2X Master/MultiMAX PCR Kit

The Best Choice of 2X Master for Multiplex PCR.
For standard and specialized end-point Multiplex PCR applications without the need for optimization

RUO *Research Use Only* REF *25501* Σ 100 2°C 8°C

DESCRIPTION

MPF Technology is iNtRON's innovative and original technology which is adapted to manufacture PCR 2X Master. It is a platform technology that guarantees the effective function of multiplex PCR and rapid amplification of PCR products using any thermal cycler. The **2X Master/MultiMAX PCR Kit** based on this MPF Technology is designed to enable end-point multiplex PCR to have simultaneous amplification of many targets in a single tube using multiple pairs of primers. The **2X Master/MultiMAX PCR Kit** is adapting "All-in-one type" which has all components for PCR amplification in the format of 2x concentrated master mix solution. This kit is very convenient to use with high specificity and sensitivity. The **2X Master/MultiMAX PCR Kit**, is optimized for simultaneous amplification of multiple targets. The quality is guaranteed.

CHARACTERISTICS

- **High sensitivity and specificity :** Maximum 27plex amplification
- **High yield and reproducibility :** specifically Stabilized bound primers
- **Easy to use :** Simple and Fast
- **Perform multiplex PCR with minimal optimization**

KIT CONTENTS

Contents	Amount
2X Master/MultiMAX PCR Solution	0.5ml x 2 Vials
Instruction Manual	1 ea

STORAGE AND STABILITY

- Storage condition : Store the product at 2 ~ 8℃ after receiving.
- Expiration : **2X Master/MultiMAX PCR Kit** 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

- Genotyping: Deletions, mutations, SNP analysis (genetic profiling), Microsatellites
- Molecular diagnostics : Pathogens, transgenic organisms
- Forensic analysis : Human identification, paternity
- Gene expression: Quantitative, RT-PCR assays

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)

IMPORTANT NOTES BEFORE STARTING

- **2X Master/MultiMAX PCR Kit** provides a final concentration of 2 mM $MgCl_2$ which produces satisfactory results in most cases. However, if a higher Mg^{2+} concentration is needed, prepare a stock solution containing 25 mM $MgCl_2$.
- Set up reaction mixtures in separate area from the site of DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- The **2X Master/MultiMAX PCR Kit** can be used with standard quality primers that can be purchased from established oligonucleotide manufacturers. Primers should be purchased desalted or purified, for example using HPLC, and dissolved in TE (10 mM Tris·Cl, 0.1 mM EDTA, pH 8.0) to obtain a 50 or 100 μM stock solution. Primer quality is a crucial factor for successful multiplex PCR. Problems encountered in multiplex PCR are frequently due to the use of incorrect primer concentrations or low-quality primers.
- The annealing efficiency of primers to the template is an important factor in PCR. If too small amount of template is used, primers may not be able to find their complementary sequences. Too much template may lead to an increase in mis-priming events.

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 Master/MultiMAX PCR Kit** 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 Master/MultiMAX PCR Kit** is tested against predetermined specifications to ensure consistent product quality.

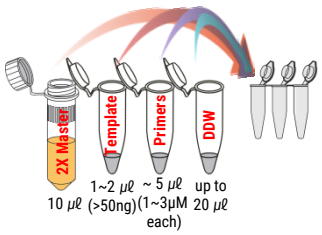
Contents	Quality Control
PCR Buffer, dNTP Mixture	Conductivity, pH, sterility, and performance in PCR are tested.
Distilled Water	Conductivity, pH, sterility, and performance in PCR are tested. Endonuclease, exonuclease, and RNase activities are tested.
2X Master/MultiMAX PCR Kit	PCR reproducibility 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)

TECHNICAL ASSISTANCE

2X Master/MultiMAX PCR Kit is intended for research use only. Prior to using it for other purposes, the user must validate the system in compliance with the applicable law, directives, and regulations. **2X Master/MultiMAX PCR Kit** is developed, designed, and sold for research purpose only. It is not intended to be used for human or animal diagnosis of diseases. Do not use internally or externally in humans or animals.

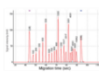
QUICK GUIDE

1. Mix the Components



2. Run the PCR Cycler

3. Electrophoresis of DNA



PROTOCOL

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

2. Add template DNA and primers into upper PCR tubes.

Note 1 : Recommended volume of template: 1 ㎕~ 2 ㎕.

