ReverTra Ace™ qPCR RT Master Mix

FSQ-201 200 reactions

Store at -20°C

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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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[1] Introduction

Description

ReverTra Ace^{TM} qPCR RT Master Mix is an efficient and convenient reagent to synthesize high quality cDNAs for real-time PCR. The master mix reagent (5x) contains the highly efficient reverse transcriptase "ReverTra Ace^{TM}", primers and buffer optimized for 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^{TM} is a mutant M-MLV reverse transcriptase that shows excellent efficiency.

Features

- 5x Master Mix reagent contains all components for reverse transcription. The Master Mix reagent will not freeze at -20°C.
- No reverse transcription control experiments (no RT-Control) can be performed.
- The master mix reagent contains random and oligo dT primers optimized for efficient reverse transcription.
- 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 abundance mRNAs.

[2] Components

The kit includes the following reagents, which can be used for 200 (FSQ-201) and 40 (FSQ-201S) 10 µl reactions. All reagents should be stored at -20°C. For extended storage, -30°C is recommended.

	FSQ-201	FSQ-201S (SAMPLE)
5x RT Master Mix	400 µL	80 µL
5x RT Maser Mix no RT-Control	40 µL	8 µL
Nuclease-free water	1000 µL x 2	400 µL

5× RT Maser Mix

This reagent is a 5x master mix that contains highly efficient reverse transcriptase "ReverTra Ace^{TM}", RNase inhibitor, oligo dT primer, random primer, $MgCl_2$ and dNTPs .

Notes

Be aware that "5x RT Master Mix" and "5x RT Master Mix II" in ReverTra Ace^{TM} qPCR RT Master Mix with gDNA remover (Code No. FSQ-301) are not compatible.

5× RT Maser Mix no-RT Control

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

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 directly as template with this kit. RNA prepared using acid guanidium-phenol-chloroform (AGPC) or the spin-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 abundance mRNAs. However, poly(A)$^+$ RNA should be treated carefully because it is more sensitive to RNase than total RNA.

2. Reverse transcription

(1) 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 5x RT Master Mix.

(2) Preparation of the reaction solution

Prepare the following reagents on ice.

5x RT Master Mix	2 μL
RNA template	1 pg – 1 μg
Nuclease-free Water	X 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 no RT-Control” should be used instead of 5x RT Master Mix. A control experiment without reverse transcription is useful to prove whether amplicons originate from cDNA and/or genomic DNA.

-The reaction volume can be increased according to need.

-Master mix reagents should be spun-down prior to use due to high viscosity.

-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.

(3) Incubate at 37°C for 15 min.

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

(5) Heat to 98°C for 5 min.

(6) 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.

-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

<Materials and Methods>

cDNA synthesis

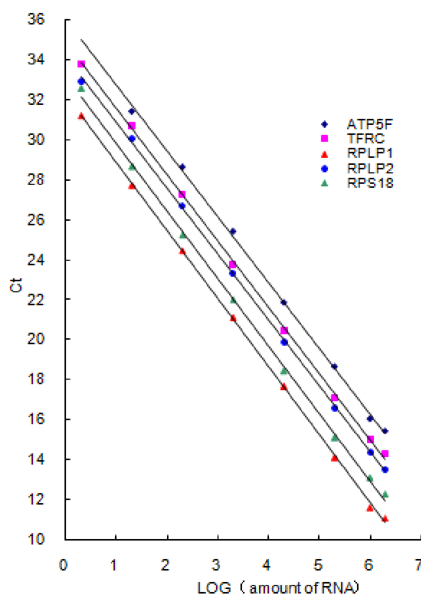
Reagent: ReverTra Ace™ qPCR RT Master Mix (Code No.FSQ-201)
Template: HeLa total RNA 2 pg-2 μg /20 μL reaction

Real-time PCR

Reagent: THUNDERBIRD™ SYBR® qPCR Mix (Code No.QPS-201)
Template: cDNA 2 μL /20 μL reaction (cDNA solution: 10%)
Targets: Typical house-keeping genes
Real-time cycler: Applied Biosystems 7900HT

<Results>

Template RNA (pg)	Log (RNA amount)	Ct of qPCR				
		ATP5F	TFRC	RPLP1	RPLP2	RPS18
2	0.301		33.76	31.16	32.89	32.54
20	1.301	31.43	30.73	27.70	30.05	28.65
200	2.301	28.64	27.29	24.44	26.72	25.22
2,000	3.301	25.41	23.79	21.12	23.31	21.98
20,000	4.301	21.86	20.43	17.69	19.88	18.42
200,000	5.301	18.65	17.09	14.14	16.59	15.10
1,000,000	6.000	16.03	15.03	11.63	14.37	13.09
2,000,000	6.301	15.42	14.28	11.11	13.53	12.28
/20µl	Slope	-3.280	-3.303	-3.384	-3.284	-3.368
	R2	0.999	0.999	1.000	1.000	0.999
	Eff.	101.8%	100.8%	97.5%	101.6%	98.1%



High linearity and no crossing over of the standard curves of five housekeeping genes suggest that the reagent shows high performance in a broad concentration range.

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[5] 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

[6] 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 genomic DNA in RNA template	Redesign the primers to prevent amplification from genomic DNA. Or treat the template RNA with DNase I prior to reverse transcription.
	Primer dimer formation	Optimize the PCR conditions or redesign the primers. HPLC-grade primers sometimes improve PCR specificity.

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[7] 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 for Real-time PCR with gDNA remover **ReverTra Ace™ qPCR RT Master Mix with gDNA remover**	200 reactions	FSQ-301

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