Maxime RT-PCR PreMix Kit

for 20μl rxn

Cat. No. 25131 (96 tubes)

DESCRIPTION

Normal RT-PCR method is that RT and PCR procedure used by DNA polymerase for cDNA synthesis procedure are reacted in each tubes. However, this method is very uncomfortable and can have cross contamination by sample's carry over. For these problems and uncomfortability, *Maxime* RT-PCR PreMix Kit made a product that is include every container for DNA polymerase and each reaction mixture for you can do first-strand cDNA synthesis and PCR from total RNA or mRNA template continually in a tube.

Maxime RT-PCR PreMix Kit is the product that contains every container for each reaction can do in each tube for doing first-strand cDNA synthesis and PCR form total RNA or mRNA template. *Maxime* RT-PCR PreMix Kit uses OptiScript RT system, so accuracy and high efficience RT-reaction can do from 50fg to 500ng template RNA, and it is developed with the best condition of synthesis first-strand cDNA, so it is useful for check a low copy of DNA transcription. In addition, it block PCR from unspecific binded primer or primer-dimer by *i*-StarTaq DNA polymerase contains hot-start PCR.

STORAGE

Store at -20°C; under this condition, it is stable for at least a year.

CHARACTERISTICS

- Ready to use: only RNA template, Primer and RNase-free water are needed
- High efficiency & specificity
  : It includes OptiScript RT System, it can do high efficiency of RT reaction, and specificity amplification is occur, because hot-start PCR by *i*-StarTaq DNA Polymerase.
- Stable for over 1 year at -20℃
- Time-saving and cost-effective

CONTENTS

- *Maxime* RT-PCR PreMix (for 20μl rxn) 96 tubes

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

PROTOCOL

1. Add template RNA and specific primer into the *Maxime* RT-PCR PreMix tubes.
   Note : Use the same amounts of gene specific primers as usual PCR reaction or two fold reverse primer recommended.

Example **Total 20μl reaction volume**

RT reaction mixture		Concentration
Template RNA	Total RNA	below 500ng
	Poly (A) RNA	0.05-0.1ug
Forward primer		10-20pmole
Reverse primer		10-20pmole
RNase-free water		Up to 20μl
Total reaction volume		Total 20μl Rxn volume

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

2. Add RNase-free water into the *Maxime* RT-PCR PreMix tubes to a total volume of 20μl.
3. Dissolve the blue pellet by pipetting.
   Note : If the mixture lets stand at RT for 1-2min after adding water, the pellet is easily dissolved.
4. (Option) Add mineral oil.
   Note : This step is unnecessary when using a thermal cycler that employs a top heating method (general methods).
5. Perform RT-PCR reaction of samples as following process using PCR machine.

RT-PCR cycle		Temp.	Time
1 Cycles	Reverse transcription reaction	45℃	30 min.
	Inactivation of RTase	94℃	5 min.
25-40 Cycles	Denaturation	94℃	20-60 sec.
	Annealing	45-68℃	20-60 sec.
	Extension	72℃	1 min / kb
Final extension		72 ℃	5 min.

6. Load samples on agarose gel without adding a loading-dye buffer and perform electrophoresis.

EXPERIMENTAL INFORMATION

• Comparison with different company kit

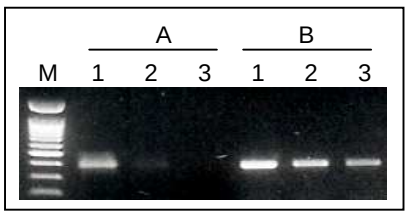

Fig.1. Comparison of *Maxime* RT-PCR PreMix Kit and Company A's RT-PCR PreMix system Kit by diagnosis of Newcastle disease (common type).
$10^{8.0}EID_{50}$ /0.1㎖ of allantoic fluid were 10-fold dilution, then total RNA were isolated using Viral Gene- spin™ Viral DNA/RNA Extraction Kit (Cat.No. 17151). From total RNA, the synthesized first strand cDNA and PCR reaction were performed using ONE-STEP RT-PCR PreMix Kit and *Maxime* RT-PCR PreMix Kit.
A, Company A; **B,** iNtRON's *Maxime* RT-PCR PreMix Kit
Lane M, 100bp Ladder DNA Marker; **lane 1,** 10^{-6} dilution; **lane 2,** 10^{-7} dilution; **lane 3,** 10^{-8} dilution

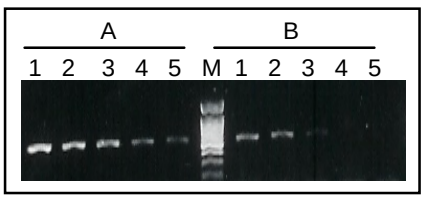

Fig.2. Comparison of *Maxime* RT-PCR PreMix and Company A's RT-PCR PreMix system by amplifying 570bp DNA fragment (GAPDH).
Total RNA was purified from SNU-1 using easy-BLUE™ Total RNA Extraction Kit (Cat. No. 17061). And then, RT-PCR reaction was performed using *Maxime* RT-PCR PreMix and different company's RT-PCR PreMix Kit.
A, iNtRON's *Maxime* RT-PCR PreMix Kit; **B,** Company A
Lane M, 100bp Ladder DNA Marker; **lane 1,** 2ng total RNA; **lane 2,** 200pg total RNA; **lane 3,** 20pg total RNA; **lane 4,** 2pg total RNA; **lane 5,** 200fg total RNA