2X Master mix UDG-PCR System

The Best Choice of 2X Master for Hot-Star PCR
Prevention of cross/carryover contamination

RUO *Research Use Only* REF *25189* Σ 100 -22 ℃ -18 ℃

INTRODUCTION

2X Master mix UDG-PCR System is ready-to-use Hot-start PCR pre-mixes are the innovation for convenience of your routine PCR. The 2X Master mix UDG-PCR System is an optimized, ready-to-use PCR mixture of components and uracil DNA glycosylase (UDG). UDG and dUTP are included in the mixture to prevent the reamplification of cross/carry-over PCR products between reactions. dUTP in the mix ensures that any amplified DNA will contain uracil. UDG removes uracil residues from single- or double-stranded DNA, preventing dU-containing DNA from serving as template in future PCRs.

2X Master mix UDG-PCR System is the product what is mixed every component. This is the product that can get the best result with the most convenience system. PCR reactions can be directly loaded onto an agarose gel without the additional need of loading buffer and dyes.

KIT CONTENTS

Label	Volume
2X Master mix UDG-PCR System	1 ml

† Spin down before use

STORAGE AND STABILITY

- Storage condition : Store the product at -20℃
- Expiration date : The solution is stable for 2 year from the date of shipping when stored and handled properly.

APPLICATIONS

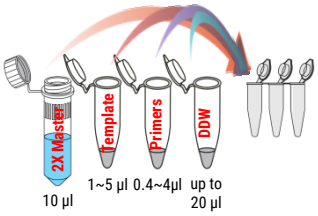
- High-throughput PCR
- Hot-start PCR
- Routine diagnostic PCR requiring high reproducibility
- DNA sequencing template preparation

NOTICE BEFORE USE

The 2X Master mix UDG-PCR System is intended for research use only. This product is not intended for the diagnosis, prevention, or treatment of disease. All due care and attention should be exercised in the handling of the products. Do not use internally or externally in humans or animals. Please observe general laboratory precaution and utilize safety while using this kit.

QUICK GUIDE

1. Mix the Components



2. Run the PCR Cycler

3. Electrophoresis of DNA

PROTOCOL

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the 2X Master mix UDG-PCR System mix. For multiple reactions, scale-up volume of reaction components proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

1. **Thaw the 2X Master mix UDG-PCR System mix, at room temperature. Mix thoroughly and then place on ice immediately after thawing.**
2. **Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.**
3. **The following table shows recommended component volumes:**

Component	20 µl Reaction	Final Concentration
2X Master mix UDG-PCR System mix	10 µl	1X
Forward Primer (10 µM)	0.2 – 2.0 µl	0.1 – 1.0 µM
Reverse Primer (10 µM)	0.2 – 2.0 µl	0.1 – 1.0 µM
Template DNA	1.0 – 5.0 µl	< 250 ng
DNase/RNase free Water	Up to 20 µl	-

* In general, use primers greater than 0.5 µM for sensitivity and less than 0.5 µM for specificity.
* Recommended amount of template per PCR reaction
: < 50 ng plasmid, < 500 – 1000 ng genomic DNA, < 2 µl of a 100 µl single plaque eluate

4. **Ensure reactions are mixed thoroughly by pipetting of gentle vortexing followed by a brief spin in a microcentrifuge.**

* (Optional) Overlay reactions with one-half volume PCR-grade mineral oil when not using heated lid on thermal cycler.

5. **Transfer tubes into a PCR instrument and run as following table.**

Steps	Temp.	Time	Cycle(s)
UDG reaction	50℃	2min	1
Initial Denaturation	95℃	5min	1
Denaturation	95℃	10 - 60 sec	25 - 40
Annealing	50℃ - 65℃	10 - 60 sec	
Elongation	72℃	60 sec / kb	
Final Extension	72℃	5min.	1

* Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

6. **After cycling, maintain the reactions at 4℃ or store at - 20℃ until ready for analysis.**

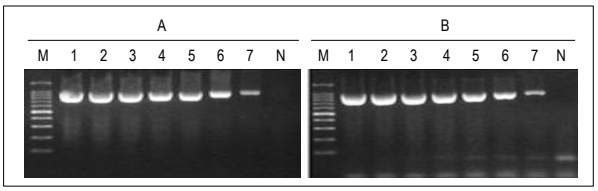
TROUBLE SHOOTING GUIDE

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. Hot start function is not activated	• Check whether PCR was started with an initial denaturation step at 95°C for 5 min.
F. Incorrect annealing temperature or time	• Decrease annealing temperature by 2℃ increments.
G. Incorrect denaturation temperature or time	• Annealing time should be between 1 - 2 min, Adjust the time in increments of 5 sec.
H. Extension time too short	• Increase the extension time by increments of 30 sec.
I. Primer design is not optimal	• Review primer design.
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.

EXPERIMENT INFORMATION

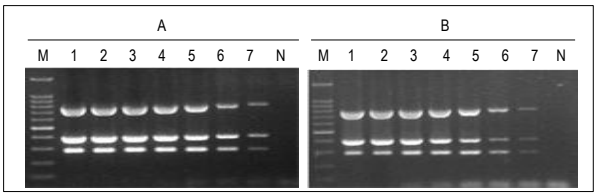
❖ Comparison test for sensitivity

Uniplex PCR for λDNA (1 kbp) is performed. Comparing to competitor's product, 2X Master mix UDG-PCR System is higher than competitor's product.



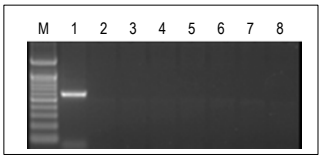
A, 2X Master mix UDG-PCR System; **B,** Competitor
Lane M, 100 bp DNA Marker; **lane N**, Negative control; **lane 1,** 200 pg gDNA, **Lane 2,** 40 pg gDNA; **lane 3,** 8 pg gDNA; **lane 4,** 1.6 pg gDNA; **Lane 5,** 320 fg gDNA; **lane 6,** 64 fg gDNA; **lane 7,** 12.8 fg gDNA

Multiplex PCR for E59 is performed. Comparing to competitor's product, 2X Master mix UDG-PCR System is higher than competitor's product.



A, 2X Master mix UDG-PCR System; **B,** Competitor
Lane M, 100 bp DNA Marker; **lane N**, Negative control; **lane 1,** 50 ng gDNA, **Lane 2,** 10 ng gDNA; **lane 3,** 2 ng gDNA; **lane 4,** 400 pg gDNA; **Lane 5,** 80 pg gDNA; **lane 6,** 16 pg gDNA; **lane 7,** 3.2 pg gDNA

UDG activation is performed.



Lane M, 100 bp DNA Marker;
lane 1, Control (not added UDG);
Lane 2 ~ Lane 8, 2X Master mix UDG-PCR System (7 times repeat test)

ORDERING INFORMATION

Product Name	Amount	Cat. No.
Fast DNA-spin™ Plasmid DNA Purification Kit	50 col.	17095
Fast DNA-spin™ Plasmid DNA Purification Kit	200 col.	17013
DNA-spin™ plasmid DNA Purification kit (Cap Column Type)	50 col.	17096
DNA-spin™ plasmid DNA Purification kit (Cap Column Type)	200 col.	17098
G-spin™ Total DNA Extraction Mini Kit	50 col.	17045
G-spin™ Total DNA Extraction Mini Kit	200 col.	17046
G-spin™ Genomic DNA Extraction Kit (for Bacteria)	50 col..	17121
2X PCR Master mix Sloution (i-StarTaq)	0.5 ml * 2 vials	25266

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Review date : 2017. 04. 13