Maxime™ PCR PreMix Kit (i-Pfu)

QUICK GUIDE

96 tube : 25185

1. KIT DESCRIPTION

iNtRON's Maxime™ PCR PreMix Kit is a ready-to-use PCR premix containing i-Pfu DNA Polymerase, dNTPs, reaction buffer, and gel loading buffer in a single pre-aliquoted PCR tube. i-Pfu DNA Polymerase possesses 3′→5′ exonuclease (proofreading) activity, supporting high-fidelity DNA synthesis. Users only need to add template DNA, primers, and sterile distilled water for PCR amplification. The included gel loading buffer allows direct loading of PCR products onto an agarose gel without additional loading dye.

2. CHARACTERISTICS

- **All-in-One System :** Contains i-Pfu DNA Polymerase, dNTPs, reaction buffer, and gel loading buffer in a pre-aliquoted tube.
- **High-Fidelity Amplification** : 3′→5′ exonuclease (proofreading) activity supports accurate DNA synthesis.
- **Long PCR Capability** : Suitable for amplification of long DNA fragments.
- **Direct Gel Loading :** PCR products can be loaded directly onto an agarose gel without additional loading dye.
- **Versatile Applications :** Highly suitable for standard PCR, high-throughput routine screening, gene expression analysis, and direct electrophoresis workflows.

3. KIT CONTENTS AND STORAGE

Contents	25185
Maxime PCR PreMix (i-pfu)	12 Strips/pk (96 Tube)

- **Storage Condition :** Store strictly at -20°C immediately upon receipt.
- **Shelf Life & Stability :** Guaranteed to maintain stable performance through the indicated Expiration Date when stored and handled under recommended conditions.

4. CONSIDERATIONS BEFORE USE

- **Research Use Only (RUO) :** For research use only. Not for diagnostic or clinical procedures.
- **Storage & Handling :** Store strictly at -20°C. Avoid repeated freeze-thaw cycles.
- **Contamination Control :** Use clean gloves and filter pipette tips to prevent cross-contamination.
- **PreMix Dissolution :** Ensure complete pellet dissolution; briefly spin down if necessary prior to use.
- **Template Integrity :** Use high-purity DNA free of inhibitors (phenol, ethanol, EDTA, genomic contaminants).

5. PROTOCOLS

1) Prepare the reaction mixture by adding components into the pre-aliquoted PCR tube as follows :

- Template DNA : 1 ~ 2 µl
(Recommended : λ DNA -10 pg~20 ng, Plasmid DNA : 10pg ~ 100ng, Genomic DNA : 0.1 ~ 1µg for single copy)
- Forward Primer (10 pmol/µl) : 1 µl
- Reverse Primer (10 pmol/µl) : 1 µl
- Sterile Distilled Water (D.W.) : 16~17 µl (final vol : 20 µl)

NOTE : Since the PreMix already contains enzyme and buffer components, adjust the volume of distilled water according to the template and primer volumes.

2) 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.

3) Perform PCR of samples.

[SUGGESTED CYCLING PARAMETERS]

Tube		Temp.	Time ≤ 2 kb	Time > 2kb
Initial denaturation		94°C	2min	
30-40 Cycles	Denaturation	94°C	20sec	
	Annealing	Tm-dependent*	10sec	
	Extension	72°C	1min/kb	2min/kb
Final extension		72°C	2~5min	

*Annealing temperature: 3–5°C below the lower primer Tm.

4) Load the PCR products directly onto an agarose gel without adding loading dye, and perform electrophoresis.

6. TROUBLESHOOTING GUIDE

Refer to the following troubleshooting guide for possible causes and recommended actions when unexpected PCR results are observed.

Observation	Possible Cause	Recommendation
Little or no PCR product	Insufficient template or poor template quality	Check template concentration and quality
	Primer concentration not optimal	Optimize primer concentration
	Incorrect cycling conditions	Optimize annealing temperature, cycle number, and extension time
	Extension time too short	Increase extension time according to amplicon length.
Non-specific bands	Annealing temperature too low	Increase annealing temperature
	Excessive template	Reduce template amount
	Excessive primer concentration	Reduce primer concentration
Primer-dimer formation	Primer concentration/design not optimal	Optimize primer concentration or primer design
Smeared bands	Poor template quality or excessive DNA	Use purified DNA and reduce template amount
	Excessive cycle number	Reduce the number of PCR cycles

7. PCR OPTIMIZATION NOTES

- Annealing temperature should be optimized according to primer Tm.
- Extension time should be adjusted according to amplicon length.
- Longer targets may require extended elongation time.
- Template and primer concentrations may require optimization depending on the target.
- Use high-quality template DNA, particularly for long-range amplification.

8. CAUTIONS

- Wear appropriate personal protective equipment when handling samples and reagents.
- Use aerosol-resistant filter tips to minimize cross-contamination.
- Use separate areas for PCR setup and post-PCR analysis whenever possible.
- Avoid contamination of reagents with amplified DNA.
- Dispose of biological samples and PCR products according to applicable laboratory procedures.

