cDNA Synthesis and RT-PCR

ExcelRT™ Reverse Transcriptase

ExcelRT™ Reverse Transcription Kit

ExcelRT™ One-Step RT- PCR Kit

ExcelRT™ One-Step RT- qPCR Kit

RNAok™ RNase Inhibitor

RP1000 (200 U/µl, 20,000 U)
RP1300 (100 Rxn)
RP1400 (100 Rxn)

Description

The ExcelRT™ Reverse Transcriptase is a recombinant Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase – an RNA dependent DNA polymerase capable of generating first strand cDNA using an RNA template. It is designed to reduce RNase H activity and create better thermal stability. The ExcelRT™ Reverse Transcriptase is able to routinely synthesize first strand cDNA > 8 kb at 37~50°C.

Additional Kit Format

The ExcelRT™ Reverse Transcription Kits contain all components to synthesize high quality first strand cDNA. The kits contain ExcelRT™ Reverse Transcriptase, RNAok™ RNase Inhibitor, oligo (dT)$_{20}$ and random hexamers, which are used to synthesize cDNA from poly(A) tailed mRNA and total RNA, respectively. The RP1400 ExcelRT™ Reverse Transcription Kit II is supplied with Oligo (dT)/Random Primer Mix that is optimal for highly efficient synthesis of short chain cDNA suitable for real-time PCR.

Features

• High yield
• Thermostable, up to 50°C, during first strand synthesis
• High processivity, generating cDNA up to 8 kb
• Reduced RNase H ribonuclease activity
• No detectable 3'→ 5' exonucleolytic proofreading function
• Thermal stable for at least 4 weeks when stored at 4°C
• Suitable for real-time PCR
• Contains all components for reverse transcription (RP1300 and RP1400)
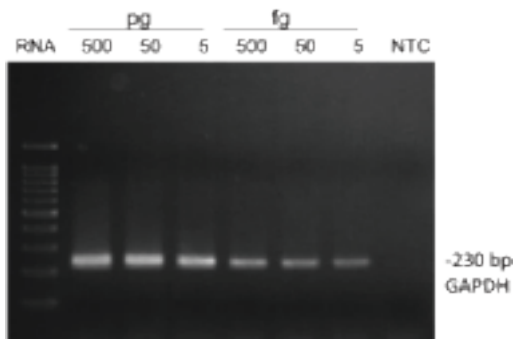• Time saving for short chain cDNA synthesis (RP1400)

Fig. 1. ExcelRT™ Reverse Transcriptase can synthesize 1st strand cDNA using only 5 fg of total RNA in solution. The bands in the gel represent the results of GAPDH (230 bp) from two step RT-PCR reaction. (M: DM2100)

Contents

RP1000
ExcelRT™ Reverse Transcriptase

Component	Volume
ExcelRT™ Reverse Transcriptase (200 U/µl)	100 µl
5X RT Buffer	1 ml
0.1 M DTT	500 µl

RP1300
ExcelRT™ Reverse Transcription Kit

Component	Volume
Reverse Transcriptase (200 U/µl)	100 µl
RNase Inhibitor (20 U/µl)	100 µl
5X RT Buffer (DTT)	500 µl
dNTPs (10 mM each)	200 µl
Oligo (dT)$_{20}$ (50 µM)	100 µl
Random Hexamers (100 µM)	100 µl
DEPC-Treated H$_2$O	2 x 1 ml

RP1400
ExcelRT™ Reverse Transcription Kit II

Component	Volume
RTase/RI Enzyme Mix	100 µl
5X RT Buffer (DTT/dNTPs)	500 µl
Oligo (dT)/Random Primer Mix	100 µl
DEPC-Treated H$_2$O	2 x 1 ml

Unit definition

One unit is defined as the amount of enzyme that will incorporate 1 nM of dTTP into acid-insoluble material in 10 minutes at 37°C using Poly (A) • oligo(dT)$_{25}$ as a template-primer.

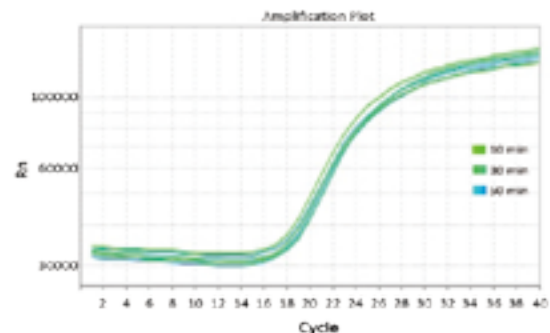### Storage

-20°C   for 24 months

Fig. 2. cDNA synthesized in 10 minutes is suitable for real-time PCR analysis. cDNA was synthesized in 10, 30, and 50 min from reverse transcription reaction. The amplification plot represents the results of 18S rRNA analyzed by using the ExcelTaq™ 2X Q-PCR Master Mix (Cat. No. TQ1100) for real-time PCR amplification.

