2x PCR Master mix Solution (i-pfu)

The Best Choice of 2X Master for mutagenesis studies.

RUO *Research Use Only* REF *25186* Σ 100 2°C 8°C

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 thermo-stable DNA polymerase studied. For routine PCR, where simple detection of an amplification productor 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.

2x PCR Master mix Solution (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.

2x PCR Master mix Solution (i-pfu) is the product what is mixed every component : *i*-Pfu DNA Polymerase, dNTP mixture, reaction buffer, and so on-in one tube. 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.

CHARACTERISTICS

- **High Fidelity :** presence of 3'→5' exonuclease (proofreading)
- **Low Error :** the lowest error rate of any thermo-stable 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
- **Construct as various reaction volume**
- **Stable for 18 months at 4℃**
- **Time-saving and cost-effective**

KIT CONTENTS

Contents	Amount
2X PCR Master mix Solution (i-pfu)	0.5 ml x 2 Vials
Instruction Manual	1 ea

- Component

i-pfu DNA Polymerase (2.5 U/㎕)	2.5 U
dNTPs	2.5 mM each
PCR Reaction Buffer	1x
Gel Loading buffer	1x

STORAGE

- Storage condition : Store the product at 2 ~ 8℃ after receiving.
- Expiration : **2X PCR Master mix Solution (i-pfu)** can be stored for up to 18 months without showing any reduction in performance and quality under appropriate storage condition. The expiration date is labeled on the product box.

APPLICATIONS

- General PCR
- Template amplification for sequencing
- Blunt Cloning etc

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

All products undergo the thorough quality control test and are warranted to perform as described when used correctly. Immediately any problems should be reported. At iNtRON we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. If you have any questions or experience any difficulties regarding the **2X PCR Master mix Solution (i-pfu)** or iNtRON products in general, please do not hesitate to contact us. iNtRON customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at iNtRON. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques. Satisfaction guarantee is conditional that the customer should provide full details of the problem to iNtRON within 60 days, and returning the product to iNtRON for examination.

QUALITY CONTROL

In accordance with iNtRON's ISO-certified Total Quality Management System, each lot of **2X PCR Master mix Solution (i-pfu)** is tested against predetermined specifications to ensure consistent product quality.

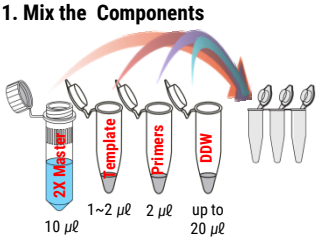
Contents	Quality Control
PCR Buffer, dNTP Mixture	Conductivity, pH, sterility, and performance in PCR are tested.
Distilled Water	Conductivity, pH, sterility, and performance in PCR are tested. Endonuclease, exonuclease, and RNase activities are tested.
2X PCR Master mix Solution (i-pfu)	PCR reproducibility assay: The PCR reproducibility assay reactions are performed in using 3 batches.
Process Inspection	Accuracy of aliquot process was validated Appearance of Master mix solution (housing, sealing contamination)

ADDITIONAL REQUIRED EQUIPMENT

- Distilled water
- Primers
- Pipettes and pipette tips (aerosol resistant)
- Thermal cycler
- Mineral oil (only if the thermal cycler does not have a heated lid)

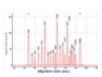
QUICK GUIDE

1. Mix the Components



2. Run the PCR Cycler

3. Electrophoresis of DNA



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PROTOCOL

1. **Dispense 10 ㎕ of 2X PCR Master mix Solution (in case of total 20 ㎕ PCR reaction) / 25 ㎕ of 2X PCR Master mix Solution (in case of total 50 ㎕ PCR reaction) into PCR tubes.**

2. **Add template DNA and gene specific primers into upper PCR tubes.**
   Note 1 : Appropriate amounts of DNA template samples
   - cDNA : 0.5-10% of first RT reaction volume
   - Plasmid DNA : 10 pg-100 ng
   - Genomic DNA : 0.1-1 ㎍ for single copy

   Note 2 : Appropriate amounts of primers
   - Primer : 5-20 pmole/㎕ each (sense and anti-sense)

3. **Add distilled water into the tubes to make total volume 20 ㎕ or 50 ㎕**
   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.

