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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
dNTP 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
TF2000	Q-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
TQ1100	ExcelTaq 2X Q-PCR Master Mix (SYBR, no ROX), 200 RXN
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
DM3100	ExcelBand 1 KB (0.25-10 kb) DNA Ladder, 500 µl
NS1000	FluoroVue Nucleic Acid Gel Stain (10,000X), 500 µl