RT **PCR** **HTP**

RP1100 (50 Rxn)

Description

The ExcelRT™ One-Step RT-PCR Kit is designed for the reverse transcription and PCR amplification of a specific target RNA from either total RNA or mRNA. The ExcelRT™ One-Step RT-PCR Kit provides the user an alternative to the lengthy two step process (first strand generation and amplification) by using a single mixture, single tube, one step reaction. The ExcelRT™ One-Step RT-PCR Kit contains a 2X reaction premix consisting of an optimized buffer, dNTPs, Mg^{2+} and enzyme stabilizer, and a blend of recombinant reverse transcriptase and *Taq* DNA polymerase. The ExcelRT™ One-Step RT-PCR Kit allows the user to complete the RT-PCR process using a thermocycler in a single reaction setting and is ideal for target RNA amplification/ analysis capable of detecting even trace amounts of target RNA.

Features

- High throughput
- High reproducibility, less pipetting errors
- High sensitivity and yields

RP1100
ExcelRT™ One-Step RT-PCR Kit

Contents

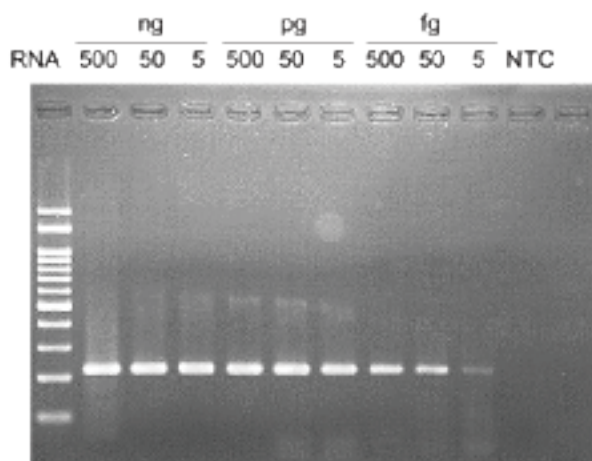| Component	Volume
2X One-Step Buffer	2 x 750 µl
Taq/RT Enzyme Mix	50 µl

Storage

-20°C   for 24 months

Fig. 1. ExcelRT™ One-Step RT- PCR Kit can synthesize PCR products with a minimal amount of total RNA in reaction solution. The bands in the gel represent the results of GAPDH (230 bp) from one-step RT-PCR reaction (M: DM2300)

ExcelRT™ One-Step RT-qPCR Kit (TaqMan, ROX)

RT Q-PCR TaqMan

RQ2110 (200 Rxn)

Description

The ExcelRT™ One-Step RT-qPCR kit (TaqMan, ROX) is designed for reverse transcription and quantitative real-time analysis of a specific target RNA by one-step reaction. The ExcelRT™ One-Step RT-qPCR kit (TaqMan, ROX), consisting of One-Step RT Enzyme Mix and 2X One-Step Master Mix, is a convenient kit designed for highly efficient cDNA synthesis and highly specific real-time PCR in a single tube. The One-Step RT Enzyme Mix contains a thermostable ExcelRT™ Reverse Transcriptase and a RNAok™ RNase inhibitor. Consequently, One-Step RT Enzyme Mix can reverse transcribe RNA to cDNA at a wide temperature range from 42 to 60°C and be active against RNase A, RNase B and RNase C. By containing specialized hot-start *Taq* DNA polymerase, which greatly reduce primer-dimer formation and can be activated within 2 minutes, the 2X One-Step Master Mix features high specificity and is suitable for fast cycle program. This master mix includes ROX reference dye for normalization of each RT-qPCR assay.

Features

- High yield
- Reverse transcription at wide temperature range (42-60°C)
- High specificity
- Suitable for fast cycle program
- ROX reference dye

Contents

RQ2110
ExcelRT™ One-Step RT-qPCR Kit (TaqMan, ROX)

Component	Volume
One-Step RT Enzyme Mix	400 µl
2X One-Step qPCR Master Mix	2 X 1 ml

Storage

Aliquot to avoid multiple freeze-thaw cycles
(stable within 30 freeze-thaw cycles)
Protect from light
-20°C   for 12 months

