Realtime PCR Master Mix

QPK-101 1 mL x 5

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.

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

[1] Introduction

Description

This product is a Taq DNA polymerase-based 2 x master mix for real-time PCR, which contains all components, except for the primer and probe. This reagent is applicable in TaqMan® assays or hybridization probe assays, in combination with each probe.

Features

-This reagent can be used in glass capillary systems (e.g. LightCycler, Roche Molecular Systems, Inc.).
-This reagent can be used in a passive reference system (e.g., ABI PRISM® 7700, Applied Biosystems, Inc.). The passive reference dye does not affect any other systems.
-Hot Start technology with anti-Taq DNA polymerase antibodies enables high specificity and reproducible amplification.

[2] Components

This reagent includes the following components for 200 reactions (QPK-101), 50 µl total reaction volume:

<QPK-101>

Realtime PCR Master Mix	1 ml x 5

Notes:
This reagent can be stored at 4°C for 2 months and protected from light. For longer storage, this reagent should be kept at -20°C and protected from light.

[3] Primer/Probe design

1. Primer conditions

Primers should be designed according to the following guidelines:

-Primer length: 20-30 mer
-GC content of primer: 40-60%
-Target length: ≤ 200 bp (optimally, ≤ 150 bp)

Notes:
Longer targets (>200 bp) reduce efficiency and specificity of amplification. The ideal optimal target length range is 50-150 bp.

2. Probe conditions

Probes should be designed in accordance with the manufacture's protocol for each individual assay system.

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[4] Detection

This reagent can be used for general detection devices, such as:

-LineGene (Bioer Technology co., ltd.)

This reagent can also be used in detection equipment using glass capillaries or passive reference, such as:

LightCycler (Roche Molecular Systems)

ABI PRISM® 7000, 7700, and 7900 (Applied Biosystems)

Note: The passive reference mode of detectors should be set at "ROX".

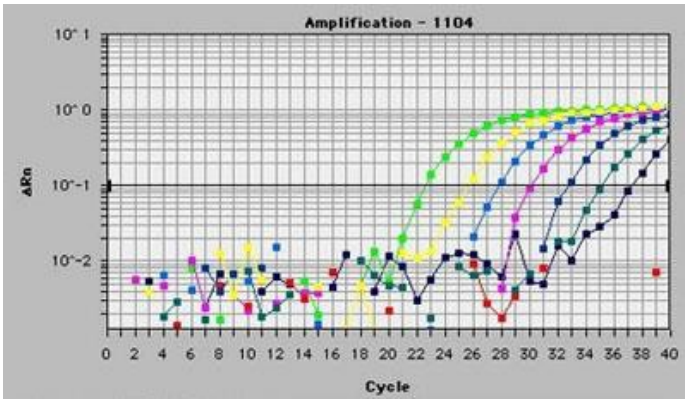

Fig. 1 Detection of β-actin expression by TaqMan® assay
Device: ABI PRISM® 7700 Target: β-actin
Assay method: TaqMan® probe Template: cDNA from total RNA (HeLa cell)

[5] Specimen

1. cDNA

-Reverse transcription reactions from total or poly $(A)^+$ RNA may be used directly, or after dilution, for real-time PCR. Purified cDNA by phenol/chloroform extraction and ethanol precipitation may also be used. Oligo dT and random primers are suitable for the reverse transcription reaction.
-Up to 20% of the synthesized cDNA solution from the ReverTra Ace® qPCR RT Kit (Code NO. FSQ-101) may be added to the PCR reaction solution directly, without purification.

2. Genomic DNA

-Purified DNA, which would be used for general PCR, is also suitable for real-time PCR. In the case of mammalian genomic DNA, 1-10 ng genomic DNA is sufficient for real-time PCR.

[6] Protocol

1. TaqMan® assay protocol using ABI PRISM® 7700

The following is a TaqMan® assay protocol to be used with ABI PRISM® 7700. For other detection devices, this protocol may require modification depending on each instruction manual.

