# THUNDERBIRD® SYBR® qPCR Mix

QPS-201T 1 mL x 1
QPS-201 1.67 mL x 3

**Store at -20°C, protected from light**

## Contents



**CAUTION**
All reagents in this kit are intended for research purposes. Do not use for diagnosis or clinical purposes. Please observe general laboratory precaution and utilize safety while using this kit.



**JAPAN**
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

**CHINA**
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

**FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.**

# [1] Introduction

## Description

THUNDERBIRD® SYBR® qPCR Mix is a Taq DNA polymerase-based highly efficient 2x Master Mix for real-time PCR using SYBR® Green I. The master mix contains all required components, except ROX reference dye and primers (50x ROX reference dye is individually supplied with this kit). The master mix facilitates reaction setup, and improves the reproducibility of experiments.

This product is an improved version of Realtime PCR Master Mix (Code No. QPK-201) and Realtime PCR Master Mix -Plus- (Code No. QPK-212). In particular, the reaction specificity and PCR efficiency is enhanced.

## Features

**-High specificity**

The specificity for the detection of low-copy targets is improved.

**-Homogeneous amplification**

The dispersion of PCR efficiency between targets is reduced by a new PCR enhancer*. (*Patent pending)

**-Broad dynamic range**

High specificity and effective amplification enable the detection of a broad dynamic range.

**-Compatibility for various real-time cyclers.**

The reagent is applicable to most real-time cyclers (i.e. Block type and glass capillary type). Because the 50x ROX reference dye is individually supplied with this kit, the kit can be applied to real-time cyclers that require a passive reference dye.

**-Hot start PCR**

The master mix contains anti-Taq DNA polymerase antibodies for hot start technology. The antibodies are easily inactivated in the first denaturation step, thereby activating the DNA polymerase.

## About the SYBR® Green I detection system

The SYBR® Green I assay system utilizes fluorescent emission when SYBR® Green is intercalated into double-stranded DNA. The signal depends on the amount of amplified DNA. However, this system cannot distinguish between target and non-specific amplicons. Therefore, melting curve analysis is necessary after amplification.

# [2] Components

This kit includes the following components for 40 reactions (QPS-201T) and 200 reactions (QPS-201), with a total of 50 µl per reaction. All reagents should be stored at -20 °C.

<QPS-201T>

| | |
|---|---|
| THUNDERBIRD® SYBR® qPCR Mix | 1 ml x 1 |
| 50xROX reference dye | 50 µl x 1 |

**JAPAN**
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

**CHINA**
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

<QPS-201>

| | |
|---|---|
| THUNDERBIRD® SYBR® qPCR Mix | 1.67 ml x 3 |
| 50xROX reference dye | 250 µl x 1 |

**Notes:**

-THUNDERBIRD® SYBR® qPCR Mix can be stored, protected from light, at 2-8°C for up to 3 months. For longer storage, this reagent should be kept at -20°C and protected from light. No negative effect was detected by 10 freeze-thaw cycles of THUNDERBRID™ SYBR® qPCR Mix. This reagent does not contain the ROX reference dye.

-50x ROX reference dye can be stored, protected from light, at 2-8°C or -20°C. For real-time cyclers that require a passive reference dye, this reagent must be added to the reaction mixture at a concentration of 1x or 0.1x. The master mix solution with the ROX reference dye can be stored, protected from light, at 2-8°C for up to 3 months. For longer storage, this reagent should be kept at -20°C and protected from light. The pre-mixed reagents can be prepared according to the following ratios. [5] Table 1 shows the optimal concentration of the ROX dye.

**1x Solution**

THUNDERBIRD® SYBR® qPCR Mix : 50xROX reference dye = 1.67 ml : 66.8 µl
THUNDERBIRD® SYBR® qPCR Mix : 50xROX reference dye = 1 ml : 40 µl

**0.1x Solution**

THUNDERBIRD® SYBR® qPCR Mix : 50xROX reference dye = 1.67 ml : 6.7 µl
THUNDERBIRD® SYBR® qPCR Mix : 50xROX reference dye = 1 ml : 4 µl

For real-time cyclers that do not require a passive reference dye, THUNDERBIRD® SYBR® qPCR Mix without the ROX reference dye can be used.

