ReverTra Ace® qPCR RT Master Mix with gDNA Remover

FSQ-301 200 reactions

Store at -20°C

Contents

CAUTION

All reagents in this kit are intended for research purposes. Do not use for diagnostic or clinical purposes. Please observe general laboratory safety precautions while using this kit.

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

[1] Introduction

Description

ReverTra Ace® qPCR RT Master Mix with gDNA remover is an efficient and convenient kit, consisting of master mix reagents, to synthesize high quality cDNAs for real-time PCR. The kit includes reagents for reverse transcription and for the removal of genomic DNA [DNase I treatment].

In many cases, total RNA prepared using spin-columns or acid guanidium-phenol-chloroform (AGPC) extraction methods contains small amount of genomic DNA. Any contaminating genomic DNA will be amplified along with cDNA, especially when primer pairs are designed within the same exon or from pseudogenes. Amplification from genomic DNA can result in qualitative and quantitative inaccuracies.

The protocol consists of i) a genomic DNA degradation step using “gDNA remover” and ii) a reverse transcription step. The two steps can be achieved sequentially without purification or heat inactivation of DNase I.

ReverTra Ace® is a mutant M-MLV reverse transcriptase that shows excellent efficiency.

Features

-“Genomic DNA degradation step” and “cDNA synthesis step” can be achieved sequentially in approximately 30 min.
-The master mix reagents will not freeze at -20°C.
- Control, no reverse transcription experiments (no RT-Control) can be performed with 5x RT Master Mix II no-RT control.
-The master mix reagent contains random and oligo dT primers optimized for efficient reverse transcription.
-The reverse transcription reaction can be completed in 15 min. The protocol does not contain an additional RNase H treatment step to remove residual RNA after reverse transcription (Patent Pending).
-Since the RT buffer is optimized for real-time PCR, the addition of 20% (v/v) of the synthesized cDNA solution to the PCR solution does not inhibit the PCR reaction. Therefore, this kit is suitable for the detection of low abundance mRNAs.

[2] Components

The kit includes the following reagents, which can be used for 200 (FSQ-301) and 40 (FSQ-301S) 10 µl reactions. All reagents should be stored at -20°C. For extended storage, -30°C is recommended.

	FSQ-301	FSQ-301S	(SAMPLE)
gDNA Remover	10 µl	4 µl	
4x RT Master Mix	440 µl	88 µl	
5x RT Master Mix II	400 µl	80 µl	
5x RT Maser Mix II no RT-Control	40 µl	8 µl	
Nuclease-free water	1000 µl x 2	400 µl	

gDNA remover

“gDNA remover” is an optimized DNase I solution. 4x DN Master Mix and gDNA remover should be mixed at a ratio of 50 : 1.

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4× DN Maser Mix

"4x DN Master Mix" is a buffer solution that contains RNase inhibitor. Prior to use, a 1 in 50 volume of gDNA remover should be added to 4x DN Master Mix (*e.g.* 4x DN Master Mix : gDNA remover = 440 µl : 8.8 µl or 4x DN Master Mix : gDNA remover = 88 µl : 1.8 µl).

Notes

4x DN Master Mix with gDNA remover can be stored at -20°C for at least 3 months. The mixture can be prepared in a smaller volume [*e.g.* 4x DN Master Mix: gDNA remover = 220 µl : 4.4 µl].

5× RT Maser Mix II

This reagent is a 5x master mix that contains highly efficient reverse transcriptase "ReverTra Ace®", RNase inhibitor, oligo dT primer, random primer and dNTPs.

Notes

Be aware that "5x RT Master Mix II" and "5x RT Master Mix" in ReverTra Ace® qPCR RT Master Mix (Code No. FSQ-201)" are not compatible.

