ReverTra Ace® qPCR RT Kit

FSQ-101 200 reactions

Store at -20°C

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

The ReverTra Ace® qPCR RT Kit is an efficient and convenient kit to synthesize high quality cDNAs for real-time PCR. This kit contains the highly efficient reverse transcriptase "ReverTra Ace®" and a RT buffer optimized for the highly efficient synthesis of short-chain cDNAs suitable for real-time PCR. The protocol is simple, and the reaction can be completed in 15 min.

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

Features

-The optimized RT buffer and Primer Mix enable the highly efficient reverse transcription from RNA to cDNA without additional requirements.

-The 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-expressing mRNAs.

[2] Components

The kit includes the following reagents which can be used for 200 applications of 10 µl reactions. All reagents should be stored at -20 °C.

5 x RT Buffer	400 µl
Enzyme Mix	100 µl
Primer Mix	100 µl
Nuclease-free water	1000 µl x 2

5 × RT Buffer

This buffer contains $MgCl_2$ and dNTPs.

Enzyme Mix

This solution contains the highly efficient reverse transcriptase "ReverTra Ace®" and an RNase inhibitor.

Primer Mix

This solution contains random and Oligo (dT) primers. The primer ratio has been optimized for the efficient reverse transcription of various RNA targets.

Nuclease-free water

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

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[3] Protocol

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 as template directly with this kit. RNA prepared by AGPC (Acid Guanidium-Phenol-Chloroform) or the column method contains genomic DNA, so total RNA should be treated with DNase I before transcription.

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

Poly (A)$^+$ RNA is useful 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.

2. Reverse transcription

(1)Denaturation of RNA

Incubate the RNA solution at 65 °C for 5 min, and keep on ice afterwards.

Notes

-This step is important to increase the efficiency of the reverse transcriptase reaction.
-Do not add the other components at this step.

(2)Preparation of the reaction solution

Prepare the following reagents.

Nuclease-free water	X µl
5 x RT Buffer	2 µl
RT Enzyme Mix	0.5 µl
Primer Mix	0.5 µl
RNA	0.5 pg – 1µg
Total Volume	10 µl

Notes

-The Primer Mix can be replaced by gene-specific primers. Gene-specific primers can be used at a final concentration of 0.5 pmol/µl.

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

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(3) Incubate at 37 °C for 15 min

(4) Heat at 98 °C for 5 min

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

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

Notes

-The reaction time at 37 °C can be prolonged up to 1 hr.

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

-Reverse transcription with gene-specific primers tends to lead to non-specific PCR amplification. Maintaining the reaction temperature at 42-50 °C helps reduce non-specific amplification.

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[4] 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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[5] Troubleshooting

Symptom	Cause	Solution
Low signal at real-time PCR	Low purity of RNA	Repurify the RNA sample.
	Degradation of RNA	Prepare the RNA sample again.
	Excess or small amounts of RNA	Optimal range of the RNA template for reverse transcription is from 0.5 pg to 1 μg of RNA.
	Inappropriate temperature conditions	Perform the reaction according to this instruction manual.
	Excess amount of cDNA solution compared to the total PCR reaction volume	The maximum volume ratio of the cDNA solution to the PCR solution is 20% (v/v). Reduce the volume of the cDNA solution.
Non-specific signal at real-time PCR	Non-specific annealing of gene specific primers	Change the reaction temperature from 37 °C to 42 -50 °C.

[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 **Realtime PCR Master Mix**	1 mL x 1 1 mL x 5	QPK-101T QPK-101
Real-time PCR master mix for SYBR® Green assay **SYBR® Green Realtime PCR Master Mix**	1 mL x 1 1 mL x 5	QPK-201T QPK-201
Real-time PCR master mix for SYBR® Green assay (improved version) **SYBR® Green Realtime PCR Master Mix –Plus-**	1 mL x 1 1 mL x 5	QPK-212T QPK-212
One-step real-time PCR master mix for probe assay ***RNA-direct*™ Realtime PCR Master Mix**	0.5 mL x 2 0.5 mL x 5	QRT-101T QRT-101
One-step real-time PCR master mix for SYBR® Green assay ***RNA-direct*™ Realtime PCR Master Mix**	0.5 mL x 2 0.5 mL x 5	QRT-201T QRT-201

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