## [3] Primer design

### 1. Primer conditions

Highly sensitive and quantitative data depend on primer design. The primer should be designed according to the following suggestions;

-Primer length: 20-30 mer
-GC content of primer: 40-60%
-Target length: ≤ 200 bp (optimally, 80-150 bp)
-Melting temperature (Tm) of primers: 60-65°C
-Purification grade of primers: Cartridge (OPC) grade or HPLC grade

**Notes:**

-Longer targets (>200 bp) reduce efficiency and specificity of amplification.
-Tm of the primers can be flexible, because the Tm value depends on the calculation formula.

## [4] Template DNA

The following DNA samples can be used as templates.

### 1. cDNA

Non-purified cDNA, generated by reverse transcription reactions, can be used directly for real-time PCR using THUNDERBIRD® SYBR® qPCR Mix. Up to 10% of the volume of a cDNA solution can be used for a real-time PCR reaction. However, excess volume of the cDNA may inhibit the PCR. Up to 20% (v/v) of the cDNA solution from ReverTra Ace® qPCR RT Kit (Code No. FSQ-101) can be used for real-time PCR.

### 2. Genomic DNA, Viral DNA

Genomic DNA and viral DNA can be used at up to 200 ng in 50 µl reactions.

### 3. Plasmid DNA

Although super-coiled plasmids can be used, linearized plasmid DNA produces more accurate assays. The copy number of the plasmid DNA can be calculated by the following formula.

Copy number of 1µg of plasmid DNA = $9.1 \times 10^{11}$ / Size of plasmid DNA (kb)

## [5] Protocol

### 1. Reaction mixture setup

| | Reaction volume | | Final |
|---|---|---|---|
| Reagent | 50µl | 20µl | Concentration |
| DW | X µl | X µl | |
| THUNDERBIRD® SYBR® qPCR Mix25 µl | 10 µl | 1x | |
| Forward Primer | 15 pmol | 6 pmol | 0.3 µM[*1] |
| Reverse Primer | 15 pmol | 6 pmol | 0.3 µM[*1] |
| 50X ROX reference dye | 1µl / 0.1µl | 0.4µl / 0.04µl | 1x / 0.1x[*2] |
| DNA solution | Y µl | Y µl | |
| Total | 50 µl | 20 µl | |

**JAPAN**
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

**CHINA**
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

**Notes:**

*1 Higher primer concentration tends to improve the amplification efficiency, and lower primer concentration tends to reduce the non-specific amplification. The primer concentration should be set between 0.2-0.6 μM.

*2 50xROX reference dye must be added when using real-time cyclers that require a passive reference dye, according to Table 1. Table 1 shows the optimum concentration of the ROX reference dye. This dye is not necessary for real-time cyclers that do not require a passive reference dye.

Table 1 Recommended ROX dye concentration

| Real-time cycler | Optimal dye concentration (dilution ratio) |
|---|---|
| Applied Biosystems 7000, 7300, 7700, 7900HT StepOne™, StepOnePlus™ etc. | 1x (50:1) |
| Applied Biosystems 7500, 7500Fast, Stratagene cyclers (Option) etc. | 0.1x (500:1) |
| Roche cyclers, Bio-Rad cyclers, BioFlux cyclers etc. | Not required |

**Notes:**

The ROX dye in SYBR® Green Realtime PCR Master Mix (Code No. QPK-201) and SYBR® Green Realtime PCR Master Mix -Plus- (Code No. QPK-212) correspond to 1x concentration.

## 2. PCR cycling conditions

The following table shows the recommended thermal conditions using primers designed according to the recommended primer described in [3]. Almost all targets can also be amplified using the ongoing conditions with other real-time PCR reagents.