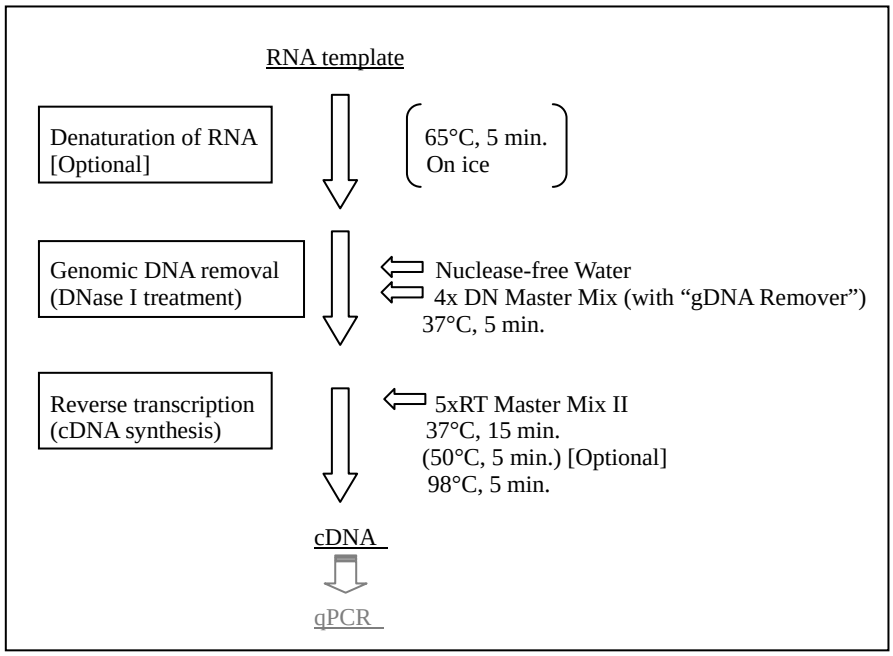
5× RT Maser Mix II no-RT Control

The composition of "5x RT Master Mix II no-RT Control" is identical to that of "5x RT Master Mix II" except that reverse transcriptase (RT) is omitted. This master mix can be used in a control experiment due to the absence of reverse transcriptase.

Nuclease-free water

This nuclease-free water has been prepared without DEPC treatment.

[3] Protocol



Flowchart of genomic DNA removal and cDNA synthesis

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1. Template RNA for reverse transcription

The following RNAs are appropriate for highly efficient reverse transcription.

(1)Total RNA
Total RNA usually contains 1-2% mRNA. Total RNA can be used directly as template with this kit.

(2)Poly(A)$^+$ RNA (mRNA)
Poly(A)$^+$ RNA is useful to detect low abundance mRNAs. However, poly(A)$^+$ RNA should be treated carefully because it is more sensitive to RNase than total RNA.

2. Reverse transcription

(1) Preparation of the "4x DN Master Mix" and "gDNA Remover" mixture.
Prior to use, a 1 in 50 volume of gDNA remover should be added to 4x DN Master Mix (*e.g.* 4x DN Master Mix : gDNA remover = 440 µl : 8.8 µl or 4x DN Master Mix : gDNA remover = 88 µl : 1.8 µl).

Notes
4x DN Master Mix with gDNA remover can be stored at -20°C for at least for 3 months. The mixture can be prepared in a smaller volume [*e.g.* 4x DN Master Mix : gDNA remover = 220 µl : 4.4 µl].

(2) Denaturation of RNA [optional]
Incubate the RNA solution at 65°C for 5 min, and then keep on ice.

Notes
- This step increases the efficiency of reverse transcription of RNA templates that form secondary structures.
-This step should be performed before adding 4x DN Master Mix.

(3) Preparation of the DNase I reaction solution:

Prepare the following reagents on ice.

4x DN Master Mix	2 µl
RNA template	0.5 pg – 0.5µg
Nuclease-free Water	X µl
Total Volume	8 µl

(4) Incubate at 37°C for 5 min.

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(5) Preparation of the for reverse transcription solution;

Prepare the following reagents on ice.

