2X PCR Master mix Solution [*i*-StarMAX™ GH]

[for Long & Hot-Start PCR]

The Best Choice of 2X Master for Long & Hot-Start PCR.
For standard and specialized proofreading, Long, Hot-Start PCR applications without the need for optimization

RUO *Research Use Only* REF *26041* Σ 100 2°C 8°C

DESCRIPTION

In case of the PCR method amplified by using general Taq DNA Polymerase, ideal length of an amplified fragment is less than 1 Kb and not greater than 3 Kb in theory. Over 10 Kb fragments can be amplified by standard PCR technique, but it is hard to get high amplification efficiency and reliable results with longer fragments. PCR technology has been developed to get reliable results and high amplification efficiency for these long PCR fragments. Discovery of pfu DNA Polymerase has had a biggest effect on widely known PCR methods for amplifying 20 Kb ~ 40 Kb. pfu DNA Polymerase is isolated from *P.furiosis* and has proof-reading function. To carry out long PCR experiment, **2x PCR Master mix Solution(*i*-StarMAX™ GH)** is can be a suitable Kit. The **2x PCR Master mix Solution(*i*-StarMAX™ GH)** is used *i*-StarMAX™ GH DNA Polymerase, which is containing Taq DNA Polymerase and thermostable DNA Polymerase with proofreading activity. The advantage of *i*-StarMAX™ GH DNA Polymerase is the outstanding PCR accuracy compared to general Taq DNA Polymerase. The *i*-StarMAX™ GH DNA Polymerase has proof-reading function which automatically stops to synthesize nucleotide sequence when incorrect PCR fragment interrupts template. But it is only applied to long PCR and there is a drawback that performance time of PCR is longer.

So, **2x PCR Master mix Solution(*i*-StarMAX™ GH)** to make up for this weakness, and then **2X PCR Master Mix Solution (*i*-StarMAX™ GH)** is designed to get the best result with simple use that all components are premixed in a tube with 2X solution mix type. After adding template DNA, primer set and D.W. only, performing PCR is possible and loading gel is done with no any other treatment because of gel loading buffer. **2X PCR Master mix Solution (*i*-StarMAX™ GH)** using *i*-StarMAX™ GH DNA Polymerase has convenience as well as high specificity and guarantees the reliable result and the reproducibility for various samples.

CHARACTERISTICS

- **Applied *i*-StarMAX™ GH DNA Polymerase**
- **Long PCR & Hot-Start PCR**

✓ Over 30 Kb fragments can be amplified by standard PCR
✓ Applied Taq Antibody

- **High Sensitivity :** Reduced or no amplification of non-specific products resulting from mis-priming during PCR.
- **High Specificity :** Hot-start PCR activity shows with a high specificity and accuracy with high amplification yield
- **High Fidelity** : presence of 3'→5' exonuclease (proofreading)
- **Ready to Use**

✓ All components premixed for PCR in a solution
✓ Adding template and primer only for PCR
✓ Gel loading dye is included so you just electrophoresis

- **Stability :** Stable for over 18 months at 2~8 ℃
- **Economic :** Time-saving and cost-effective

KIT CONTENTS

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

STORAGE AND STABILITY

- Storage condition : Store the product at 2 ~ 8℃ after receiving.
- Expiration : **2X PCR Master mix Solution(*i*-StarMAX™ GH)** 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

- Amplification of Genomic DNA PCR and cDNA target over 20 Kb
- PCR with difficult templates e.g. secondary structures or GC-rich sequences.
- Multiplex PCR
- Molecular diagnosis
- HGP (Human Genome Project), cDNA Library synthesis.
- Cloning with TA and blunt ends. etc.

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

At iNtRON we pride ourselves on the quality and availability of our technical support. iNtRON is 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 experence any difficulties regarding the **2X PCR Master mix Solution(*i*-StarMAX™ GH)**, 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. For technical assistance and more information please call iNtRON local distributors.

TECHNICAL ASSISTANCE

2X PCR Master mix Solution(*i*-StarMAX™ GH) 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 PCR Master mix Solution(*i*-StarMAX™ GH)** 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.

QUALITY CONTROL

In accordance with iNtRON's ISO-certified Total Quality Management System, each lot of **2X PCR Master mix Solution(*i*-StarMAX™ GH)** 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*-StarMAX™ GH)	PCR reproducibility assay: The PCR reproducibility assay reactions are performed in using 3 batch.
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)

IMPORTANT NOTES BEFORE STARTING

- Set up reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- The annealing efficiency of primers to the template is an important factor in PCR. If too little 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.

PROTOCOL

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

2. Add template DNA and 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 : 5-20pmol/ ㎕ each (sense and anti-sense)

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

Example

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

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 < 10Kb

PCR Steps		Temp.	Time
Initial denaturation		94-95 ℃	2~5 min
25-35 Cycles*2	Denaturation	94-95 ℃	15 sec~1 min
	Annealing*3	45-65(5 ℃ below Tm)	15 sec~1 min
	Extension*1	72 ℃	1 min / 1-1.5 Kb
Final extension		72 ℃	Optional , Normally, 2~5 min

*1 Extension time for 30s-1min sufficient for fragment < 1Kb.
*2 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.
*3 Depending on the specific Tm Primers

Cycle Program for fragments > 10Kb, Using 2 Step PCR : Primer Tm above 60℃

PCR Steps		Temp.	Time
Initial denaturation		94-95 ℃	2~5 min
20-30 Cycles	Denaturation	94-95 ℃	10 ~ 30 sec
	Annealing/Extension*1	65-68 ℃	1min / 1Kb
Final extension		72 ℃	Optional , Normally, 2~5 min

*1 Annealing/Extension at 65-68 ℃ work for most target. However, reactions may need to be tested at different temperature in increment of 1-2 ℃above and below 65-68 ℃ to find the optimal condition for your target.

