RNA-direct™ Realtime PCR Master Mix

QRT-101T 0.5 mL x 2
QRT-101 0.5 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 2 x Master Mix for "1-step real-time PCR" using a thermostable DNA polymerase derived from *Thermus thermophilus* (Tth) HB8[1)]. Tth DNA polymerase exhibits reverse transcriptase activity in the presence of Mn^{2+} ions. This system allows for "1-step real-time PCR", including reverse transcription and PCR steps. This reagent is applicable for TaqMan® assay or hybridization probe assay, in combination with each probe.

Features

-This reagent is suitable for high-throughput real-time PCR and increases reliability of product, due to lowered risk of contamination.
-This reagent can be used in systems using glass capillaries (e.g., LightCycler, Roche Molecular Systems, Inc.).
-This reagent can be used in systems using passive reference (e.g., 7700, Applied Biosystems, Inc.). The passive reference dye does not affect any other systems.
-Hot Start technology, using anti-Tth DNA polymerase antibodies, allows for high specificity and reproducible amplification.

[2] Components

This reagent includes the following components for 40 reactions (QRT-101T) and 100 reactions (QRT-101), with a total of 50 µl per reaction. All reagents should be stored at -20 °C.

<QRT-101T>

RNA-direct™ Real-time PCR Master Mix	0.5 ml x 2
50 mM $Mg(OAc)_2$	0.2 ml x 1

<QRT-101>

RNA-direct™ Real-time PCR Master Mix	0.5 ml x 5
50 mM $Mg(OAc)_2$	0.5 ml x 1

Notes:

This reagent can be stored at 4°C for up to 2 months, 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, 50-150 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 to the guidelines for each assay system.

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[4] Detection

This reagent can be used with the following devices:

1. Normal devices, such as:

-LineGene, Bioer etc.

2. Devices using a glass capillary or a passive reference, such as:

-LightCycler, Roche Molecular Systems
-7000, 7700, and 7900, Applied Biosystems

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

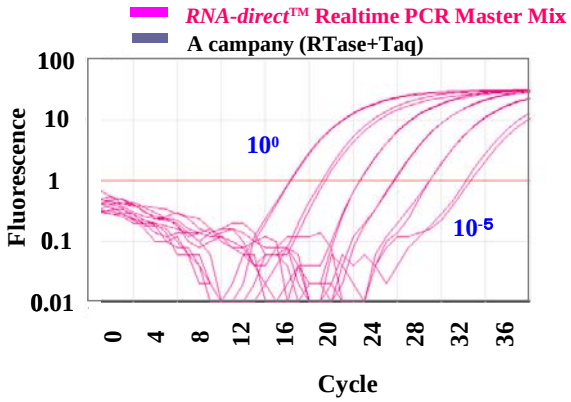

Fig. 1 Detection of β-actin expression by TaqMan® assay
Device: LineGene, Bioer Target: β-actin
Assay method: TaqMan® probe Template: cDNA from poly(A)$^+$ RNA (HeLa cell)

[5] Specimen

The following RNAs are appropriate for highly efficient 1-step real-time PCR.

1. Total RNA

Total RNA typically contains 1-2% mRNA, which can be used as template directly with this kit. RNA prepared by AGPC (Acid Guanidium-Phenol-Chloroform) or the column method contains genomic DNA; therefore, the total RNA should be treated with DNase I prior to transcription.

2. Poly(A)$^+$ RNA (mRNA)

Poly(A)$^+$ RNA can be used to detect low-level expressing mRNA. However, poly(A)$^+$ RNA should be treated carefully, because Poly(A)$^+$ RNA is more sensitive to RNase than total RNA.

[6] Protocol

1. TaqMan® assay protocol using Applied Biosystems 7900HT

The following is a TaqMan® assay protocol using 7900HT. If using other detection devices, this protocol should be slightly altered, according to each instruction manual.