Reacted solution from (4)	8 µl
5x RT Master Mix II	2 µl
Total Volume	10 µl

Notes

-The master mix reagent contains oligo dT and random primers. Do not use with specific primers.

-For control experiments, "5x RT Master Mix II no RT-Control" should be used instead of 5x RT Master Mix II. A control experiment without reverse transcription is useful to prove whether amplicons originate from cDNA and/or genomic DNA.

-This kit contains nuclease-free water for 200 reverse transcription reactions. The kit does not contain sufficient nuclease-free water for the dilution of RNA samples. Nuclease-free water prepared without DEPC-treatment is recommended for the dilution of RNA samples.

-The reaction volume can be increased according to need.

(6) Incubate at 37°C for 15 min

(7) Incubate at 50°C for 5 min [optional]

(8) Heat at 98°C for 5 min

(9) Store the reacted solution* at 4°C or – 20°C

*This solution can be used directly or after dilution for real-time PCR.

Notes

-"ReverTra Ace®" excels at high reaction temperatures (up to 50°C). This step may increase the efficiency of the reverse transcription.

-Up to 20% of the synthesized cDNA solution can be added to the PCR reaction solution.

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[4] Application data

(1) Efficiency of genomic DNA removal.

<Materials and Methods>

cDNA synthesis

Reagent: ReverTra Ace® qPCR RT Master Mix with gDNA remover (Code No.FSQ-301)

Template: HeLa total RNA 0.5 µg /10 µl reaction

Experiment conditions: The experiments were preformed with the following conditions.

	4x DN Master Mix [DNase I treatment]	5x RT Master Mix [Reverse transcription]
A	gDNA Remover (-)*	RTase (-)**
B	gDNA Remover (-)*	RTase (+)
C	gDNA Remover (+)	RTase (-)**
D	gDNA Remover (+)	RTase (+)

* 4xDN Master Mix without gDNA Remover

**5x RT Master Mix II no-RT Control

Real-time PCR

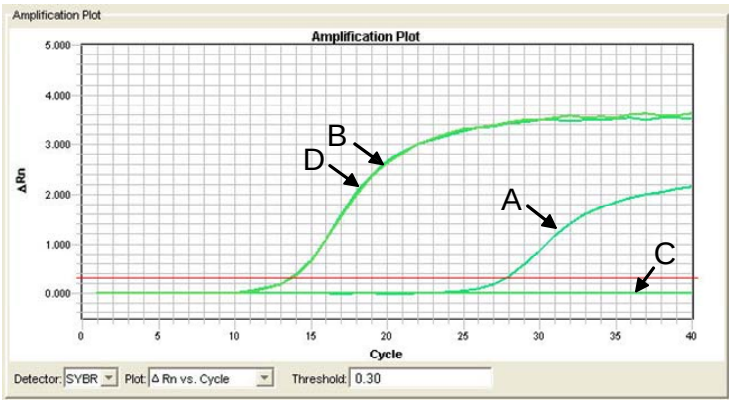
Reagent: THUNDERBIRD® SYBR® qPCR Mix (Code No.QPS-201)

Template: cDNA 2 µl/20 µl reaction (cDNA solution: 10%)

Target: β-actin (188 bp)

Real-time cycler: Applied Biosystems 7900HT

<Results>



No signal for the “C experiment” indicates that the contaminating genomic DNA in the RNA template was completely removed by “gDNA remover”.