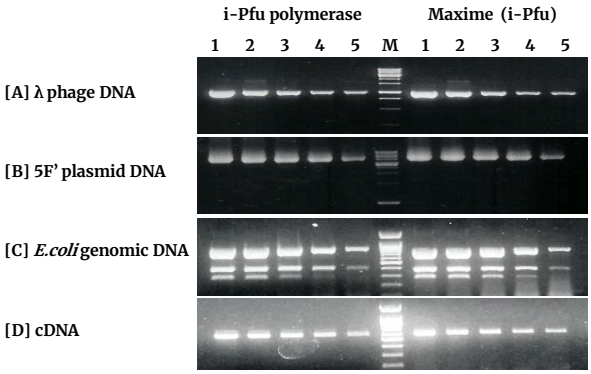
9. ADDITIONAL REQUIRED EQUIPMENT

- Thermal cycler
- Micropipettes and aerosol-resistant tips
- PCR-grade water
- Forward and reverse primers
- Agarose gel electrophoresis equipment

10. EXPERIMENTAL INFORMATION

1) Versatile amplification performance

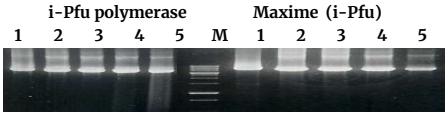
Maxime™ PCR PreMix Kit (i-Pfu) was evaluated using various DNA templates and target sizes. Amplification was performed with λ phage DNA (1 kb), 5F′ plasmid DNA (4.5 kb), E. coli genomic DNA for multiplex PCR (780, 420, and 280 bp), and cDNA (570 bp) over serially diluted template concentrations. Maxime™ PCR PreMix Kit (i-Pfu) showed amplification performance comparable to i-Pfu DNA Polymerase across the tested templates.



Panel A. 1 kb amplification from λ phage DNA, **lane M,** 1 kb DNA ladder; **lanes 1–5**, 200 pg, 20 pg, 2 pg, 200 fg, and 20 fg, respectively.
Panel B. 4.5 kb amplification from 5F′ plasmid DNA, **lane M,** 1 kb DNA ladder; **lanes 1–5**, 10 pg, 1 pg, 100 fg, 10 fg, and 1 fg, respectively.
Panel C. Multiplex amplification from *E. coli* genomic DNA-fyuA (780 bp), tsh (420 bp), and irp2 (280 bp) **lane M,** 100 bp DNA marker; **lanes 1–5,** 50 ng, 10 ng, 2 ng, 400 pg, and 80 pg of genomic DNA, respectively.
Panel D. 570 bp amplification from cDNA, **lane M,** 100 bp DNA ladder; **lanes 1–5,** serially diluted cDNA at $(1/2)^3$, $(1/2)^4$, $(1/2)^5$, $(1/2)^6$, and $(1/2)^7$, respectively.

2) Long-range amplification

Maxime™ PCR PreMix Kit (i-Pfu) showed comparable long-range amplification performance to i-Pfu DNA Polymerase using a 9 kb SLT plasmid DNA target.

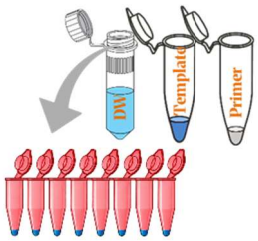


Lane M, 1 kb DNA marker; **lanes 1–5**, 250 ng, 50 ng, 10 ng, 2 ng, and 400 pg DNA, respectively.

11. EXPLANATION OF SYMBOLS

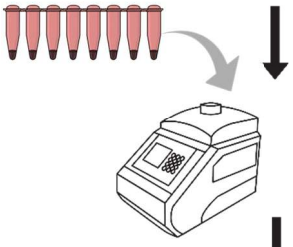
LOT	Batch code	REF	Catalogue number
Σ	Contains sufficient for <n> tests		Temperature limit
	Date of manufacture		Manufacturer
	Use-by date	RUO	Research Use Only
i	Consult instructions for use		Caution

Visual Protocol

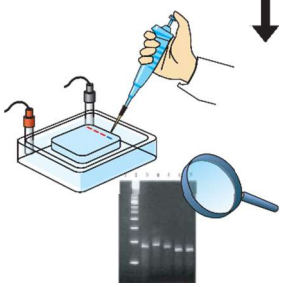


- Add to each PreMix tube :
  Template DNA (1~2 µl)
  +Forward primer + Reverse primer
  (10 pmol/µl , each 1 µl)
  +D.W. (up to 20 µl total)

- Mix thoroughly.
  Ensure the blue dried reagent is fully dissolved before PCR.



- PCR amplification



- Direct gel loading :
  Load directly onto an agarose gel
  (No additional loading dye required)