

**Maxime PCR PreMix Kit (*i*-StarTaq)**for 20 $\mu$ l rxn

Cat. No. 25165 (96 tubes)

**PROTOCOL**

- Add template DNA and primers into *Maxime* PCR PreMix tubes (*i*-StarTaq).
  - Note 1** : Recommended volume of template and primer : 3 $\mu$ l-5 $\mu$ l
  - Appropriate amounts of DNA template samples
    - cDNA : 0.5-10% of first RT reaction volume
    - Plasmid DNA : 10pg-100ng
    - Genomic DNA : 0.1-1 $\mu$ g for single copy
  - Note 2** : Appropriate amounts of primers
    - Primer : 5-20pmol/ $\mu$ l each (sense and anti-sense)
- Add distilled water into the tubes to a total volume of 20 $\mu$ l.

Example	Total 20 $\mu$ l reaction volume
PCR reaction mixture	Add
Template DNA	1 ~ 2 $\mu$ l
Primer (F : 10pmol/ $\mu$ l)	1 $\mu$ l
Primer (R : 10pmol/ $\mu$ l)	1 $\mu$ l
Distilled Water	16 ~ 17 $\mu$ l
<b>Total reaction volume</b>	<b>20<math>\mu</math>l</b>

**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.

- 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.
- (Option) Add mineral oil.
  - Note** : This step is unnecessary when using a thermal cycler that employs a top heating method(general methods).
- Perform PCR of samples.
- Load samples on agarose gel without adding a loading-dye buffer and perform electrophoresis.

**SUGGESTED CYCLING PARAMETERS**

PCR cycle	Temp.	PCR product size			
		100-500bp	500-1000bp	1Kb-5Kb	
Initial denaturation	94 °C	2min	2min	2min	
30-40 Cycles	Denaturation	94 °C	20sec	20sec	20sec
	Annealing	50-65 °C	10sec	10sec	20sec
	Extension	65-72 °C	20-30sec	40-50sec	1min/Kb
Final extension	72 °C	Optional. Normally, 2-5min			

**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. Hot start PCR technique was developed as a method to minimize the deleterious effects of mispriming at lower temperatures during PCR. In a PCR reaction, even short incubations at temperatures below the optimum annealing temperature for a particular set of primers can result in mispriming, elongation and the subsequent formation of spurious bands.

*Maxime* PCR PreMix Kit (*i*-StarTaq) is the product what is mixed every component : *i*-StarTaq™ 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**

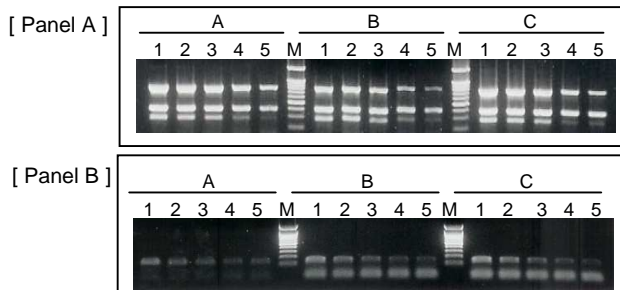
- Sensitivity : reduced or no amplification of non-specific products resulting from mispriming during PCR.
- Specificity : generating fragments of high specificity and high yield.
- Flexibility : available for various DNA template including cloned fragment, phage DNA, mammalian genomic DNA and etc.
- Ready to use: only template and primers are needed
- Stable for over 1 year at -20 °C
- Time-saving and cost-effective

**CONTENTS**

- Maxime* PCR PreMix (*i*-StarTaq; for 20 $\mu$ l rxn) 96 tubes.

Component in 20 $\mu$ l reaction	
<i>i</i> -StarTaq™ DNA Polymerase(5U/ $\mu$ l)	2.5U
dNTPs	2.5mM each
Reaction Buffer(10x)	1x
Gel Loading buffer	1x

**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*-StarTaq™ and *i*-StarMaster mix PCR PreMix**

**Fig.1. Comparison with *Maxime* PCR PreMix (*i*-StarTaq), *i*-StarTaq™ and *i*-StarMaster mix PCR PreMix Kit**

**A**, *i*-StarTaq™ DNA Polymerase; **B**, *i*-StarMaster mix PCR Kit; **C**, *Maxime* PCR PreMix (*i*-StarTaq)

**[ Panel A ]** Comparison with *i*-StarTaq™ DNA Polymerase, *i*-StarMaster mix PCR PreMix Kit and *Maxime* PCR PreMix (*i*-StarTaq) by amplifying *fyuA* (780bp), *tsh* (420bp) and *Irp2* (280bp) from variable amounts of *E.coli* gDNA

Aliquots of 3 $\mu$ l in 20 $\mu$ l reaction are loaded on 1% agarose gel.

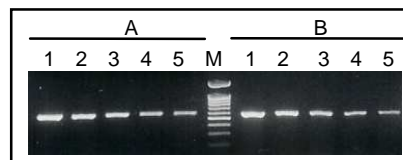
**Lanes M**, 100bp Marker; **lanes 1**, 50ng gDNA; **lane 2**, 10ng gDNA; **lane 3**, 2ng gDNA; **lane 4**, 400pg gDNA; **lane 5**, 80pg gDNA

**Note** : The PCR process is covered by patents issued and applicable in certain countries. iNtRON Biotechnology does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

**[ Panel B ]** Comparison with *i*-StarTaq™ DNA Polymerase, *i*-StarMaster mix PCR PreMix Kit and *Maxime* PCR PreMix (*i*-StarTaq) by amplifying TNF- $\alpha$  (131bp) from variable amounts of K562 gDNA

Aliquots of 5 $\mu$ l in 20 $\mu$ l reaction are loaded on 1% agarose gel.

**Lanes M**, 100bp Marker; **lanes 1**, 50ng gDNA; **lane 2**, 25ng gDNA; **lane 3**, 12.5ng gDNA; **lane 4**, 6.25ng gDNA; **lane 5**, 3.125ng gDNA

**• Comparison with different company kit**

**Fig.2. Comparison of *Maxime* PCR PreMix (*i*-StarTaq) and Company A's hot start PreMix system 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). After diluting the cDNA mixture as indicates, the RT-PCR reaction was performed.

**A**, Company A; **B**, iNtRON's *Maxime* PCR PreMix (*i*-StarTaq)

**Lane M**, 100bp Ladder DNA Marker; **lane 1**, undiluted cDNA; **lane 2**, 1/2 diluted cDNA; **lane 3**, 1/4 diluted cDNA; **lane 4**, 1/8 diluted cDNA; **lane 5**, 1/16 diluted cDNA