(1) Preparation of reaction solution

Component	Volume	Final Concentration
Nuclease-free water	X µl	
RNA-direct™ Realtime PCR Master Mix	25 µl	1x
50 mM $Mn(OAc)_2$	2.5 µl	2.5 mM
10 pmol/µl (10 µM) Primer #1	1.5 µl	0.3 µM
10 pmol/µl (10 µM) Primer #2	1.5 µl	0.3 µM
5 pmol/µl (5 µM) TaqMan® probe	2 µl	0.2 µM
Template RNA	Y µl	
Total RNA		<2.5 µg/50µl
Poly(A)$^+$ RNA		<500ng/50µl
Total volume	50 µl	

Notes:

-Primer and probe concentrations can be further optimized, if needed. The optimal ranges of primer and probe are 0.2-0.6 µM and 0.05-0.3µM, respectively. In the case of commercially available primers or probes, those recommended conditions should be used.

-The final concentration of $Mn(OAc)_2$ should be adjusted to 2-3.5 mM. Lower Mn concentrations result in decreased non-specific amplification; higher Mn concentrations result in increased amplification efficiency.

-Nuclease-free water prepared without DEPC-treatment is recommended.

(2) Cycling conditions

The following is a cycling condition for the “standard mode” of 7900HT.

<2-step cycle>		
Denaturation:	90 °C, 30 sec.	
RT*:	61 °C, 20 min.	
Pre-denaturation:	95 °C, 1 min.	
Denaturation:	95 °C, 15 sec.	⌐ 45 cycles
Extension:	60 °C, 1 min. (data collection)	⌙

*The temperature of reverse transcription (RT) should be set below Tm of primers. The temperature can be optimized between 50 and 61 °C.

Notes

-The PCR Master Mix contains anti-Tth DNA polymerase antibodies for Hot Start PCR. The first denaturation step (90°C, 30 sec.) is sufficient to inactivate the antibodies. Do not prolong this denaturation step.

2. TaqMan® assay protocol using Roche LightCycler™

The following is a TaqMan® assay protocol using the Roche LightCycler™. In the case of other detection devices, this protocol should be slightly altered accordingly.

(1) Preparation of reaction solution

Component	Volume	Final Concentration
Nuclease-free water	X µl	
RNA-direct™ Real-time PCR Master Mix	10 µl	1x
50 mM $Mn(OAc)_2$	1 µl	2.5 mM
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
5 pmol/µl (5 µM) TaqMan® probe	0.8 µl	0.2 µM
Template RNA	Y µl	
Total RNA		<1 µg/20 µl
Poly(A)$^+$ RNA		<200 ng/20 µl
Total volume	20 µl	

Notes

-Primer and probe concentrations can be further optimized, if needed. The optimal ranges of primer and probe are 0.2-0.6 µM and 0.05-0.3 µM, respectively. In the case of commercially available primers or probes, those recommended conditions should be used.

-The final concentration of $Mn(OAc)_2$ should be adjusted to 2-3.5 mM. Lower Mn concentrations result in decreased non-specific amplification; higher Mn concentrations result in increased amplification efficiency.

-Nuclease-free water prepared without DEPC-treatment is recommended.

(2) Cycling conditions

The following conditions are recommended:

<2-step cycle>		
Denaturation:	90 °C, 30 sec.	
RT*:	61 °C, 20 min.	
Pre-denaturation:	95 °C, 30 sec.	
Denaturation:	95 °C, 0 sec.	↰ 45 cycles
Extension:	60 °C, 45 sec. (data collection)	

*The temperature of reverse transcription (RT) should be set below Tm of primers. The temperature can be optimized between 50 and 61 °C.

Notes

-The PCR Master Mix contains anti-Tth DNA polymerase antibodies for Hot Start PCR. The first denaturation step (90 °C, 30 sec.) is sufficient to inactivate the antibodies. Do not prolong this denaturation step.

-The temperature transition rate can be set to 20 °C/sec. Poor amplification may be improved by changing the temperature transition rate to 2 °C/sec.

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[7] Related Protocol

1. DNase I treatment of total RNA

Total RNA prepared by general methods contains genomic DNA. Genomic DNA can be eliminated by the following method.

(1) Mix the following reagents.

Nuclease-free water	X µl
Total RNA (<10 µg)	Y µl
10 x DNase I Buffer [*e.g.* 100 mM Tris-Cl, 20 mM $MgCl_2$(pH 7.5)]	1 µl
RNase-free DNase I (10 U/µl)	0.5 µl
Total volume	10 µl

(2) Incubate on ice for 10-30 min.

