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Instruction manual ReverTra Ace® -α- 0810 F0937K

ReverTra Ace -α- ®

FSK-101 100 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.

JAPAN
TOYOBO CO., LTD.
Tel(81)-6-6348-3888
www.toyobo.co.jp/e/bio
tech_osaka@toyobo.jp

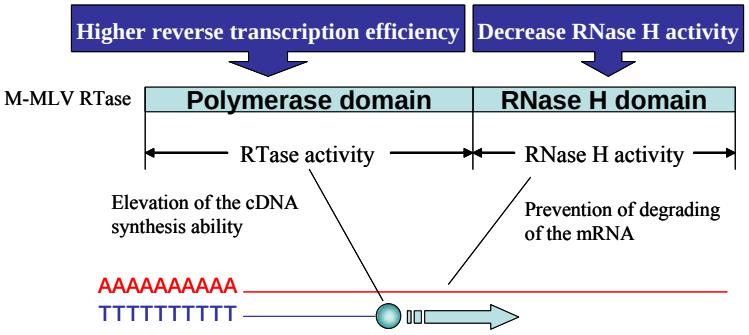
CHINA
TOYOBO Bio-Technology, CO., LTD.
Tel(86)-21-58794900.4140

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

[1] Introduction

Description

ReverTra Ace-α-® is an efficient and convenient kit to synthesize high quality cDNA. This kit contains the highly efficient reverse transcriptase "ReverTra Ace®", as well as other components optimized for the synthesis of long cDNAs suitable for RT-PCR. ReverTra Ace® is an M-MLV reverse transcriptase that has been improved by point mutation technology. ReverTra Ace® has two mutations in the polymerase and RNase H domains.



Features

-This kit contains all components for reverse transcription.
-This kit enables the synthesis of ≧ 14 kb cDNA.

[2] Components

The kit includes the following reagents which can be used for 100 reactions. All reagents should be stored at -20 °C.

ReverTra Ace®	100 µl
5xRT buffer (contains 25 mM Mg^{2+})	400 µl
RNase inhibitor (10 U/µl)	100 µl
dNTPs mixture (10 mM)	200 µl
RNase-free H_2O	1200 µl
Oligo (dT)20 (10 pmol/µl)	100 µl
Random primers (25 pmol/µl)	100 µl
Control Primer F (10 pmol/µl)	50 µl
Control Primer R (10 pmol/µl)	50 µl
Positive control RNA (10^5 copies/µl)	50 µl

Sequence of primers

-Oligo (dT)20: 5'-$(dT)_{20}$-3'
-Random Primer: 5'-$(dN)_9$-3'
-Control Primer F (G3PDH): 5'-ACCACAGTCCATGCCATCAC-3' (20mer)
-Control Primer R (G3PDH): 5'-TCCACCACCCTGTTGCTGTA-3' (20mer)

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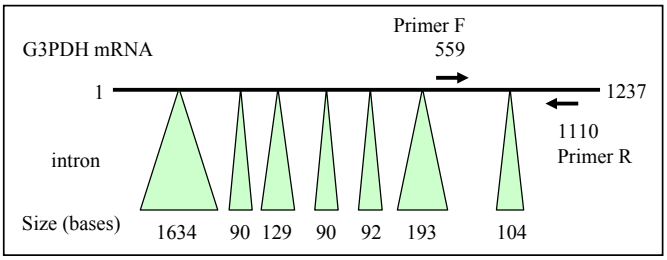
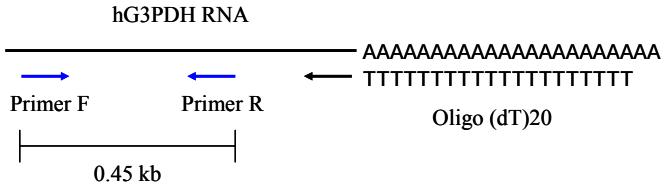
Control Primer F and R

These primers have been designed from exons 7 and 8 of the Glyceraldehyde-3 -Phosphate Dehydrogenase (G3PDH or GAPDH) gene. The predicted size of the PCR product from cDNA is approximately 450 bp.
G3PDH is a housekeeping gene expressed in mammalian tissues, and the expression level of G3PDH mRNA is similar among almost all tissues. G3PDH expression is not affected by some inducers, such as cytokines and phorbol esters. Therefore, G3PDH mRNA can be used as an internal control in most tissues, such as human, mouse, rat, pig, etc.



Positive Control RNA

The positive control RNA has been prepared by an *in vitro* transcription method, using a linearized plasmid bearing the human G3PDH gene. The transcript has a 22-mer poly(A)$^+$ tail. An approximately 450-bp PCR product is produced from this RNA with the control primers F and R. The concentration of the positive control RNA has been adjusted to 10^5 copies/µl.