(2) Comparison of cDNA yields

<Materials and Methods>

cDNA synthesis

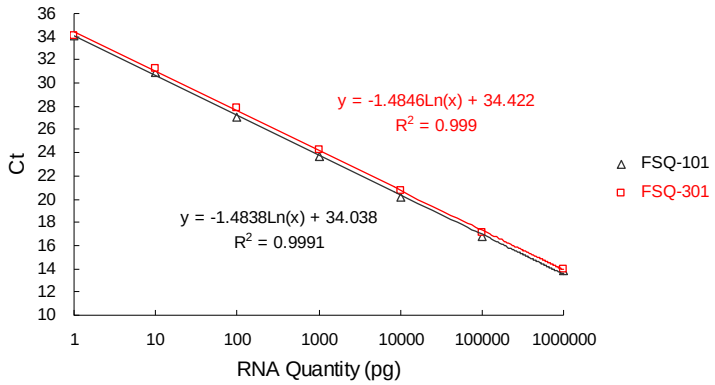
Reagents: -ReverTra Ace® qPCR RT Master Mix with gDNA remover (Code No.FSQ-301)
-ReverTra Ace® qPCR RT Kit* (Code No. FSQ-101)
*Previous version of the kit without gDNA remover.

Template: HeLa total RNA 1 pg, 10 pg, 100 pg, 1 ng, 10 ng, 100 ng, 1 µg /20 µl reaction**
**Genomic DNA (100 ng) was added to the experiments using FSQ-301.

Real-time PCR

Reagent: THUNDERBIRD® SYBR® qPCR Mix (Code No.QPS-201)
Template: cDNA 2 µl/20 µl reaction (cDNA solution: 10%)
Target: GAPDH (65 bp)
Real-time cycler: Applied Biosystems 7900HT

<Results>



Despite the genomic DNA contamination, the results of ReverTra Ace® qPCR RT Master Mix with gDNA remover (Code No.FSQ-301) correlate highly with those of the ReverTra Ace® qPCR RT Kit* (Code No. FSQ-101). Both reagents showed highly linear standard curves in a broad concentration range.

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[5] Troubleshooting

Symptom	Cause	Solution
Low signal after real-time PCR	Low purity of RNA	Repurify the RNA sample.
	Degradation of RNA	Prepare fresh RNA sample. Diluted RNA templates have a tendency to degrade and to adsorb on the vessel walls. RNA template for the reaction should be prepared from a highly concentrated stock prior to use.
	Excess or small amount of RNA	The recommended RNA concentration range for reverse transcription is from 1 pg to 1 μg in a 10 μl reaction. However, the optimal concentration of RNA template should be determined for each case.
	Secondary structure of RNA template	The efficiency of reverse transcription of RNAs that form secondary structures tends to be low. Incubation at 65°C for 5 min and quenching prior to the reaction is usually effective on such templates. Also, the additional step of 50°C for 5 min after the reaction at 37°C for 15 min might be effective for such difficult templates.
	Inappropriate temperature conditions	Perform the reaction according to this instruction manual.
	Excess amount of cDNA solution compared to the total PCR reaction volume	Reduce the cDNA solution to less than 10%.
Amplification in no-RT control reaction	Contamination of an excess amount of genomic DNA in RNA template	Repurify the RNA template. Contaminating genomic DNA of up to approximately 50 ng (per 10 μl reaction) can be treated. An excess amount of genomic DNA in the RNA template can result in incomplete degradation
	Primer dimer formation	Optimize the PCR conditions or redesign the primers. HPLC-grade primers sometimes improve PCR specificity.

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[6] Related products

Product name	Package	Code No.
High efficient revers transcriptaase **ReverTra Ace®**	10,000 U	TRT-101
RNase inhibitor (Recombinant type)	2,500 U	SIN-201
Real-time PCR master mix for probe assay **THUNDERBIRD® Probe qPCR Mix**	1.67 mL x 3	QPS-101
Real-time PCR master mix for SYBR® Green assay **THUNDERBIRD® SYBR® qPCR Mix**	1.67 mL x 3	QPS-201
High efficient cDNA synthesis kit for Real-time PCR **ReverTra Ace® qPCR RT Kit**	200 reactions	FSQ-101
High efficient cDNA synthesis master mix Real-time PCR **ReverTra Ace® qPCR RT Master Mix**	200 reactions	FSQ-201

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