When satisfactory results are not obtained or the Tm value of the primers is lower than 60°C, a 3-step cycle should be used. The detailed conditions are described in [5] 2-3.

| <2-step cycle> | Temperature | Time | Ramp | |
|---|---|---|---|---|
| Pre-denaturation | 95°C | 20-60 sec[*1]. | Maximum | |
| Denaturation: | 95°C | 1-15 sec[*2]. | Maximum | ↰ 40 cycles |
| Extension: | 60°C[*3] | 30-60 sec[*4]. | Maximum | |
| | (data collection should be set at the extension step) | | | |
| Melting / Dissociation Curve Analysis[*5] | | | | |

[*1] Due to the anti-Taq antibody hot start PCR system, the pre-denaturation can be completed within 60 sec. The pre-denaturation time should be determined according to the recommendations of each real-time cycler. If the optimal pre-denaturation time cannot be determined, the time should be set at 60 sec.

**JAPAN**
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

**CHINA**
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

**Table 2** The recommended pre-denaturation time on each real-time cyclers

| Real-time cycler | Pre-denaturation time |
|---|---|
| High speed cycler (e.g. Applied Biosystems 7500Fast) | 20 sec |
| Capillary cycler (e.g. Roche LightCycler™ 1.x, 2.0) | 30 sec |
| General real-time cyclers (e.g. Applied Biosystems 7700, 7500, 7900HT [normal block], StepOne™, StepOnePlus™ Agilent cyclers, BioFlux cyclers) | 60 sec |

*2 The following table shows the optimal denaturation times for each real-time cycler. If the optimal denaturation time cannot be determined, the time should be set at 15 sec.

**Table 3** The recommended denaturation time on each real-time cyclers

| Real-time cycler | Pre-denaturation time |
|---|---|
| High speed cycler (e.g. Applied Biosystems 7500Fast) | 3 sec |
| Capillary cycler (e.g. Roche LightCycler™ 1.x, 2.0) | 5 sec |
| General real-time cyclers (e.g. Applied Biosystems 7700, 7500, 7900HT [normal block], StepOne™, StepOnePlus™ Agilent cyclers, BioFlux cyclers) | 15 sec |

*3 Insufficient amplification may be improved by decreasing the extension temperature, and non-specific amplification (e.g. abnormal shapes of the amplification curve at low template concentrations) may be reduced by increasing the extension temperature. The extension temperature should be set at 56-64°C.

*4 If the target size is smaller than 300 bp, the extension time can be set at 30 sec on almost all real-time cyclers. Instability of the amplification curve or variation of data from each well may be improved by setting the extension time at 45-60 sec. Some real-time cyclers or software need over 30 sec for the extension step. In these cases, the time should be set according to each instruction manual (e.g. Applied Biosystems 7000/73000: ≥ 31 sec; Applied Biosystems 7500: ≥ 35 sec.).

*5 The melting curve analysis should be performed according to the recommendations of each real-time cycler.

**JAPAN**
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

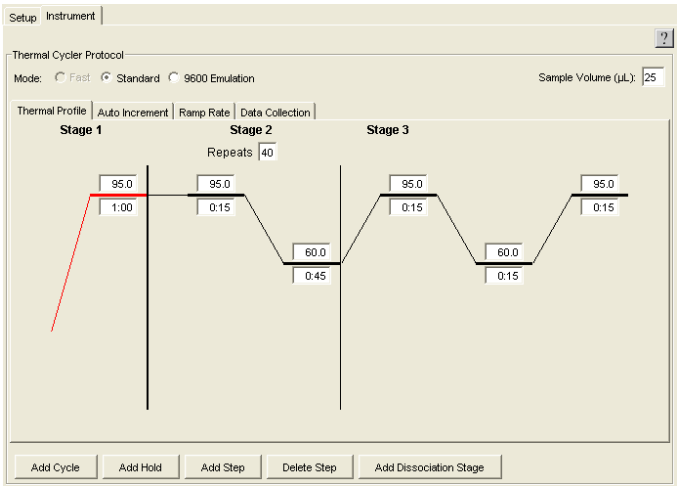
**CHINA**
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

## 2-1. Real-time PCR conditions using Applied Biosystems 7900HT

(Normal block type, software version 2.2.2)

The following is an example of a SYBR® Green I assay using Applied Biosystems 7900HT.

