ISO 9001/14001 Certified Company

ONE-STEP RT-PCR PreMix Kit

Cat. No. 25101 50 Rxns

DESCRIPTION

The ONE-STEP RT-PCR PreMix Kit is designed for easy, convenient and sensitive RT-PCR (cDNA synthesis and PCR) from RNA templates. Each tube of ONE-STEP RT-PCR PreMix Kit contains all components (required to synthesize your single str anded cDNA and its PCR reaction). Because the kit contains OptiScript™ RT Syst em, you can perform highly efficient and specific reverse transcription reaction. O ptiScript™ RT System designed for reverse transcription of any RNA quantity form 1pg to 2 μg . And the kit contains *i- StarTaq*™ DNA polymerase, you can perform hot-start PCR procedure, eliminate extension from nonspecifically annealed prime rs and primer-dimers in the first cycle ensuring highly specific and efficient PCR. The kit contains also stabilizing buffer, the activity of contained enzymes (re verse transcriptase, *Taq* DNA polymerase) maintained for long time.

STORAGE

Store at -20℃.

KIT CONTENTS

ONE-STEP RT-PCR PreMix 50 Rxn

Component in 20μl reaction
OptiScript™ RT System
RT-PCR buffer (10×)
dNTPs
i-StarTaq™ DNA polymerase
Stabilizing buffer

CHARACTERISTICS

- The kit contains all the reagents required for the synthesis of cDNA and its ampl ification, you can perform easily RT-PCR reaction.
- OptiScript™ RT System are included in the iNtRON's ONE-STEP RT-PCR PreMix K it and provide highly efficient and specific reverse transcription.
- *i-StarTaq*™ DNA polymerase included in the iNtRON's ONE-STEP RT-PCR PreMix Kit provides hot-start PCR for highly specific amplification.
- The kit also contains stabilizing buffer, the stability of contained enzymes maint ained for a long time.

PROTOCOL

1. Dispense 8μl of ONE-STEP RT-PCR PreMix Kit into PCR tubes.
2. Add RNA templates and gene specific primers into the upper PCR tubes. **Note :** Use the same amounts of gene specific primers as usual PCR reaction or two fol d reverse primer recommended.
3. Add distilled water into the tubes to a total volume of 20μl.
4. Mix the mixture thoroughly.
5. (Option) Add mineral oil.
   Note : This step is unnecessary when using a thermal cycler that employs a top heating method (general methods)
6. Perform RT-PCR reaction of samples as following process using PCR machine.

ONE CYCLE	
Reverse transcription reaction	45℃ / 30min
Denaturation of RNA : cDNA hybrid	94℃ / 5min
3-STEP CYCLING	
Denaturation	94℃ / 20-60sec.
Annealing	45-68℃ / 20-60sec.
Extension	72℃ / 1min/kb
Number of cycles : 25-40	
ONE CYCLE	
Final Extension	72℃ / 5min

REACTION COMPONENTS FOR RT-PCR

Components	Volume / reaction	Final concentration
ONE-STEP RT-PCR PreMix Kit	8.0μl / tube	–
Template RNA	Variable	
Forward primer	Variable	0.5pM
Reverse primer	Variable	0.5pM
RNase inhibitor(optional)	Variable	5-10 units/reaction
RNase-free water	Variable	
Total volume	20.0μl	

* Use the same amount of reverse primer or two fold reverse primer.

TECHNICAL INFORMATION

EXPERIMENTAL INFORMATION

The high sensitivity and specificity for ONE-STEP RT-PCR PreMix Kit is provided by OptiScript™ RT System and *i-StarTaq*™ DNA Polymerase. The OptiScript™ RT System is developed for all reverse transcription with very small amount of RNA. The *i-StarTaq*™ DNA Polymerase provides all the advantages of a hot start for PCR. Developed ONE-STEP RT-PCR PreMix Kit of agony sensitivity by above two elements and experiment result is as following.

• *Sensitivity and specificity*

< Element I : OptiScript™ RT System >

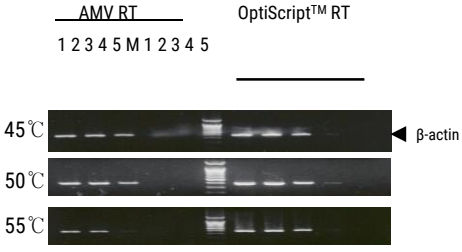

Fig. 1. RT-PCR amplification with ONE-STEP RT-PCR PreMix Kit
Total RNA was purified from human cancer cell lines(SNU 5) using easy-BLUE™ Total RNA Extraction Kit (Cat. No. 17061). And then, the first strand of cDNA was synthesized using the indicated RT reaction temperatures with AMV Reverse Transcriptase (Cat.No. 27021) and Optiscript™ RT System respectively. After diluting the cDNA mixture, the RT-PCR reaction was performed for the expression of β-Actin (400bp) gene.