(1) Preparation of reaction solution

Component	Volume	Final Concentration
PCR grade water	14 µl	
Realtime PCR Master Mix	25 µl	1x
10 pmol/µl (10 µM) Primer #1	2 µl	0.4 µM
10 pmol/µl (10 µM) Primer #2	2 µl	0.4 µM
5 pmol/µl (5 µM) TaqMan® probe	2 µl	0.2 µM
Template DNA	5 µl	
Total volume	50 µl	

Notes

-The primer and probe concentrations may require optimization. The optimal ranges for the primer and probe are 0.2-1 µM and 0.05-0.3 µM, respectively. In the case of commercially available primers or probes, recommended conditions from those companies should be used.

(2) Cycling conditions

The following condition is recommended:

<2-step cycle>		
Pre-denaturation:	95°C, 1 min.	
Denaturation:	95°C, 15 sec.	↰ 40 cycles
Extension:	60°C, 1 min. (data collection)	

Notes

-The extension temperature should be adjusted in accordance with the Tm of primers and probes.

-The pre-denaturation condition described above is sufficient for inactivation of the anti-Taq DNA polymerase antibodies used in Hot Start PCR. To prevent unexpected and inappropriate results, do not prolong the pre-denaturation period. Fifteen seconds is also sufficient for denaturation during each cycle.

-Data collection step should be longer than 30 seconds.

-If commercially available primers or probes are employed, the recommended conditions from each company should be used.

2. Hybridization probe assay protocol using Roche LightCycler™

The hybridization probe assay is based on fluorescence resonance energy transfer (FRET) using two adjacent probes: <probe 1>, an acceptor fluorescent dye (e.g., LC-Red640) and <probe 2>, a donor fluorescence dye (e.g. FITC) at the 5' and 3'ends, respectively.

The following is a hybridization assay protocol using the Roche LightCycler™. In the case of other detection devices, this protocol should be modified accordingly.

(1) Preparation of reaction solution

Component	Volume	Final Concentration
PCR grade water	5.6 µl	
Realtime PCR Master Mix	10 µl	1x
10 pmol/µl (10 µM) Primer #1	0.6 µl	0.3 µM
10 pmol/µl (10 µM) Primer #2	0.6 µl	0.3 µM
10 pmol/µl (10 µM) Hybridization probe 1 (5' acceptor)	0.8 µl	0.4 µM
10 pmol/µl (10 µM) Hybridization probe 2 (3' donor)	0.4 µl	0.2 µM
Template DNA	2 µl	
Total volume	20 µl	

Notes

-The primer and probe concentrations can be further optimized, if needed. In the case of commercially available primers or probes, recommended conditions from each manual should be followed.

(2) Cycling conditions

The following condition is recommended:

<3-step cycle>		
Pre-denaturation:	95°C, 30 sec.	
Denaturation:	95°C, 5 sec.	↩ 40 cycles
Annealing:	50°C, 15 sec. (data collection)	
Extension:	75°C, 30 sec.	

Notes

-The annealing temperature should be set below the probe Tm value. The extension temperature should be greater than the probe Tm value.

-The pre-denaturation condition described above is sufficient for inactivation of the anti-Taq DNA polymerase antibodies used in Hot Start PCR. To prevent unexpected and inappropriate results, do not prolong the pre-denaturation period. Five seconds is also sufficient for denaturation during each cycle.

-Data should be collected during the annealing step.

-If commercially available primers or probes are employed, the recommended conditions from each company should be used.

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3. One-step RT-PCR using reverse transcriptase

This reagent can be used for a one-step TaqMan® assay using reverse transcriptase. The following is a one-step protocol using this reagent and a high-efficiency reverse transcriptase, "ReverTra Ace® (Code No. TRT-101)".