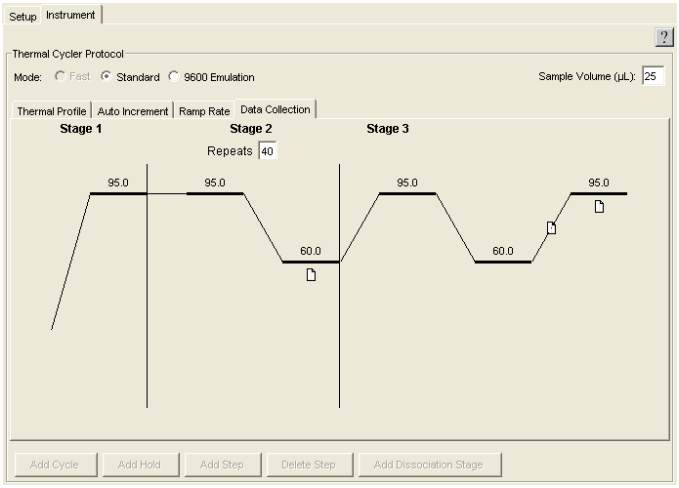
(1) The cycling parameters should be set according to the following "Thermal Cycler Protocol" window under the "Instrument" tab. When adding the melting curve analysis, click the "Add Dissociation Stage" button.




**Notes:**

- Appropriate sample volumes should be set.
- ≥ 45 sec is necessary for the extension step.

(2) Click the "Data collection" tab.




(3) Insert the PCR plate

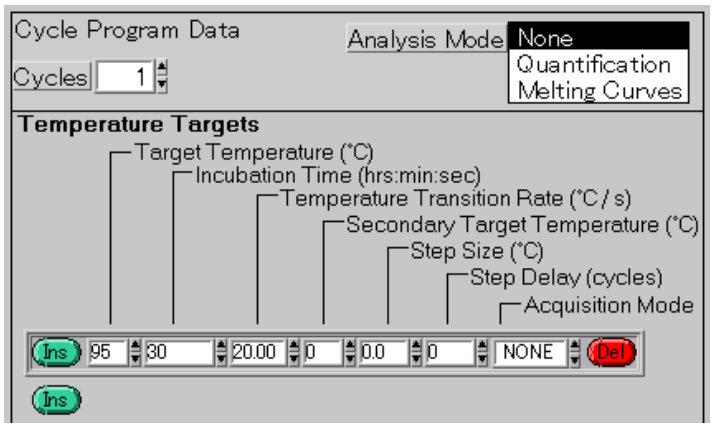
(4) Start the program

## 2-2. Real-time PCR conditions using Roche LightCycler 1.1

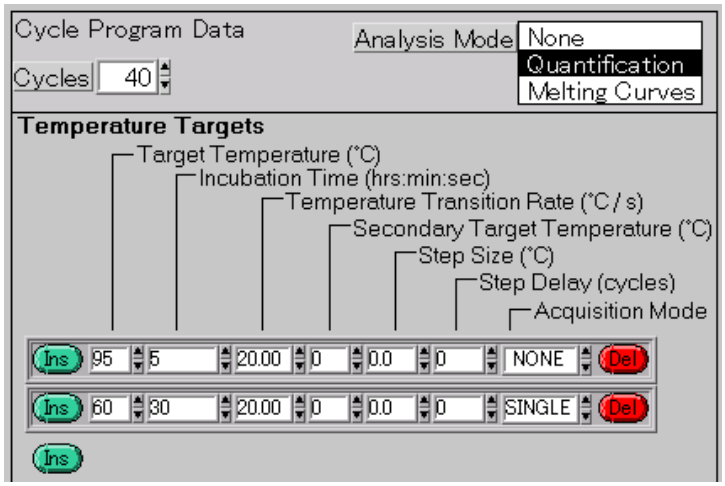
(Software version 3.5)

The following is an example of a SYBR® Green I assay using Roche LightCycler 1.1.

(1) The cycling parameters should be set according to the following window. Analysis and Acquisition mode of the initial denaturation step must be set at “None”.