< Element II : *i-StarTaq*™ DNA Polymerase

> M *i- Taq*™ *i-StarTaq*™

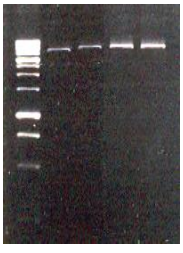

Fig. 2. Amplification of 4.5kb cloned gene in vector using *i-Taq*™ DNA polymerase or *i-StarTaq*™ DNA polymerase.
i-StarTaq™ DNA polymerase is more efficient in amplifying moderate long DNA fragments than common *Taq* DNA polymerase. 8 l of the PCR reactions was loaded onto a 0.8% agarose gel.
Lane M, 1Kb Ladder DNA Marker; **lane 1,2,** *i-Taq*™; **lane 3 , 4** *i-StarTaq*™ DNA Polymerase

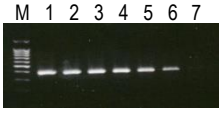

Fig. 3. RT-PCR amplification with ONE-STEP RT-PCR PreMix Kit
Total RNA was purified from human cancer cell lines using easy-BLUE™ Total RNA Extraction Kit (Cat. No. 17061).
And then, ONE-STEP RT-PCR reaction performed for β-Actin (400bp) gene from total RNA using ONE-STEP RT-PCR PreMix Kit, respectively. A dilution series of viral RNA was prepared as indicated.
Lane M, 100bp Ladder DNA Marker; **lane 1**, 1 ㎍ total RNA; **lane 2**, 10ng total RNA; **lane 3**, 1ng total RNA; **lane 4**, 100pg total RNA; **lane 5**, 10pg total RNA; **lane 6**, 1pg total RNA; **lane 7**, Negative control

• *RT-PCR from different virus*

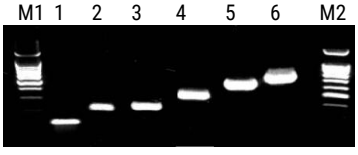

Fig. 4. RT-PCR amplification.
Total RNA was purified from virus using Viral Gene-spin™ Viral DNA/RNA Extraction Kit (Cat.No. 17151) and easy-BLUE™ Total RNA Extraction Kit (Cat. No. 17061). And then, the first strand cDNA was synthesized and its PCR reaction using ONE-STEP RT-PCR PreMix Kit.
Lane M1, 100bp Ladder DNA Marker; **lane M2**, 1Kb Ladder DNA Marker; **lane 1**, Newcastle Disease Virus HN (120bp); **lane 2**, Hog Cholera Virus NCR (421bp); **lane 3**, Infectious Bursal Disease Virus VP2 (500bp); **lane 4**, Bfl-1 (570bp), Bcl-2 family; **lane 5**,Human β-Actin (890bp); **lane 6**, hnRNP (900bp), alternative splicing factor

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
No PCR product or very little PCR product	Insufficient amount of template RNA	- Increase amount of RNA template in the reaction.
	Template RNA degraded	- Prepare fresh RNA template, being careful to prevent RNase activity. - Check RNA preparation by gel electrophoresis.
	Too much template RNA	- Decrease amount of RNA template; note that too high amount of RNA will affect/inhibit performance of RT-PCR.
	Template secondary structure prevented effective first strand cDNA synthesis	- Briefly denature the RNA template at 94°C (1 min) before adding reverse transcriptase. - Caution : Do not incubate reverse transcriptase or RNase Inhibitor at this elevated temperature, as they will be inactivated.
	Template secondary structure Inhibits effective formation of full-length products	- If GC content of RNA is high (>60%), increase denaturation temperature or denaturation time in PCR cycles
	Pipetting error or missing reagent	- Check the concentrations and storage temperature of reagent. Repeat the reaction.
	RT-PCR of long fragments	- Increase the concentraion of template RNA.
Product is smeared	Primer concentration not optimal or primers degraded	- A primer concentration of 0.6μM is recommended. However, if the desired results are not obtained using this condition, perform and check the RT-PCR with different primer concentrations from 0.5-1.0μM
	Secondary amplification product(s)	- Optimize primer concentration. - Decrease number of cycles.
Nonspecific product bands		- Check and perhaps decrease concentration of template.
	Too much starting template	- Check the concentration of the starting RNA template.
	Too many cycles	- Reduce the number of cycles in steps of 3 cycles.
	Annealing temperature too low	- Increase annealing temperature during PCR to increase specificity of amplification.
	Contaminating DNA in sample	- Your RNA sample may be contaminated by another RNA or DNA sample. As a control, perform PCR alone, omitting the RT step; if the sample is free of DNA, no product should be generated.
	Starting conditions for reverse-transcriptase reaction in correct	- Make sure that thermal cycler is preheated to 45℃ before placing samples in it.
	PCR annealing temperature too low	- Increase annealing temperature in increments of 2℃.

RELATED PRODUCTS

Product Name	Cat.No.
Maxime RT-PCR PreMix Kit	25131
easy-BLUE™ Total RNA Extraction Kit	17061
RNA-spin™ Total RNA Extraction Kit for Cell/Tissue	17211
Viral Gene-spin™ Viral DNA/RNA Extraction Kit	17151