

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. This procedure is reacted in each tube. However, this method is very uncomfortable and can have cross contamination by sample's carry over. For these problems and discomfortability, Maxime RT-PCR PreMix Kit made a product that includes every component for RT-PCR reaction: reverse transcriptase, DNA polymerase, and each reaction mixture for you to 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 component for each reaction to do in each tube for doing first-strand cDNA synthesis and PCR from total RNA or mRNA template. Maxime RT-PCR PreMix Kit uses OptiScript RT system, so accuracy and high efficiency 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 checking a low copy of DNA transcription. In addition, it blocks PCR from unspecific binding of primer or primer-dimer by *i*-StarTaq™ DNA Polymerase. *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 ensured, because hot-start PCR by *i*-StarTaq™ DNA Polymerase.

- Stable for over 1 year at -20 °C
- 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
TPs
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.

* **Note**: This example serves as a guideline for PCR amplification. Optimal reaction conditions such as amount of template DNA and amount of primer, may vary and must be individually determined.

2. Add RNase-free water into the Maxime RT-PCR PreMix tubes to a total volume of 20 µl.
 Do not calculate the dried components

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 °C	30 min.
	Inactivation of RTase	94 °C	5 min.
25-40 Cycles	Denaturation	94 °C	20-60 sec.
	Annealing	45-68 °C	20-60 sec.
	Extension	72 °C	1 min / kb
Final extension		72 °C	5 min.

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

EXPERIMENTAL INFORMATION

Comparison with different company kit

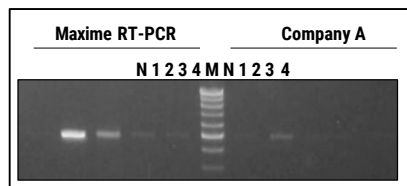


Fig.1. Comparison of Maxime RT-PCR PreMix Kit and Supplier A's RT-PCR PreMix system Kit by diagnosis of Infectious Bursal Disease Virus.

10⁶ EID₅₀ / 0.1 ml of fluid were 10-fold dilution, then total RNA were isolated using Viral Gene-spin™ Viral DNA/RNA Extraction Kit (Cat.No. 17151). From total viral genome, the synthesized first strand cDNA and PCR reaction were performed using Maxime RT-PCR PreMix Kit and different company's RT-PCR PreMix Kit.
Lane M, SiZer-100bp DNA Marker; **lane 1**, 10⁻⁵ dilution; **lane 2**, 10⁻⁶ dilution; **lane 3**, 10⁻⁷ dilution; **lane 4**, 10⁻⁸ dilution; **lane N**, Negative control

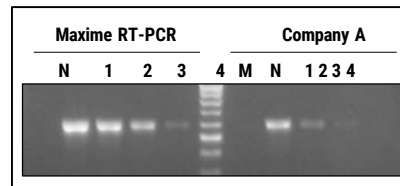


Fig.2. Comparison of Maxime RT-PCR PreMix and Supplier A's RT-PCR PreMix system by amplifying 570bp DNA fragment (GAPDH).

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.
Lane M, SiZer-100bp DNA Marker; **lane 1**, 2ng total RNA; **lane 2**, 200pg total RNA; **lane 3**, 20pg total RNA; **lane 4**, 2pg total RNA; **lane N**, Negative control