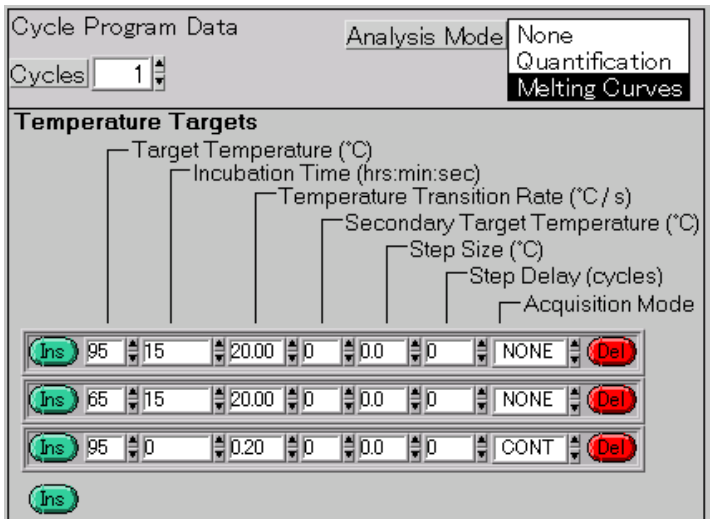

(2) The cycling parameters should be set according to the following window. Analysis mode of the PCR step must be set at “Quantification”. And Acquisition modes of 95°C and 60°C must be set at “None” and “Single”, respectively.



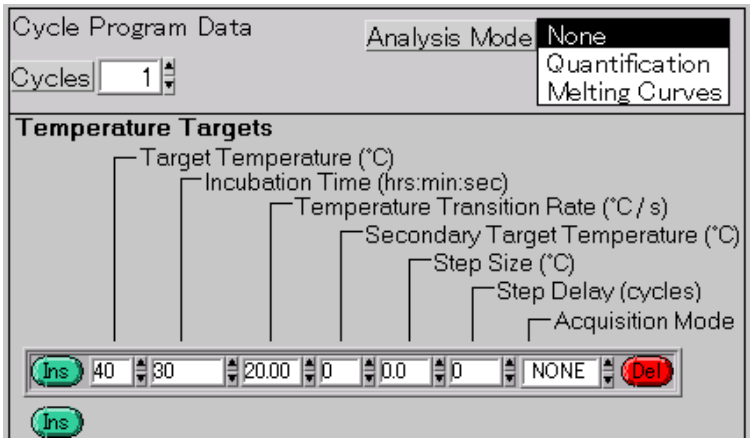

**JAPAN**
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

**CHINA**
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

(3) [Melting curve analysis] The cycling parameters should be set according to the following window. The analysis mode must be set at “Melting curves”. Acquisition modes of 95°C (first) and 65°C must be set at “Non”. Acquisition mode of the second 95°C must be set at “CONT”.




(4) The cycling parameters should be set according to the following window. Analysis and Acquisition mode of the cooling step must be set at “Non”.




(5) Insert the capillaries in the carousel, and start the cycling program.

**JAPAN**
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

**CHINA**
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

## 2-3. 3-step cycle

In the event of the 2-step cycle failing, the following 3-step cycle may improve results. In the following cases, the 3-step cycle conditions may improve the result efficiently.

-The Tm of the primer is lower than 60°C.
-The target is longer than 300 bp.
-PCR efficiency is low.

| <3-step cycle> | Temperature | Time | Ramp | |
|---|---|---|---|---|
| Pre-denaturation | 95°C | 20-60 sec[*1]. | Maximum | |
| Denaturation: | 95°C | 1-15 sec[*1]. | Maximum | 40 cycles |
| Annealing | 55-65°C[*2] | 5-30 sec[*3] | Maximum | |
| Extension: | 72°C | 30-60 sec[*4]. | | |
| | (data collection should be set at the extension step) | | | |
| Melting / Dissociation Curve Analysis[*5] | | | | |

[*1] The denaturation step should be determined according to [5] 2.

[*2] The annealing temperature should be set at primer's Tm-5°C. A higher annealing temperature may improve non-specific amplification.

