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Product Information

ExcelRT™ series

Reverse Transcriptase

RP1000	**20,000 units**	
	Reverse Transcriptase	100 µl
	5X RT Buffer	1 ml
	0.1 M DTT	500 µl

Storage

-20°C for 24 months

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.

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

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

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

5X RT Buffer

250 mM Tris-HCl (pH 8.3 at 25°C), 375 mM KCl and 15 mM $MgCl_2$

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.

First Strand Synthesis Condition

1. Denature (Mixture A):

Total RNA	X µl (1 pg~2 µg)
Primers 100 µM $d(T)_{20}$	0.5 µl
or 10 µM specific primers	
or 100 ng/µl random hexamers	
dNTP*	1.0 mM (each)
DEPC-treated H_2O	to 10 µl final vol.

Mix well; incubate at 70°C/5 minutes
Place on ice for at least 1 minute

*High concentration of stock dNTP (10 mM, each) is recommended, allowing greater RNA volume be added in the event of low RNA yield.

2. First Strand DNA Buffer (Mixture B) per reaction:

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

5X RT reaction Buffer	4 µl
DEPC-treated H_2O	3 µl
0.1M DTT	1 µl
RNase inhibitor	1 µl
ExcelRT™ Reverse Transcriptase	1 µl
Final volume	10 µl

First Strand Synthesis Condition (continued)

3. First Strand cDNA synthesis:

Mixture A (RNA + Primers + dNTP)	10 µl
Mixture B (First Strand DNA Buffer)	10 µl
Final Volume	20 µl
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 µl 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

cDNA	2~10 µl
Forward primer	0.1 – 0.5 µM
Reverse primer	0.1 – 0.5 µM
10 × *Taq* buffer	5 µl
dNTPs	0.2 mM each
Taq DNA polymerase	0.25 µl (1.25 U)
H_2O	to 50 µl
Total volume	50 µl

Recommended PCR Program

94°C	2 min	
94°C	30 sec	25 ~ 40 cycles
50~68°C*	30 sec	
72°C	30 sec/kb	
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
CV1000	GetClone PCR Cloning Vector, 20 RXN
DM1100	ExcelBand 50 bp DNA Ladder, 500 µl
DM2100	ExcelBand 100 bp DNA Ladder, 500 µl
DM2300	ExcelBand 100 bp+3K DNA Ladder, 500 µl
DM3100	ExcelBand 1 KB (0.25-10 kb) DNA Ladder, 500 µl
DM3200	ExcelBand 1 KB Plus (0.1-10 kb) DNA Ladder, 500 µl
DM4100	ExcelBand XL 25 kb DNA Ladder, Broad Range (up to 25 kb), 500 µl
DL5000	FluoroDye DNA Fluorescent Loading Dye (Green, 6×), 1 ml
NS1000	FluoroVue Nucleic Acid Gel Stain (10,000X), 500 µl
TF1000	SMO-HiFi DNA Polymerase, 100 U
TP1000	ExcelTaq Taq DNA Polymerase, 500 U × 1
TP1200	ExcelTaq 5× PCR Master Dye Mix, 200 RXN
TP1260	ExcelTaq 5× Fluorescent PCR Master Mix, 200 RXN
TP2100	ExcelTaq Blood Direct PCR Master Mix Kit, 200 RXN
RP1100	ExcelRT One-step RT-PCR Kit, 50 RXN
RI1000	RNAok RNase Inhibitor, 2000 U