2x PCR Master mix Solution (i-StarTaq™)

The Best Choice of 2X Master for Hot-Star PCR

RUO *Research Use Only* REF *25166* 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.
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.
2x PCR Master mix Solution (i-StarTaq™) is the product what is mixed every component: i-StarTaq™ 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

- **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 18 months at 4℃**
- **Time-saving and cost-effective**

KIT CONTENTS

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

- Component

i-StarTaq™ DNA Polymerase (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-StarTaq™)** 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

- Pathogen detection
- Genomic DNA PCR
- Hot-Star PCR
- Real-time PCR
- High GC rich, repeat region PCR
- TA vector cloning
- LOH or MSI analysis related PCR, 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-StarTaq™)** 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-StarTaq™)** is tested against predetermined specifications to ensure consistent product quality.

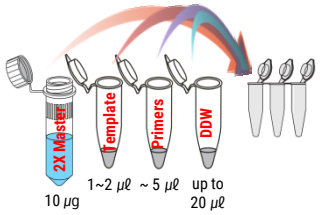
Contents	Quality Control
PCR Buffer, dNTP Mixture	Conductivity, pH, sterility, and performance in PCR are tested.
Distilled Water	Conductivity, pH, sterility, and performance in PCR are tested. Endonuclease, exonuclease, and RNase activities are tested.
2X PCR Master mix Solution (i-StarTaq™)	PCR reproducibility 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)

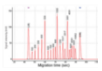
QUICK GUIDE

1. Mix the Components



2. Run the PCR Cycler

3. Electrophoresis of DNA



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-StarTaq™)	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**

	Temp.	PCR product size (Time)			Cycle No.
		100 ~ 500 bp	500 ~ 1,000 bp	1 Kb ~ 5 Kb	
Initial Denaturation	94℃	2 min	2 min	2 min	1
Denaturation Annealing Extension	94℃ 50 ~ 65℃ 65 ~ 72℃	20 sec 10 sec 20 ~ 30 sec	20 sec 10 sec 40 ~ 50 sec	20 sec 20 sec 1 min/Kb	30-40
Final extension	72℃ 4℃	2 ~ 5min Hold	2 ~ 5min Hold	2 ~ 5min 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.

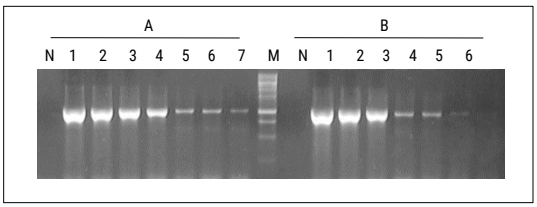
TROUBLESHOOTING GUIDE

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. • Check whether PCR was started with an initial denaturation step at 95°C for 5 min.
E. Hot start function is not activated	•Decrease annealing temperature by 2℃ increments. •Annealing time should be between 1 - 2 m Adjust the time in increments of 5 s.
F. Incorrect annealing temperature or time	•Increase the extension time by increments of 30 s.
G. Incorrect denaturation temperature or time	•Review primer design.
H. Extension time too short	•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.
I. Primer design is not optimal	
J. cDNA concentration	
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. •Always use high-quality, purified DNA templates.
D. Quality of template DNA is too low	•Dispose of reagents, make fresh reagents, then repeat the PCR.
E. Carryover contamination	

EXPERIMENT INFORMATIONS

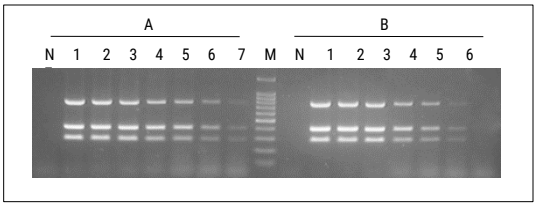
❖ Comparison test for sensitivity

To compare the sensitivity of 2X PCR Master mix Solution (i-StarTaq™) with competitor's product. 1 Kb DNA fragment PCR is performed with human genomic DNA diluted by concentration. The data shows that 2X PCR Master mix Solution (i-StarTaq™) is highly sensitive comparing to competitor's product.



A, 2x PCR Master mix Solution(i-StarTaq™); **B,** Competitor
Lane M, 1 Kb DNA Marker; **lane NC,** Negative control; **lane 1,** 50 ng DNA; **lane 2,** 25 ng DNA, **lane 3,** 12.5 ng DNA, **lane 4,** 6.25 ng DNA, **lane 5,** 3.125 ng DNA; **lane 6,** 1.56 ng DNA; **lane 7,** 0.753 ng DNA

To compare the sensitivity of 2X PCR Master mix Solution (i-StarTaq™) with competitor's product, multiplex PCR for fyuA(780 bp), tsh(420 bp) and Irp2(280 bp) is performed with *E.coli* genomic DNA diluted by concentration. The data shows that 2X PCR Master mix Solution (i-StarTaq™) is highly sensitive comparing to competitor's product.



A, 2x PCR Master mix Solution(i-StarTaq™); **B,** Competitor
Lane M, 100 bp DNA Marker; **lane NC**, Negative control; **lane 1,** 100 ng gDNA, **Lane 2,** 50 ng DNA; **lane 3,** 25 ng gDNA; **lane 4,** 12.5 ng gDNA; **Lane 5,** 6.25 ng gDNA; **lane 6,** 3.125 ng gDNA; **lane 7,** 1.5625 ng gDNA