Cycle Program for fragments > 10Kb, Using 3 Step PCR : Primer Tm below 60℃

PCR Steps		Temp.	Time
Initial denaturation		94-95 ℃	2~5 min
20-30 Cycles	Denaturation	94-95 ℃	10 ~ 30 sec
	Annealing	45-65(5 ℃ below Tm)	10 ~ 30 sec
	Extension	65-72 ℃*1	1 min / 1 Kb
Final extension		72 ℃	Optional , Normally, 2~5 min

*1 Extension at 65 ℃ work for most target. However, reactions may need to be tested at temperature in 1-2 ℃ increments starting at 65 ℃ and going up to 72 ℃ to find the optimal condition for your target.

7. Load samples on agarose gel without adding a loading-dye buffer and perform electrophoresis.

Note: RedSafe ™ Nucleic Acid Staining Solution (iNtRON, Cat. No. 21141) and Agarose LE (iNtRON, Cat. No. 32034) are recommended.

EXPERIMENT INFORMATIONS

❖ Performance of 2X PCR Master mix Solution(*i*-StarMAX™ GH) in Various size PCR

In various size PCR, 2X PCR Master mix Solution(*i*-StarMAX™ GH) provides the detection of PCR band with high sensitivity and amplification yield than 2X PCR Master mix Solution(*i*-StarMAX™ II).

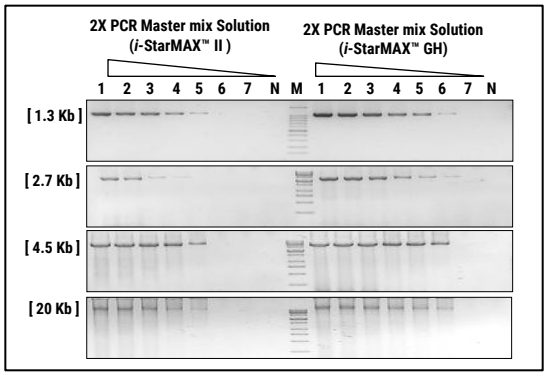

Fig 1. PCR amplification of 1.3/2.7/4.5/20 Kb with 2X PCR Master mix Solution (i-StarMAX™ II) and 2X PCR Master mix Solution (i-StarMAX™ GH)

[1.3 Kb]
Lane M, SiZer™-100 DNA Marker Solution(Cat. No. 24073); **lane N**, Negative Control
Lane 1, 100 ng K562 gDNA; **lane 2,** 10 ng K562 gDNA; **lane 3,** 1 ng K562 gDNA; **lane 4,** 100 pg K562 gDNA; **lane 5,** 10 pg K562 gDNA; **lane 6,** 1 pg K562 gDNA; **lane 7,** 100 fg K562 gDNA
[2.7 Kb]
Lane M, SiZer™-1000 DNA Marker Solution(Cat. No. 24074); **lane N**, Negative Control
Lane 1, 100 ng K562 gDNA; **lane 2,** 10 ng K562 gDNA; **lane 3,** 1 ng K562 gDNA; **lane 4,** 100 pg K562 gDNA; **lane 5,** 10 pg K562 gDNA; **lane 6,** 1 pg K562 gDNA; **lane 7,** 100 fg K562 gDNA
[4.5 Kb]
Lane M, SiZer™-1000 DNA Marker Solution(Cat. No. 24074); **lane N**, Negative Control
Lane 1, 100 ng 5F plasmid DNA; **lane 2,** 10 ng 5F plasmid DNA; **lane 3,** 1 ng 5F plasmid DNA; **lane 4,** 100 pg 5F plasmid DNA; **lane 5,** 10 pg 5F plasmid DNA; **lane 6,** 1 pg 5F plasmid DNA; **lane 7,** 100 fg 5F plasmid DNA
[20 Kb]
Lane M, SiZer™-1000 DNA Marker Solution(Cat. No. 24074); **lane N**, Negative Control
Lane 1, 10 ng λDNA; **lane 2,** 1 ng λDNA; **lane 3,** 100 pg λDNA; **lane 4,** 10 pg λDNA; **lane 5,** 1 pg λDNA ; **lane 6,** 100 fg λDNA; **lane 7,** 10 fg λDNA

TROUBLESHOOTING GUIDE

Symptoms & Possible Causes	Comments & Suggestions
Little or no product	
1) Pipetting error or missing reagent	•Repeat the PCR. Check the concentrations and storage conditions of the kit, primers and template.
2) 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).
3) 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
4) Insufficient number of cycles	•Increase the number of cycles in increments of 5 cycles.
5) Hot Start function is not activated	•Check whether PCR was started with an initial denaturation Step at 95℃ for 5 min.
6) Incorrect annealing temperature or time	•Decrease annealing temperature by 2℃ increments.
Multi bands	
1) Annealing templerature too low	•Increase annealing temperature in 2℃ steps.
2) Primer design or concentration not optimal	•Review primer design •Titrate primer concentration

RELATED PRODUCTS

Product Name	Cat.No.
i-Taq™ DNA Polymerase	25021 / 25022
i-StarTaq™ DNA Polymerase	25161 / 25162
i-MAX II DNA Polymerase	25261
i-StarMAX™ II DNA Polymerase	25173
i-pfu DNA Polymerase	25181
TankKlen MDx DNA Polymerase	26020
TankStar MDx DNA Polymerase	26001
i-StarTaq™ GH DNA Polymerase	26030 / 26031