(3) Purify the treated RNA according to the following step.

DNase I-treated RNA
↓ ← Add nuclease-free water (adjust volume to 100 µl)
↓ ← Add 100 µl TE-saturated phenol

Vortex
Keep on ice for 5 min
↓ Centrifuge at 12,000 rpm for 5 min

Supernatant
↓ ← Add 100 µl chloroform: isoamyl alcohol (24:1), Vortex
↓ Centrifuge at 12,000 rpm for 5 min at 4 °C

Supernatant
↓ ←Add 100 µl 5 M ammonium acetate + 200 µl isopropanol
+ [5 µl 2 mg/ml glycogen* (for coprecipitation) : optional]
Vortex
Incubate at - 20 °C for 30 min
↓ Centrifuge at 12,000 rpm for 10-15 min at 4 °C

Discard supernatant
Precipitate
↓ ←Add 1 ml 70% ethanol
↓ Centrifuge at 12,000 rpm for 5 min

Discard supernatant
Precipitate
↓ ←Dissolve in appropriate volume of nuclease-free water

RNA solution

*Molecular biology grade

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[8] Troubleshooting

Symptom	Cause	Solution
No amplification	Incorrect setting of detector mode 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 primer or probe concentration according to instructions (see [6]).
	Inappropriate design of primers or probes.	Confirm specificity and Tm of primers and probes.
	Inappropriate cycle conditions.	Confirm Tm of the primers and probes.
	Low purity or quality of samples	Check the purity or quality of sample DNA.
	Low concentration of $Mn(OAc)_2$.	Increase the Mn concentration to 3.5 mM.
Variation in detection	Failure or malfunction of device	Check the device
	Low quality of sample DNA.	Repurify DNA sample by phenol/chloroform extraction and ethanol precipitation, or other method.
	Inappropriate concentration of primers or probes.	Optimize primer or probe concentration according to the instructions (see [6]).
	Inappropriate design of primers or probes.	Confirm specificity and Tm of primers and probes.
	Inappropriate cycle conditions.	Confirm Tm of the primers and probes.
	Variation of dispensed volume	Increase the reaction volume
Signals in blank reactions	Contamination ofamplicons or sample DNAs.	Use fresh PCR grade water. Re-make primer solution, probe solution, and master mix.

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[9] Related products

Product name	Package	Code No.
High efficient cDNA synthesis kit for real-time PCR **ReverTra Ace® qPCR RT Kit**	200 rxns	FSQ-101
High efficient cDNA synthesis master mix for real-time PCR **ReverTra Ace® qPCR RT Master Mix**	200 rxns	FSQ-201
High efficient cDNA synthesis master mix for real-time PCR with genomic DNA remover **ReverTra Ace® qPCR RT Master Mix with gDNA remover**	200 rxns	FSQ-301
High efficient revers transcriptaase **ReverTra Ace®**	10,000U	TRT-101
RNase inhibitor (Recombinant type)	2,500U	SIN-201
Realtime PCR master mix for probe assay **THUNDERBIRD® Probe qPCR Mix**	1mLx1 1.67mLx3	QPS-101T QPS-101
Realtime PCR master mix for SYBR® Green assay **THUNDERBIRD® SYBR qPCR Mix**	1mLx1 1.67mLx3	QPS-201T QPS-201
Realtime PCR master mix for probe assay **Realtime PCR Master Mix**	1mLx1 1mLx5	QPK-101T QPK-101
Realtime PCR master mix for SYBR® Green assay **SYBR® Green Realtime PCR Master Mix**	1mLx1 1mLx5	QPK-201T QPK-201
One-step realtime PCR master mix for SYBR® Green assay ***RNA-direct*™ SYBR® Green Realtime PCR Master Mix**	0.5mLx2 0.5mLx5	QRT-201T QRT-201

[10] References

1) Reverse transcription and DNA amplification by a *Thermus thermophilus* DNA polymerase. Myers T. W. and Gelfand D. H. , *Biochemistry*, 30: 7661-6 (1991)

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