Example

PCR reaction mixture	20 ㎕ Rxn	50 ㎕ Rxn
2X PCR Master mix Solution (i-pfu)	10 ㎕	25 ㎕
Template DNA	1 ~ 2 ㎕	1 ~ 2 ㎕
Primer (F : 10 pmol/㎕)	1 ㎕	1 ㎕
Primer (R : 10 pmol/㎕)	1 ㎕	1 ㎕
Distilled Water	6 ~ 7 ㎕	21 ~ 22 ㎕
Total reaction volume	**20 ㎕**	**50 ㎕**

4. **Mix the mixture well by pipetting or voltexing then spin down the mixture by brief centrifugation.**

5. **(Option) Add mineral oil.**
   Note: This step is unnecessary when using a thermal cycler that employs a top heating (general methods) .

6. **Perform PCR of samples.**
   Note : SUGGESTED CYCLING PARAMETERS

*** Cycle program for fragments ≤ 2 Kb**

	Temp.	Time	Cycle No.
Initial Denaturation	94℃	2 min	1
Denaturation Annealing Extension	94℃ 50 ~ 65℃ 65 ~ 72℃	20 sec 10 sec 30 sec ~ 1 min / kb	30 – 40
Final extension	72℃ 4℃	2 ~ 5 min Hold	1

*** Cycle program for fragments ≥ 2 Kb**

	Temp.	Time	Cycle No.
Initial Denaturation	94℃	2 min	1
Denaturation Annealing Extension	94℃ 50 ~ 65℃ 65 ~ 72℃	20 sec 10 sec 1 min 30 sec ~ 2 min / kb	30 ~ 40
Final extension	72℃ 4℃	2 ~ 5 min hold	1

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.

7. **Load samples on agarose gel without adding a loading-dye buffer and perform electrophoresis.**
   Note: > 2% agarose (Agarose LE, iNtRON, Cat. No. 32034) gel is recommended.

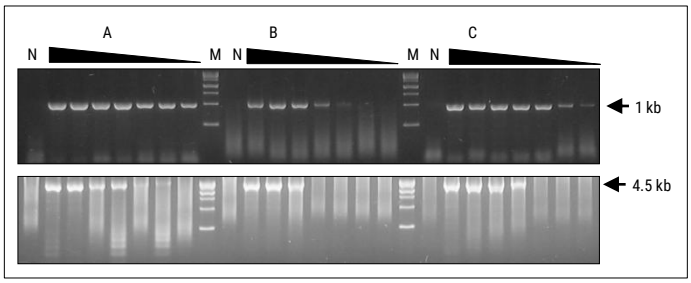
TROUBLESHOOTING GUIDE

Symptoms & Possible Causes	Comments & Suggestions
Little or no product	
A. Pipetting error or missing reagent	•Repeat the PCR. Check the concentrations and storage conditions of the kit, primers and template.
B. Primer concentration is not optimal or primers degraded	•Repeat the PCR with different primer concentrations from 0.1–0.5 μM of each primer (in 0.1 μM increments).
C. Problems with starting template	•Check the concentration, storage conditions, and quality of the starting template. If necessary, make new serial dilutions of template nucleic acid from stock solutions. Repeat the PCR using the new dilutions
D. Insufficient number of cycles	•Increase the number of cycles in increments of 5 cycles.
E. Incorrect annealing temperature or time	•Decrease annealing temperature by 2℃ increments.
F. Incorrect denaturation temperature or time	•Annealing time should be between 1 - 2 m Adjust the time in increments of 5 s.
G. Extension time too short	•Increase the extension time by increments of 30 s.
H. Primer design is not optimal	•Review primer design.
I. cDNA concentration	•For RT-PCR, take into consideration the efficiency of reverse transcriptase reaction which averages 10–30%. As RT reaction Mix is known to be a PCR inhibitor. The added volume of the cDNA should not exceed 10% of the final PCR volume.
Dimer or Product bands are smeared	
A. Primer concentration is not optimal or primers degraded	•Repeat the PCR with different primer concentrations from 0.1–0.5 μM each.
B. Primer design is not optimal	•Primer (in 0.1 μM increments). Review primer design.
C. Cycle number is too high	•Reduce the cycle number in increments of three cycles.
D. Quality of template DNA is too low	•Always use high-quality, purified DNA templates.
E. Carryover contamination	•Dispose of reagents, make fresh reagents, then repeat the PCR.

EXPERIMENT INFORMATIONS

❖ Comparison with different company's Kit

To compare the sensitivity with competitor's product after serial dilution of a various DNA template. 1Kb & 4.5 Kb DNA fragment PCR were performed. The sensitivity of 2X PCR Master mix Solution (i-pfu) is higher than competitor's product.



A, 2x PCR Master mix Solution(i-pfu); **B,** Competitor A; **C,** Competitor B
Lane M, 100 bp DNA Marker, 1 Kb DNA Marker; **lane N,** Negative control