Maxime RT PreMix Kit

for 20μl rxn

Oligo $(dT)_{15}$ Primer
Cat. No. 25081 (96 tubes)

Random Primer
Cat. No. 25082 (96 tubes)

DESCRIPTION

Maxime RT PreMix Kit is the product what is mixed every component for cDNA synthesis in each tube for 1 rxn PCR. This product's specific character is that you can simply synthesis cDNA just with a PCR machine by 2 steps. This means that we can synthesis cDNA with simple protocol -do just cDNA synthesis temperature- without the procedure that after add a primer to RNA, do either boiling or heating at 75℃, 5 min. for loosen the RNA 2nd structure. We can get the cDNA synthesis rate which is same with a existed method's result by add just RNA primer and D.W. to *Maxime* RT PreMix Kit PCR tube, and than playing a PCR machine at 45℃, 60min. and at 95℃, 5min.
And also, it has merit that *Maxime* RT PreMix Kit has two kinds of product – oligo $(dT)_{15}$ primer and random primer- for choose the product for each using type. The product that finished a cDNA synthesis reaction can do PCR directly by using *Maxime* PCR PreMix Kit.

STORAGE

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

CHARACTERISTICS

- Ready to use: only RNA template and D.W. are needed
- easy and speed protocol : Just 2 steps
- Stable for over 1 year at -20℃
- Time-saving and cost-effective

CONTENTS

- *Maxime* RT PreMix (Oligo dT Primer; for 20μl rxn) 96 tubes
- *Maxime* RT PreMix (Random Primer; for 20μl rxn) 96 tubes

PROTOCOL

1. Add template RNA and distilled water into the *Maxime* RT PreMix tubes (Oligo dT or Random primer) to a total volume of 20μl.

Example **Total 20μl reaction volume**

RT reaction mixture		Concentration
Template RNA	Total RNA	0.1-1ug
	Poly (A) RNA	0.05-0.1ug
Distilled Water (treated by DEPC)		Up to 20μl
Total reaction volume		Total 20μl Rxn volume

2. Dissolve the clear pellet by pipetting.

   Note : If the mixture lets stand at RT for 1-2min after adding water, the pellet is easily dissolved.

3. Perform the cDNA synthesis reaction as follows using PCR machine;

Reaction Step	Temp.	Time
cDNA Synthesis	45℃	60 min
RTase inactivation step	95 ℃	5min

4. **(Option)** Dilute the reactant above by adding 20-50μl sterile water into a tube containing the cDNA obtained at RT reactant.
   Note : Minimum amount of cDNA is optimal condition for PCR reaction. Therefore dilution of cDNA is recommended.

5. Proceed to PCR reaction.
   Note : Perform the PCR using *Maxime* PCR PreMix series (Cat.No.25165, 25185, 25025, 25166, 25186, 25027) as follows;
   5-1) Transfer 0.2-1μl of RT product (synthesized cDNA) to *Maxime* PCR PreMix tube.
   Note : This RT product's usage serves as a guideline for PCR amplification. Optimal reaction conditions such as amount of template RT product and amount of primer may vary and must be individually determined. If you use the diluted RT product (cDNA), transfer 1-5μl of RT product (synthesized cDNA) to *Maxime* PCR PreMix tube. Just recommended.
   5-2) Perform PCR cycles according to the PCR condition.

EXPERIMENTAL INFORMATION

• Comparison with different company kit

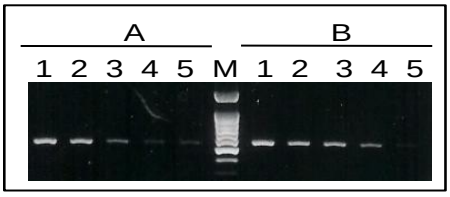

Fig.1. Comparison of *Maxime* PCR PreMix (Oligo dT Primer) and Company A's RT PreMix system by amplifying 570bp DNA fragment (GAPDH).
A, Company A; **B,** iNtRON's *Maxime* RT PreMix (Oligo dT Primer)
Total RNA was purified from mouse cells using easy-BLUE™ Total RNA Extraction Kit (Cat. No. 17061). And then, the first strand of cDNA was synthesized using *Maxime* RT PreMix Kit (Oligo dT Primer) and company's A RT PreMix Kit. After diluting the cDNA mixture as indicates, the PCR reaction was performed using *Maxime* PCR PreMix Kit
Lane M, 100bp Ladder DNA Marker; **lane 1,** $(1/2)^3$ diluted cDNA**; lane 2,** $(1/2)^4$ diluted cDNA; **lane 3,** $(1/2)^5$ diluted cDNA; **lane 4,** $(1/2)^6$ diluted cDNA; **lane 5,** $(1/2)^7$ diluted cDNA

• Comparison with Maxime RT PreMix Oligo dT and Random Primer

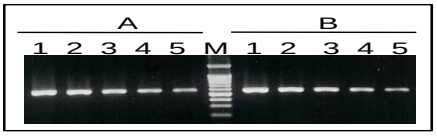

Fig.2. Comparison of *Maxime* PCR PreMix (Oligo dT Primer) and *Maxime* RT PreMix (Random Primer) by amplifying 570bp DNA fragment (GAPDH).
A, *Maxime* RT PreMix (Oligo dT Primer); **B,** *Maxime* RT PreMix (Random Primer)
Lane M, 100bp Ladder DNA Marker; **lane 1,** undiluted cDNA; **lane 2,** $(1/2)^2$ diluted cDNA**; lane 3,**$(1/2)^3$ diluted cDNA**; lane 4,** $(1/2)^4$ diluted cDNA; **lane 5,** $(1/2)^5$ diluted cDNA

• Comparison with Power cDNA Kit and Maxime RT PreMix

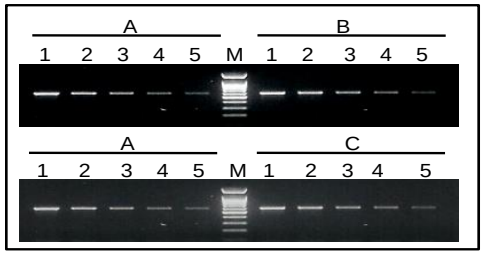

Fig.3. Comparison of *Maxime* PCR PreMix (Oligo dT Primer and Random Primer) and Power cDNA Synthesis Kit (Manual method) by amplifying 570bp DNA fragment (GAPDH).
Total RNA was purified from mouse cells using easy-BLUE™ Total RNA Extraction Kit (Cat. No. 17061). And then, the first strand of cDNA was synthesized using Power cDNA Synthesis Kit (Cat. No. 25011) and *Maxime* RT PreMix Kit (Oligo dT Primer & Random Primer). After diluting the cDNA mixture as indicates, the PCR reaction was performed using *Maxime* PCR PreMix Kit
A, iNtRON Power cDNA Synthesis Kit; **B,** *Maxime* RT PreMix (Oligo dT Primer); **C,** *Maxime* RT PreMix (Random Primer)
Lane M, 100bp Ladder DNA Marker; **lane 1,** $(1/2)^3$ diluted cDNA**; lane 2,** $(1/2)^4$ diluted cDNA; **lane 3,** $(1/2)^5$ diluted cDNA; **lane 4,** $(1/2)^6$ diluted cDNA; **lane 5,** $(1/2)^7$ diluted cDNA

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