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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_2O	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 $MgCl_2$, 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 µl (1 ng~2 µg)
Oligo (dT)/Random Primer Mix	1 µl
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 cDNA buffer (Mixture B) per reaction:**
(Master Mix can be prepared before or during the denaturing step)

5X RT Buffer (DTT/dNTPs)	4 µl
DEPC-Treated H_2O	5 µl
RTase/RI Enzyme Mix	1 µl
Final volume	10 µl

3. **First strand cDNA synthesis:**

Mixture A (RNA + Primers)	10 µl
Mixture B (First strand cDNA buffer)	10 µl
Final volume	20 µl
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_2O	to 20 µl
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 µl
Forward primer	0.1 – 0.5 µM
Reverse primer	0.1 – 0.5 µM
10X *Taq* Buffer	5 µl
dNTPs	0.2 mM each
Taq DNA polymerase	0.25 µl (1.25 units)
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

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
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
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
DM3100	ExcelBand 1 KB (0.25-10 kb) DNA Ladder, 500 µl
NS1000	FluoroVue Nucleic Acid Gel Stain (10,000X), 500 µl