(1) Preparation of diluted RTase solution. <This solution is used in (2)>

Component	Volume	Final Concentration
PCR grade water	28 µl	
RNase inhibitor (40 U/µl) <Code No. SIN-201>	4 µl	5 U/µl
ReverTra Ace® (100 U/µl) <Code No.TRT-101>	1 µl	3 U/µl
Total volume	33 µl	

(2) Preparation of reaction solution

Component	Volume	Final Concentration
PCR grade water	9 µl	
Realtime PCR Master Mix	25 µl	1x
10 pmol/µl (10 µM) Primer #1	2 µl	0.4 µM
10 pmol/µl (10 µM) Primer #2	2 µl	0.4 µM
5 pmol/µl (5 µM) TaqMan® probe	2 µl	0.2 µM
Diluted RTase solution (ReverTra Ace® 3 U/µl)	5 µl	0.3 U/ µl
Template DNA	5 µl	
Total volume	50 µl	

Notes

-Because this method tends to result in non-specific amplifications, the conditions should be optimized. Primer and probe concentrations can be further optimized, if needed. In the case of commercially available primers or probes, recommended conditions from each manual should be employed.

-The reverse transcriptase concentration can be increased to 1 U/µl (final concentration).

(2) Cycling conditions

The following is a cycling condition using ABI PRISM® 7700:

<2-step cycle>		
Reverse transcription	42°C, 20 min.	
Pre-denaturation :	95°C, 5 min.	
Denaturation :	95°C, 15 sec.	↰ 40 cycles
Extension :	65°C, 1 min. (data collection)	

Notes

-The reverse transcription step should take place at 42°C for 20 minutes. The pre-denaturation step should be at 95°C for 5 minutes to inactivate the antibodies against Taq DNA polymerase and reverse transcriptase.

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[7] Troubleshooting

Symptom	Cause	Solution
No amplification	Incorrect mode setting of detector for the fluorescence dye.	Confirm the detector setting.
	Incorrect setting for data collection.	Confirm the data collection setting.
	Incorrect setting for sample position.	Reposition the sample tubes.
	Inappropriate concentration of primers or probes.	Optimize the concentration of primers or probes according to instructions (see [6]).
	Inappropriate design of primers or probes.	Confirm specificity and Tm values of the primers and probes.
	Inappropriate cycle conditions.	Confirm the Tm values of the primers and probes.
	Low purity or quality of samples	Check the purity or quality of sample DNA.
Variation in detection	Failure or malfunction of the device	Check the device
	Low quality sample DNA.	Repurify the sample DNA by phenol/chloroform extraction and ethanol precipitation, or other method.
	Inappropriate concentration of primers or probes.	Optimize the concentration of primers or probes according to instructions (see [6]).
	Inappropriate design of primers or probes.	Confirm specificity and Tm values of the primers and probes.
	Inappropriate cycle conditions.	Confirm the Tm values of the primers and probes.
	Variation of dispensed volume.	Increase reaction volume.
Signals in blank reactions	Contamination of amplicons or sample DNAs.	Use fresh PCR grade water, and remake the primer solution, probe solution, and master mix.

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[8] Related products

Product name	Package	Code No.
High efficient cDNA synthesis kit for real-time PCR **ReverTra Ace® qPCR RT Kit**	200 reactions	FSQ-101
High efficientrevers transcriptaase **ReverTra Ace®**	10,000 U	TRT-101
RNase inhibitor (Recombinant type)	2,500 U	SIN-201
Real-time PCR master mix for SYBR® Green assay **SYBR® Green Realtime PCR Master Mix**	1 mL x 5	QPK-201
Real-time PCR master mix for SYBR® Green assay (improved version) **SYBR® Green Realtime PCR Master Mix –Plus-**	1 mL x 5	QPK-212
One-step real-time PCR master mix for probe assay ***RNA-direct*™ Realtime PCR Master Mix**	0.5 mL x 5	QRT-101
One-step real-time PCR master mix for SYBR® Green assay ***RNA-direct*™ SYBR® Realtime PCR Master Mix**	0.5 mL x 5	QRT-201

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