

Maxime RT PreMix Kit

for 20 μ l 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

RNA, do either boiling or heating at 75 $^{\circ}$ C, 5 min. for loosen the RNA 2nd cDNA synthesis temperature- without the procedure that after add a primer to 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 $^{\circ}$ C, 60min. and at 95 $^{\circ}$ C, 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 $^{\circ}$ 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 $^{\circ}$ C
- Time-saving and cost-effective

CONTENTS

- Maxime RT PreMix (Oligo dT Primer; for 20 μ l rxn) 96 tubes
- Maxime RT PreMix (Random Primer; for 20 μ l 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 20 μ l.

Example : Total 20 μ l reaction volume

RT reaction mixture	Concentration	
Template RNA	Total RNA	0.1-1 μ g
	Poly (A) RNA	0.05-0.1 μ g
Distilled Water (treated by DEPC)		Up to 20 μ l
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 $^{\circ}$ C	60 min
RTase inactivation step	95 $^{\circ}$ C	5min

4. (Option) Dilute the reactant above by adding 20-50 μ l 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 0.2-1 μ l 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 1-5 μ l 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

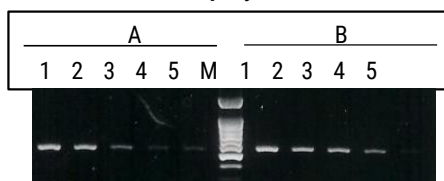


Fig.1. Comparison of Maxime PCR PreMix (Oligo dT Primer) and Company A's RT PreMix Kit by amplifying 570bp DNA fragment (GAPDH).

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 indicated, 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

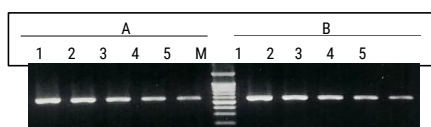


Fig.2. Comparison of Maxime PCR PreMix (Oligo dT Primer) and Maxime RT PreMix (Random Primer) by amplifying 570bp DNA fragment (GAPDH).

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

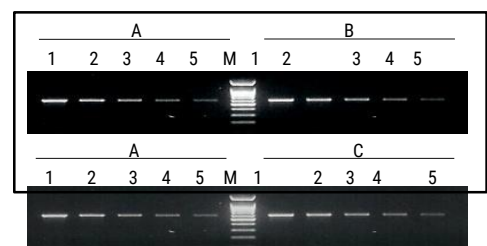


Fig.3. Comparison of Maxime PCR PreMix (Oligo dT Primer and Random Primer) and Power cDNA Synthesis Kit (Manual method) by amplifying 570bp DNA fragment (GAPDH).

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 indicated, 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

iNTRON BIOTECHNOLOGY

www.intronbio.com/ / info@intronbio.com

T. (0505)550-5600 / F. (0505)550-5660