



SMOBIO[®]

Small Bio, Smart Tool

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Product Information

ExcelRT™ series

Reverse Transcription Kit

RP1300 100 RXN

Reverse Transcriptase (200 U/μl)	100 μl
RNase Inhibitor (20 U/μl)	100 μl
5X RT Buffer (DTT)	500 μl
dNTPs Mix (10 mM each)	200 μl
Oligo (dT) ₂₀ (50 μM)	100 μl
Random Hexamers (100 μM)	100 μl
DEPC-Treated H ₂ O	1 ml x2

Storage

-20°C for 24 months

Description

ExcelRT™ Reverse Transcription Kit 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 oligo (dT)₂₀ and random hexamers, which are used to synthesize cDNA from poly(A) tailed mRNA and total RNA, respectively.

Features

- Contains all components for reverse transcription
- High yield
- Thermostable, up to 50°C, during first strand synthesis
- High processivity, generating cDNA up to 8 kb
- Reduced RNase H ribonuclease activity

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.

Storage Buffer

Reverse Transcriptase: 20 mM Tris-HCl (pH 7.5), 200 mM NaCl, 0.1 mM EDTA, 1 mM DTT, stabilizer and 50% (v/v) glycerol

RNase Inhibitor: 40 mM HEPES-KOH (pH 7.5), 100 mM KCl, 8 mM DTT, 0.1 mM EDTA, stabilizer and 50% (v/v) glycerol

5X RT Buffer (DTT)

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

First Strand cDNA Synthesis Condition

1. Denature (Mixture A):

Total RNA X μ l (1ng~2 μ g)

dNTP Mix (10 mM each) 1 μ l

Primers 50 μ M Oligo (dT)₂₀
or 100 μ M Random Hexamers
or 10 μ M Gene Specific Primers 1 μ l

DEPC-Treated H₂O 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) 4 μ l

DEPC-Treated H₂O 4 μ l

RNAok™ RNase Inhibitor 1 μ l

ExcelRT™ Reverse Transcriptase 1 μ l

Final volume 10 μ l

First Strand cDNA Synthesis Condition (continued)

3. First strand cDNA synthesis:

Mixture A (RNA + primers + dNTPs) 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
Keep at 4°C

5. RNA removal[#]: add 1 μ l RNase H into each reaction
37°C/20 minutes

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

* For random hexamers, an additional 10 minutes of incubation at 25°C is suggested.

[#] Optional step recommended for long range RT-PCR reaction.

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

CK1000	Champion E. coli Transformation Kit
RI1000	RNAok RNase Inhibitor, 2000 U
RP1000	ExcelRT Reverse Transcriptase, 20,000 U
RP1100	ExcelRT One-step RT-PCR Kit, 50 RXN
RP1400	ExcelRT Reverse Transcription Kit II, 100 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
TQ1110	ExcelTaq 2X Q-PCR Master Mix (SYBR, ROX), 200 RXN
TQ2110	ExcelTaq 2X Q-PCR Master Mix (TaqMan, ROX), 200 RXN
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.