SMOBIO
Small Bio, Smart Tool

www.smobio.com

Product Information

ExcelRT Reverse Transcriptase

RP1000	**20,000 units**	
	ExcelRT Reverse Transcriptase	100 µl
	5X RT Buffer	1 ml

Storage

-20°C ≥ 24 months

Description

The ExcelRT Reverse Transcriptase is a recombinant Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase – a RNA dependent DNA polymerase capable of generating the first strand cDNA using RNA template. Designed to reduce RNase H activity and better thermal stability, the ExcelRT Reverse Transcriptase can synthesize routinely 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.
- Processivity up to 8 kb.

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), 50 mM DTT, 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 template-primer.

First Strand Synthesis Condition

1. Denature (Mixture A):

Total RNA	X µl (1pg~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)

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

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

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

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

5X RT reaction Buffer	4 µl
DEPC-treated H_2O	4 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, additional 10 minutes incubation at 25°C is suggested.
[#]Optional Step recommended for long range RT-PCR reaction.

Recommended PCR Condition

(SMOBiO's TP1000 ExcelTaq DNA Polymerase)

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 enzyme	0.25 µl (1.25units)
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 condition varies according to primers' thermodynamic properties.

Related Products

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
DS1000	FluoroStain DNA Fluorescent Staining Dye (Green, 10,000×), 500 µl
TF1000	ExcelTaq SMO-HiFi DNA Polymerase, 1 U/ µl, 100 U × 1
TP1000	ExcelTaq DNA Polymerase, 500 U × 1
TP1200	ExcelTaq 5× PCR Master Dye Mix, 200 RXN
TP1260	ExcelTaq 5× Fluorescent PCR Master Mix Kit, 200 RXN
TP2000	ExcelTaq Blood Direct DNA Polymerase, 5 U/ µl, 500 U × 1
TP2100	ExcelTaq Blood Direct PCR Master Mix Kit, 200 RXN

For Research use only
2013 ver. 1.2.0