Fig. 2. ExcelRT™ One-Step RT-qPCR Kit can quantitatively analyze target RNA at a wide temperature range (42-60°C). The amplification plot of one-step RT-qPCR with reverse transcription at temperature range from 42 to 60°C, analyzed by using RQ2110 ExcelRT™ One-Step RT-qPCR Kit (A) or kit from brand N (B) for RT-qPCR amplification. The overlapped amplification curves display that ExcelRT™ One-Step RT-qPCR Kit preforms successfully cDNA synthesis at wide temperature range.
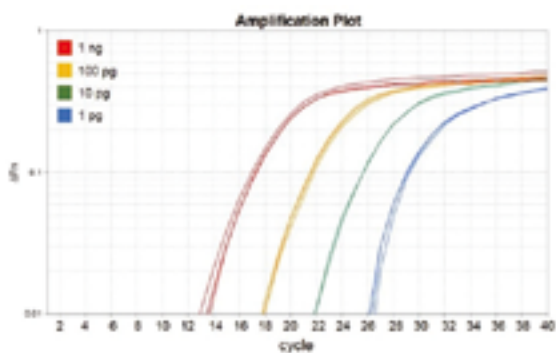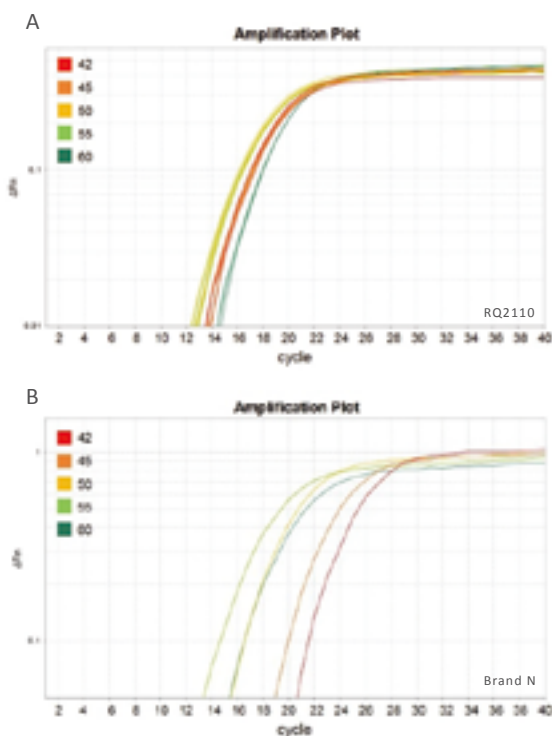
Fig. 1. ExcelRT™ One-Step RT-qPCR Kit can quantitatively analyze target RNA from a wide range of RNA template input. The amplification plot of one-step RT-qPCR with total RNA templates ranging from 1 pg to 1 ng in quantity, analyzed by using RQ2110 ExcelRT™ One-Step RT-qPCR Kit (TaqMan, ROX) for RT-qPCR amplification.

RNAok™ RNase Inhibitor

RI1000 (20 U/µl, 2000 U x 1)
RI1001 (20 U/µl, 2000 U x 5)

Description

RNAok™ RNase Inhibitor is a recombinant mammalian RNase inhibitor that is purified by affinity chromatography from *E. coli*. This protein inhibits pancreatic-type ribonucleases, RNase A, B, and C by binding strongly to RNases in a noncompetitive mode at a 1:1 ratio. RNAok™ RNase Inhibitor does not inhibit eukaryotic RNases T1, T2, U1, U2, CL3 as well as prokaryotic RNases I and H. RNAok™ RNase Inhibitor is compatible with RT-PCR enzymes such as AMV, M-MLV and ExcelRT™ Reverse Transcriptase or *Taq* DNA polymerase. Extensive quality control tests ensure RNAok™ RNase Inhibitor is free of unwanted contaminants that can plague other commercially available preparations of RNase inhibitors.
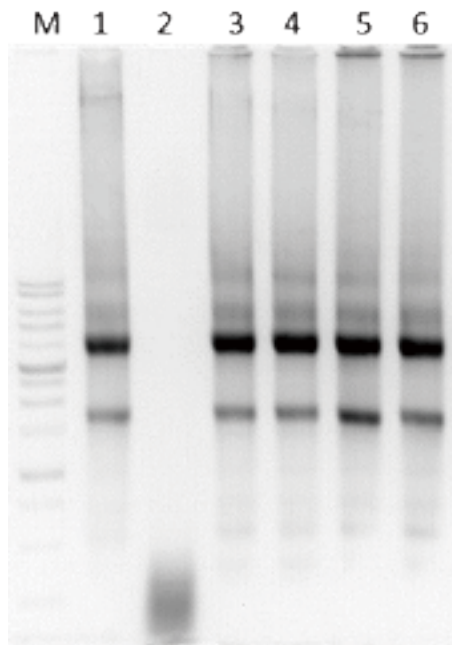
Application
• RT-PCR
• cDNA Synthesis
• *in vitro* transcription

Usage Recommendation
Add RNAok™ RNase Inhibitor to transcription, translation, and cDNA synthesis reactions at a final concentration of 1 Unit/µl.

Storage Buffer
20 mM HEPES-KOH (pH 7.6), 50 mM KCl, 8 mM DTT, stabilizer, 50% (v/v) glycerol

Unit Definition
One unit is defined as the amount of RNAok™ RNase Inhibitor required to inhibit the activity of 5 ng of RNase A by 50%.

Storage
-20°C   for 24 months

Fig. 1. RNAok™ RNase Inhibitor inhibits RNase A activity. Total HeLa RNA (1 µg) was incubated with 1U/ 1 µl of RNAok™ RNase Inhibitor and 0.4 ng RNase A for 30 min at 37°C. The result shows that RNAok™ RNase Inhibitor can protect RNA against 0.4 ng RNase A. M: DM3100, Lane 1: total HeLa RNA (1 µg), Lane 2: total HeLa RNA with RNase A (no inhibitor added), Lane 3: total HeLa RNA with RNase A and inhibitor from brand A, Lane 4-6: total HeLa RNA with RNase A and RNAok™ RNase Inhibitor.