Note 2 : 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 3 : 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 ㎕.

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	Add
Template DNA	1 ~ 2 ㎕
Primer mix (final conc. 1 ~ 3μM each)	~ 5 ㎕
2X Master/MultiMAX PCR Solution	10 ㎕
Distilled Water	Up to 20 ㎕
Total reaction volume	20 ㎕

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

PCR Steps		Temp.	PCR product size		
			100-500bp	500-1000bp	1kb-2.5kb
Initial denatulation		95 ℃	5-10 min	5-10 min	5-10 min
30-40 Cycles	Denaturation	95 ℃	20 sec	20 sec	20 sec
	Annealing	50-65 ℃	1 min	1 min	1 min
	Extension	68-72 ℃	1 min	1 min	1 min/kb
Final extension		72 ℃	Optional , Normally, 2~5 min		

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 UltraPhor (iNtRON, Cat.No. 32037) gel is recommended.

TECHNICAL ADVICE

❖ Design of Multiplex PCR Primers

A prerequisite for successful multiplex PCR is the design of optimal primer pairs.

A. Melting temperature

• The melting temperature of primers used for multiplex PCR should be at least 60℃. The melting temperature of primers can be calculated using the formula below:

Tm= 2°C x (number of [A+T]) + 4°C x (number of [G+C])

• Whenever possible, design primer pairs with similar T_m values. Functionality and specificity of all primer pairs should be checked in individual reactions before combining them in a multiplex PCR assay.

• Primers for multiplex PCR should have a GC content of 40–60%.

• Primers for multiplex PCR should be 21–30 nucleotides in length.

B. Annealing temperature

• If possible, perform a gradient PCR to determine the optimal annealing temperature

C. Sequence

• Avoid complementarily 2 or 3 bases at the 3' ends of primer pairs to reduce primer-dimer formation.

• Avoid mismatches between the 3' end of the primer and the target-template sequence.

• Avoid runs of 3 or more G and/or C at the 3' end.

• Avoid complementary sequences within primers and between primer pairs.

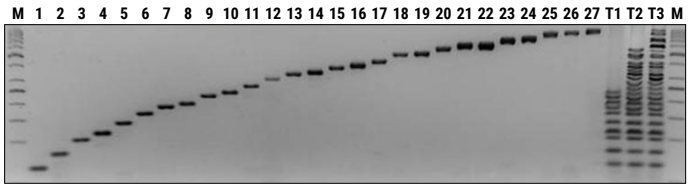
TROUBLESHOOTING GUIDE

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℃ 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	•Increase the cycle number in increments of three cycles.
J. cDNA concentration	•Using the same cycling conditions, repeat the multiplex PCR adding 5% DMSO.
K. Cycle number is too low	
L. Template with a high degree of secondary structure	
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	•Adjust the time in increments of 10 s.
F. Denaturation time is too short or too long	

EXPERIMENT INFORMATIONS

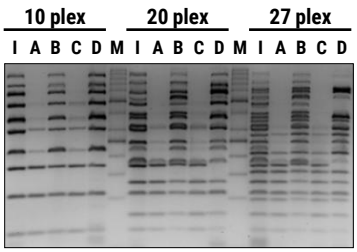
❖ High yield and reproducibility

More compatible to mulitplex PCR (up to 27 Plex) - 2X Master/MultiMAX PCR Kit is stable and efficient primer annealing.



Single PCR and Multiplex PCR was implemented using 2X Master/MultiMAX PCR Kit. PCRs were performed using the human genomic DNA and 27 pairs of primers.
Lane 1-27, single PCR amplicons; Lane T1,10plex PCR; LaneT2, 20plex PCR;
Lane T3, 27plex PCR; Lane M, SiZer™-50 plus DNA Marker Solution (Cat. No. 24072)

❖ Comparison of PCR efficiency



Comparison of amplification 2X Master/MultiMAX PCR Kit and competitor Multiplex PCR kits.
The DNA Polymerase of 2X Master / MultiMAX PCR Kit has hot start PCR activity. The kit show high specific and high sensitivity amplification.

Lane I, 2X Master/MultiMAX PCR Kit; lane A ~ D, competitor A ~ D's Multiplex PCR kits