[3] RT Primers

The following primers can be used for reverse transcription:

1. Oligo (dT) <Oligo (dT)20 primer is supplied by this kit>
   This primer can be applied only to poly (A)$^+$ RNA.

2. Random Primer <Random primer (9 mer) is supplied by this kit >
   This primer can be applied to various types of RNAs (e.g., total RNA, poly (A)$^+$ RNA, tRNA, rRNA, and viral RNA). Because of the low Tm of this primer, the reverse transcription reaction should include a pre-incubation step (30°C for 10 min) to allow for sufficient annealing to the template RNA.

3. Gene specific primer
   Primers complimentary for mRNA can be used.

[4] 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 and can be used directly as the template 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 prior to transcription.

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

Poly (A)$^+$ RNA is useful for detecting 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) Preparation of the reaction solution

Component	Volume	Final concentration
RNase-free H_2O	(11-X) µl	-
5 x RT buffer	4 µl	1x
dNTP mixture (10 mM each)	2 µl	1 mM
RNase inhibitor (10 U/µl)	1 µl	0.5 U/µl
Primer	1 µl	
Random primer (25 pmol/µl)		1.25 pmol/µl
Oligo (dT)20 (10 pmol/µl)		0.5 pmol/µl
Specific primer (10 pmol/µl)		0.5 pmol/µl
RNA	X µl	
Total RNA		≤ 1µg
mRNA [Poly (A)$^+$ RNA]		10-100 ng
Positive Control RNA		10^5 copies/µl (X=1 µl)
ReverTra Ace®	1 µl	
Total Volume	20 µl	

(2) (Incubate at 30 °C for 10 min.) [In case of Random Primer only]

(3) Incubate at 42 °C for 20 min.

(4) Heat at 99 °C for 5 min.

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

Notes

-The heating step is necessary for dissociation of the DNA/RNA complex and increased PCR efficiency

-This kit contains RNase-free H_2O for 100 reverse transcription reactions, but not for the dilution of RNA samples. An RNase-free H_2O, prepared without DEPC-treatment, is recommended for dilution of RNA samples.

[5] Applicaions for PCR

The synthesized cDNA produced with this kit can be used as a template for efficient PCR amplification. cDNA synthesis of ≥ 14 kb-targets have been confirmed.

It is known that residual RNA in cDNA or genomic DNA solutions inhibits amplification through Mg^{2+} chelation. Therefore, PCR should be performed using a template DNA containing the following amounts of RNA, depending on each PCR reagent:

- KOD -Plus- (Code. KOD-201) : ≤ 100 ng RNA/50µl PCR reaction
- KOD FX (Code. KFX-101) : ≤ 200 ng RNA/50µl PCR reaction
- Blend Taq (Code. BTQ-101) : ≤ 200 ng RNA/50µl PCR reaction
- Blend Taq -Plus-(Code. BTQ-201) : ≤ 200 ng RNA/50µl PCR reaction
- KOD Dash (Code. LDP-101) : ≤ 1 µg RNA/50µl PCR reaction

PCR fidelity tends to decrease with excess amounts of Mg^{2+} or dNTPs; therefore, the reacted kit solution (≤ 2 µl) should be applied to the PCR solution for amplification with high fidelity enzymes, such as KOD -Plus- or KOD FX.

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[6] 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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[7] Troubleshooting

Symptom	Cause	Solution
Low efficiency	Low purity of RNA	Re-purify the RNA sample.
	RNA degradation	Prepare the RNA sample again.
	Insufficient reaction	Prolong the reaction time up to 1 hr.
	Excess or small amounts of RNA	Optimal range of RNA template for reverse transcription is ≤ 1 μg of RNA.
	Secondary structure of RNA	-Use random primer. -Incubate RNA solution at 65°C for 5 min, followed by incubation on ice prior to reaction.
	Inappropriate temperature conditions	Perform the reaction according to this instruction manual.
Low specificity	Non-specific annealing of gene-specific primers	Change the reaction temperature from 42°C to 50 °C.

[8]Related products

Product name	Package	Code No.
dNTPs mixture (10mM)	2 μmoles / 0.2ml	NTP-301
High efficient revers transcriptaase **ReverTra Ace®**	10,000 U	TRT-101
RNase inhibitor (Recombinant type)	2,500 U	SIN-201
High efficient cDNA synthesis kit for real-time PCR **ReverTra Ace® qPCR RT Kit**	200 reactions	FSQ-101
High fidelity PCR enzyme (Hot Start) **KOD -Plus-**	200 U	KOD-201
High reliable PCR enzyme (Hot Start) **KOD FX**	200 U	KFX-101
High efficient DNA polymerase **KOD Dash**	250 U	LDP-101
High efficient Taq DNA polymerase **Blend Taq**	200 U	BTQ-101
High efficient Taq DNA polymerase (Hot Start) **Blend Taq -Plus-**	200 U	BTQ-201

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