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Small Bio, Smart Tool

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Product Information

ExcelRT™ series

Reverse Transcription Kit II

RP1400 100 RXN

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	1 ml x2

Storage

-20°C for 24 months

Description

ExcelRT™ Reverse Transcription Kit II is a complete, efficient and convenient kit to synthesize high quality first strand cDNA. This kit contains ExcelRT™ Reverse Transcriptase, which is able to synthesize the first strand cDNA at 37~50°C. The ExcelRT™ Reverse Transcriptase is a recombinant Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase, which is designed to reduce RNase H activity and create better thermal stability. This kit also contains RNAok™ RNase Inhibitor, which is active against RNase A, RNase B, and RNase C. This product is supplied with optimized RT Buffer and Oligo (dT)/Random Primer Mix for highly efficient synthesis of short chain cDNA suitable for real-time PCR.

Features

- Contains all components for reverse transcription
- High yield
- Thermostable, up to 50°C
- Reduced RNase H ribonuclease activity
- Suitable for real-time PCR

Application

- Generation of first strand cDNA from total RNA or mRNA.
- Suitable for generating cDNA from RNA with strong secondary structure which can be reduced at temperature up to 50°C.

RTase/RI Enzyme Mix

ExcelRT™ Reverse Transcriptase (200 U/μl), RNAok™ RNase Inhibitor (20 U/μl), 20 mM Tris-HCl (pH 7.5), 200 mM NaCl, 0.1 mM EDTA, 1 mM DTT, stabilizer, and 50% (v/v) glycerol

5X RT Buffer (DTT/dNTPs)

250 mM Tris-HCl (pH 8.3 at 25°C), 375 mM KCl, 15 mM MgCl₂, 50 mM DTT and 2.5 mM dNTPs (each)

Oligo (dT)/Random Primer Mix

50 μM oligo (dT)₂₀ and 100 μM random hexamers

First Strand cDNA Synthesis Condition

1. Denature (Mixture A):

Total RNA	X μ l (1 ng~2 μ g)
Oligo (dT)/Random Primer Mix	1 μ l
DEPC-Treated H ₂ O	to 10 μ l final vol.

Mix well; incubate at 70°C/5 minutes

Place on ice for at least 1 minute

2. First strand cDNA buffer (Mixture B) per reaction:

(Master Mix can be prepared before or during the denaturing step)

5X RT Buffer (DTT/dNTPs)	4 μ l
DEPC-Treated H ₂ O	5 μ l
RTase/RI Enzyme Mix	1 μ l

Final volume 10 μ l

3. First strand cDNA synthesis:

Mixture A (RNA + Primers)	10 μ l
Mixture B (First strand cDNA buffer)	10 μ l

Final volume 20 μ l

Incubate 25°C/10 minutes

37~50°C/50 minutes

4. Termination: 85°C/5 minutes

Store cDNA at -20°C or for immediate qPCR reaction

Recommended real-time PCR Condition

(SMOBIO's TQ1110 ExcelTaq™ 2X Q-PCR Master Mix (SYBR, ROX))

Recommended reaction mixture set up for real-time PCR

cDNA	2 µl*
Forward primer	50~400 nM**
Reverse primer	50~400 nM**
2X Q-PCR Master Mix	10 µl
H ₂ O	to 20 µl
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Total volume	20 µl

Recommended real-time PCR Program

Two-step cycle

Steps	Temp.	Time	Cycles
Template denature and enzyme activation	95°C	10 min [#]	1
Denature	95°C	15 sec	40
Annealing/Extension	60°C	60 sec	
Melting curve analysis	Refer to instrument manual		

* The recommended amount of cDNA is 100 fg -100 ng. The volume of cDNA should be less than 10% of the total qPCR reaction volume.

** The PCR primer concentration for an optimal qPCR reaction may vary according to the primers' properties and template conditions.

[#]10 minutes is suggested in the first step to thoroughly denature DNA and activate enzymes.

Recommended PCR Condition

(SMOBIO's TP1000 ExcelTaq™ *Taq* DNA polymerase)

cDNA	2~10 μ l
Forward primer	0.1 – 0.5 μ M
Reverse primer	0.1 – 0.5 μ M
10X <i>Taq</i> Buffer	5 μ l
dNTPs	0.2 mM each
<i>Taq</i> DNA polymerase	0.25 μ l (1.25 units)
H ₂ O	to 50 μ l
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Total volume	50 μ l

Recommended PCR Program

94°C	2 min	} 25 ~ 40 cycles
94°C	30 sec	
50~68°C**	30 sec	
72°C	30 sec/kb	
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72°C	1 min	

** Optimal PCR conditions vary according to primers' thermodynamic properties.

Other Information

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Caution: Not intended for human or animal diagnostic or therapeutic uses.

Related Products

TQ1110	ExcelTaq 2X Q-PCR Master Mix (SYBR, ROX), 200 RXN
TQ2110	ExcelTaq 2X Q-PCR Master Mix (TaqMan, ROX), 200 RXN
TF1000	SMO-HiFi DNA Polymerase, 100 U
TF3000	G-HiFi DNA Polymerase, 100 U
TP1000	ExcelTaq Taq DNA Polymerase, 500 U × 1
TP1200	ExcelTaq 5X PCR Master Dye Mix, 200 RXN
TP5000	ExcelTaq Hot Start II DNA Polymerase, 500 U
RI1000	RNAok RNase Inhibitor, 2000 U
RP1000	ExcelRT Reverse Transcriptase, 20,000 U
RP1100	ExcelRT One-step RT-PCR Kit, 50 RXN
RP1300	ExcelRT Reverse Transcription Kit, 100 RXN
CK1000	Champion E. coli Transformation Kit
DM2100	ExcelBand 100 bp DNA Ladder, 500 µl
NS1000	FluoroVue Nucleic Acid Gel Stain (10,000X), 500 µl

The latest version of the manual can be downloaded from www.smobio.com/shop.