Maxime PCR PreMix Kit (*i*-pfu)

for 20㎕ rxn

Cat. No. 25185 (96 tubes)

DESCRIPTION

iNtRON's Maxime PCR PreMix Kit has not only various kinds of PreMix Kit according to experience purpose, but also a 2X Master mix solution.

Pfu DNA Polymerase exhibits the lowest error rate of any thermostable DNA polymerase studied. For routine PCR, where simple detection of an amplification product or estimation of the product's size is important, Taq DNA polymerase is the obvious enzyme to choose. However, when the amplified product is to be cloned, expressed or used in mutagenesis studies, Pfu DNA polymerase is a much better enzyme of choice for PCR.

Maxime PCR PreMix Kit (i-pfu) is made from iNtRON's i-Pfu DNA Polymerase. i-Pfu DNA Polymerase is a thermostable DNA polymerase purified from an E.coli strain carrying a plasmid with the cloned gene encoding Pyrococcus furiosus DNA polymerase. The enzyme catalyzes the incorporation of nucleotides into duplex DNA in the 5'=>3' direction in the presence of Mg2+ at 70-80°C. Pfu DNA Polymerase exhibits 3'=>5' exonuclease (proofreading) activity, but has no detectable 5'=>3' exonuclease activity. Maxime PCR PreMix Kit (i-pfu) is the product what is mixed every component : i-pfu DNA Polymerase, dNTP mixture, reaction buffer, and so on- in one tube for 1 rxn PCR. This is the product that can get the best result with the most convenience system. The first reason is that it has every components for PCR, so we can do PCR just add a template DNA, primer set, and D.W.. The second reason is that it has Gel loading buffer to do electrophoresis, so we can do gel loading without any treatment. In addition, each batches are checked by a thorough Q.C., so its reappearance is high. It is suitable for various sample's experience by fast and simple using method.

STORAGE

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

CHARACTERISTICS

- High Fidelity : presence of 3'→5' exonuclease (proofreading)
- Low Error : the lowest error rate of any thermostable DNA polymerase studied.
- Flexibility : available for various DNA template including cloned fragment, phage DNA, mammalian genomic DNA and etc.
- Ready to use: only template and primers are needed
- Stable for over 1 year at -20℃
- Time-saving and cost-effective

CONTENTS

- *Maxime* PCR PreMix (*i-pfu*; for 20㎕ rxn) 96 tubes.

Component in 20 ㎕ reaction	
i-Pfu DNA Polymerase(2.5U/㎕)	2.5U
dNTPs	2.5mM each
Reaction Buffer(10x)	1x
Gel Loading buffer	1x

PROTOCOL

1. Add template DNA and primers into *Maxime* PCR PreMix tubes (*i*-pfu).
   Note 1 : Recommended volume of template and primer : 3㎕~5㎕
   Appropriate amounts of DNA template samples
   - I DNA : 1-20ng
   - Plasmid DNA : 10pg-100ng
   - Genomic DNA : 0.1-1ug for single copy

   Note 2 : Appropriate amounts of primers
   - Primer : 5-20pmol/㎕ each (sense and anti-sense)
2. Add distilled water into the tubes to a total volume of 20㎕.

Example **Total 20㎕ reaction volume**

PCR reaction mixture	Add
Template DNA	1 ~ 2㎕
Primer (F : 10pmol/㎕)	1㎕
Primer (R : 10pmol/㎕)	1㎕
Distilled Water	16 ~ 17㎕
Total reaction volume	**20㎕**

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.

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

SUGGESTED CYCLING PARAMETERS

PCR cycle		Temp.	PCR product size	
			≤2kb	≥2kb
Initial denaturation		94℃	2min	2min
30-40 Cycles	Denaturation	94℃	20sec	20sec
	Annealing	50-65℃	10sec	10sec
	Extension	65-72℃	30sec ~1min/kb	1min 30sec ~2min/kb
Final extension		72 ℃	Optional. Normally, 2-5min	

Note : This CYCLING PARAMETERS serves as a guideline for PCR amplification. optimal reaction conditions such as PCR cycles, annealing temperature, extension temperature and incubation times, may vary and must be individually determined.

EXPERIMENTAL INFORMATION

- ***Comparison with i-pfu and Maxime PCR PreMix (i-pfu)***

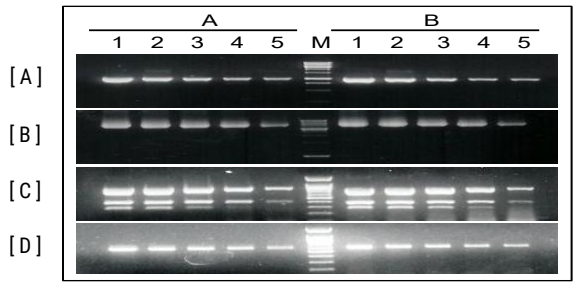

Fig.1. Comparison with *Maxime* PCR PreMix (*i*-pfu), *i*-pfu DNA Polymerase
A, *i*-pfu DNA Polymerase; **B,** *Maxime* PCR PreMix (*i*-pfu)
[A] By amplifying 1Kb fragment from variable amounts of Lambda DNA
Lanes M, 1Kb ladder; **lanes 1,** 200 pg; **lane 2,** 20 pg; **lane 3,** 2 pg; **lane 4,** 200 fg; **lane5,** 20 fg
[B] By amplifying 4.5Kb fragment from variable amounts of 5F' plasmid DNA
Lanes M, 1Kb ladder; **lanes 1,** 50ng gDNA; **lane 2,** 10ng gDNA; **lane 3,** 2ng gDNA; **lane 4,** 400pg gDNA; **lane 5,** 80pg gDNA
[C] By amplifying fyuA (780bp), tsh (420bp) and Irp2 (280bp) from variable amounts of *E.coli* gDNA
Lanes M, 100bp Marker; **lanes 1,** 50ng gDNA; **lane 2,** 10ng gDNA; **lane 3,** 2ng gDNA; **lane 4,** 400pg gDNA; **lane 5,** 80pg gDNA
[D] By amplifying 570bp fragment from variable amounts of cDNA
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

- ***The comparison of the long PCR (9kb) between i-pfu and Maxime PCR PreMix (i-pfu)***

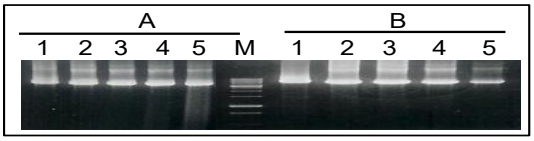

Fig.2. Comparison with *Maxime* PCR PreMix (*i*-pfu), *i*-pfu DNA Polymerase by amplifying 9Kb DNA fragment from variable amounts of SLT plasmid DNA .
A, *i*-pfu DNA Polymerase; **B,** *Maxime* PCR PreMix (*i*-pfu)
Lanes M, 1Kb Marker; **lanes 1,** 250 ng DNA; **lane 2,** 50 ng DNA; **lane 3,** 10 ng DNA; **lane 4,** 2 ng DNA; **lane 5,** 400 pg DNA

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