[*3] The annealing time should be set at 5 sec (Fast cycler), 15 sec (Normal cycler). Shorter annealing times may reduce non-specific amplification. Longer annealing times (up to 30 sec) may increase the PCR efficiency when the efficiency is low.

[*4] Shorter targets (≤300 bp) require shorter extension times (≤30 sec). However, certain cyclers require >30 sec detection time at the extension step. An unstable signal may be improved by prolonging the extension time up to 40-60 sec. Also note that some cyclers cannot have an extension time of 30 sec (Applied Biosystems 7000, 7300: ≥ 31 sec, 7500: ≥35 sec)

[*5] The melting curve analysis should be performed according to each cycler's recommendations.

**JAPAN**
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

**CHINA**
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

## [ 6 ] Related Protocol

### 1. cDNA synthesis

cDNA synthesized by various cDNA synthesis reagents can be used with THUNDERBIRD® SYBR® qPCR Mix. However, cDNA synthesized by a reagent specialized for real-time PCR can increase sensitivity.

ReverTra Ace® qPCR RT Kit (Code No. FSQ-101) is a cDNA synthesis kit suitable for real-time PCR. Here, the protocol with ReverTra Ace® qPCR RT Kit is described. However, for the detailed protocol, please refer to the instruction manual of the kit.

(1) Denaturation of RNA

Incubate the RNA solution at 65°C for 5 min and then chill on ice.

**Notes:**

-This step can be omitted. But this step may increase the efficiency of the reverse transcription of RNA, which forms secondary structures.

-Do not add 5x RT Buffer and/or enzyme solution at this step.

(2) Preparation of the reaction solution

| Reagent | Volume (amount) |
|---|---|
| Nuclease-free Water | X µl |
| 5x RT Buffer | 2 µl |
| Primer Mix | 0.5 µl |
| Enzyme Mix | 0.5 µl |
| RNA solution | 0.5 pg-1 µg |
| Total | 10 µl |

(3) Reverse transcription reaction

-Incubate at 37°C for 15 min. <Reverse transcription>

-Heat at 98°C for 2 min. <Inactivation of the reverse transcriptase>

-Store at 4°C or -20°C.*

*This solution can be used in the real-time PCR reaction directly or after dilution.

**Notes:**

The above temperature conditions are optimized for ReverTra Ace® qPCR RT Kit.

**JAPAN**
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

**CHINA**
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

## [7] Troubleshooting

| Symptom | Cause | Solution |
|---|---|---|
| Loss of linearity in the high cDNA/DNA concentration region. | Intercalation of SYBR® Green I into the template DNA. | Because SYBR® Green I is also intercalated into the template DNA, the base line tends to be higher when high concentration DNA samples are used. Diluted template should be used to obtain a correct Ct value. |
| Lost of linearity or lower signal in the low DNA/cDNA concentration region. | The template DNA is insufficient. | When the DNA/cDNA copy number is lower than 10 copies per reaction, the linearity of the reaction tends to be lost. The template concentration should be increased. |
| | Adsorption of the DNA to the tube wall. | The diluted DNA templates tend to be absorbed onto the tube wall. Dilution should be performed just prior to experiments. |
| | Competition with primer dimer formation. | Dimer formation may reduce the amplification efficiency of the target, especially for reactions at low template concentration. The reaction condition should be optimized or the primer sequences should be changed. |
| Loss of linearity of the amplification carves. | Competition with non-specific amplification. | Non-specific amplification may reduce the amplification efficiency of the target. The reaction conditions should be optimized or the primer sequences should be changed. |
| The PCR efficiency is lower than 90% (slope: <-3.6) | Inappropriate cycling conditions. | Optimize the cycling conditions according to [5]. |
| | Degradation of the primers. | Fresh primer solution should be prepared. |
| | The calculation of the PCR efficiency is inappropriate. | The Ct value on the linear region should be used to calculate PCR efficiency. |
| The PCR efficiency is higher than 110%. | The calculation of the PCR efficiency is inappropriate. | The Ct value on the linear region should be used to calculate PCR efficiency. |
| Reproducibility is not good. | Poor purification of the template DNA | Low-purity DNA may contain PCR inhibitors. Re-purify the DNA samples. |
| | Absorption of the template DNA to the tube wall. | Diluted DNA templates tend to be absorbed onto the tube wall. Dilution of the template DNA/cDNA should be performed just prior to experiments. |
| | Plasmid DNA or PCR product is used as a template. | In general, plasmid DNA or PCR product is used at low concentration. Diluted DNA templates tend to be absorbed onto the tube wall. Dilution of the template DNA/cDNA should be performed just prior to experiments. Dilution with a carrier nucleic acid solution (Yeast RNA) is also effective in improving linearity. |
| | Inappropriate thermal conditions. | Optimize the thermal conditions according to [5]. |
| | Low purity of the primers | Different lots of primers may show different results. When the lot is changed, prior testing of the primer should be performed. |

