40
95℃ 1 sec
60℃ 20 sec*8

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.

*1 - 8: Refer to <PCR reaction conditions> on page 11.

[Protocol when using the Applied Biosystems 7500/7500 Fast Real-Time PCR System]

* Follow the instructions in the user manual for each instrument.

1. Prepare a PCR reaction mixture as indicated below.

<For 1 reaction>

Reagent	Volume	Volume	Final conc.
Probe qPCR Mix, with UNG (2X)	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 II (50X)	0.2 µl	0.5 µl	0.5X *6
Template*3	2 µl	4 µl	
Sterile purified water	6.2 µl	16.5 µl	
Total	20 µl *7	50 µl *7	

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 11.)

<7500 Real-Time PCR System>

Shuttle PCR standard protocol

<Hold>
25℃ 10 min*4
Stage 1: Initial denaturation
Number of cycles: 1
95℃ 30 sec
Stage 2: PCR reaction
Number of cycles: 40
95℃ 5 sec
60℃ 34 sec*8

<7500 Fast Real-Time PCR System>

Fast protocol

<Hold>
25℃ 10 min*4
Holding stage
Number of cycles: 1
95℃ 20 sec
Cycling stage
Number of cycles: 40
95℃ 3 sec
60℃ 30 sec*8

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.

*1 - 8: Refer to <PCR reaction conditions> on page 11.

[Protocol when using the CFX96 Real-Time PCR Detection System]

* Follow the instructions in the user manual for the CFX96 Real-Time PCR Detection System.

1. Prepare a PCR reaction mixture as indicated below.

<For 1 reaction>

Reagent	Volume	Final conc.
Probe qPCR Mix, with UNG (2X)	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	

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 11.)

Shuttle PCR standard protocol

<Hold>
25℃ 10 min*4
Hold (Initial denaturation)
Number of cycles: 1
95℃ 30 sec
2-step PCR
Number of cycles: 40
95℃ 5 sec
60℃ 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.

*1 - 4: Refer to <PCR reaction conditions> on page 11.

<PCR reaction conditions>

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) Cycle number: 30 - 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 (31, 34 sec)*8	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.

*1 A final primer concentration of 0.2 μM works well in most cases. 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 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 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.

*4 When contamination from the previous reaction containing dUTP is suspected, add a step that incubates the mixture at 25℃ for 10 minutes. PCR products that were carried over from the previous experiment are degraded by the UNG activity.

*5 Use ROX Reference Dye (50X) at a final concentration of 1X.

*6 Use ROX Reference Dye II (50X) at a final concentration of 0.5X.

*7 Adjust the reaction volume according to the recommendations for the real-time PCR instrument used.

*8 Depending on the real-time PCR instrument used, it may not be possible to set the detection step within 30 sec. Set the detection step to 31 seconds or more for the Applied Biosystems 7300 Real-Time PCR System and 34 seconds or more for the Applied Biosystems 7500 Real-Time PCR System.

X. Related Products

PrimeScript™ RT reagent Kit (Perfect Real Time) (Cat. # RR037A/B)
PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (Cat. # RR047A/B)
PrimeScript™ RT Master Mix (Perfect Real Time) (Cat. # RR036A/B)*
Probe qPCR Mix (Cat. # RR391A/B)*
One Step PrimeScript™ III RT-qPCR Mix, with UNG (Cat. # RR601S/A/B)*
Thermal Cycler Dice® Real Time System III (Cat. # TP950/TP970/TP980/TP990)*
Thermal Cycler Dice® Real Time System *Lite* (Cat. # TP700/TP760)*

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