**JAPAN**
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

**CHINA**
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

| Symptom | Cause | Solution |
|---|---|---|
| Amplification from the non-template control (NTC). | Formation of primer dimer. | On the melting curve analysis, a peak at a temperature lower than that of the target peak suggests a primer dimer. The PCR cycle should be optimized according to [4] (2). If the result is not improved, the following should be performed:change the primer sequence and/or change the purification grade of the primer (HPLC grade) |
| | Contamination or carry over of the PCR products. | When the no-template control generates a peak at the same melting temperature as the target on the melting curve analysis, the amplification is caused by a carry-over or contamination. Use fresh reagents. |
| Low amplification curve signal / Unstable amplification curve signal. | Excessive amount of ROX reference dye. | Excessive amount of ROX reference dye may cause low signal. 50x ROX reference dye should be used according to [5] Table 1. |
| | Inappropriate settings of fluorescence measurement | Settings should be confirmed according to the instruction manual of each detector. |
| | Insufficient reaction volume. | Low reaction volume may cause an unstable signal. Increase the reaction volume. |
| Detection of multiple peaks on the melting curve analysis | Non-specific amplification. | Optimize the reaction conditions. If the result is not improved, the primer sequence should be changed. |
| | Formation of primer dimer. | On the melting curve analysis, a peak at a temperature lower than that of the target peak suggests a primer dimer. The PCR cycle should be optimized according to [4] (2). If the result is not improved, the following action should be performed:change the primer sequence and/or change the purification grade of the primer (HPLC grade) |

**JAPAN**
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

**CHINA**
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

## [ 8 ] Related products

| Product name | Package | Code No. |
|---|---|---|
| High efficient real-time PCR master mix<br>**THUNDERBIRD® Probe qPCR Mix** | 1mLx1<br>1.67mLx3 | QPS-101T<br>QPS-101 |
| High efficient cDNA synthesis kit for real-time PCR<br>**ReverTra Ace® qPCR RT Kit** | 200 rxns | FSQ-101 |
| High efficient cDNA synthesis master mix for real-time PCR<br>**ReverTra Ace® qPCR RT Master Mix** | 200 rxns | FSQ-201 |
| High efficient cDNA synthesis master mix for real-time PCR with genomic DNA remover<br>**ReverTra Ace® qPCR RT Master Mix with gDNA remover** | 200 rxns | FSQ-301 |
| One-step Real-time PCR master mix for probe assay<br>***RNA-direct*™ Realtime PCR Master Mix** | 0.5 mL x 2<br>0.5 mL x 5 | QRT-101T<br>QRT-101 |
| One-step Real-time PCR master mix for SYBR® Green assay<br>***RNA-direct*™ SYBR® Realtime PCR Master Mix** | 0.5 mL x 2<br>0.5 mL x 5 | QRT-201T<br>QRT-201 |

**JAPAN**
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

**CHINA**
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

**NOTICE TO PURCHASER: LIMITED LICENSE**

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,079,352, 5,789,224, 5,618,711, 6,127,155, 5,677,152, 5,773,258, 5,407,800, 5,322,770, 5,310,652, 5,994,056, 6,171,785, and claims outside the US corresponding to US Patent No. 4,889,818. 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 (such as apparatus or system claims in US Patent No. 6,814,934) 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 Center Drive, Foster City, California 94404, USA.

**JAPAN**
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

**CHINA**
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140