2X SuperGreen PCR Master Mix

Cat. no. TTC-PA31-5
Storage: Store at -20°C
Product Size: 5 mL

Introduction

The 2X SuperGreen PCR Master Mix is a 2× concentrated optimized mixture composed of Taq DNA Polymerase, the NH_4^+ buffer system, dNTPs, and $MgCl_2$. An inert green dye and a stabilizer are also present to allow direct loading of the final products onto an agarose gel for analysis. The advantages of the 2X SuperGreen PCR Master Mix include convenience and high sensitivity, specificity, and stability. It minimizes human-made errors during the PCR operating process. Additionally, the 2X SuperGreen PCR Master Mix is suitable for routine PCR reaction, amplification of complex templates such as GC rich templates and templates with secondary structure, and large-scale gene detection.

Notes

1. Choose C or G as the last base of the 3'-end of the primer;
2. Avoid continuous mismatching at the last eight bases of the 3'-end of the primer;
3. Avoid a hairpin structure at the 3'-end of the primer;
4. The temperature of the primers should be within the range of 55r; the 3'
5. Additional sequences should not be included when calculating the temperature of the primers;
6. GC content of the primers should be within the range of 40%–60%
7. The temperature and GC content of forward and reverse primers should be as similar as possible.
8. This product is suitable for gene detection, conventional PCR, colony PCR, RT-PCR, genotyping, and amplification of fragments with high GC content.

Example:

Note: The following example is only for reference. Users must set up an optimal reaction system according to their specific reaction conditions.

For a 25μL PCR reaction system: 1 kb fragment of human genomic DNA was amplified using 2X SuperGreen PCR Master Mix (if using a different reaction system, please proportionally increase or decrease the amount of reaction components referring to this system).

Protocol

1. Gently vortex and briefly centrifuge 2X Master Mix after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 25μL reaction:

Component	Volume	Final Conc.
Template (< 1 μg)	X μL	-
Forward Primer (10 μM)	1 μL	0.4 μM
Reverse Primer (10 μM)	1 μL	0.4 μM
2x Master Mix	12.5 μL	-
ddH_2O	up to 25 μL	-
Total volume	25 μL	-

3. Perform PCR using the recommended standard or fast condition outlined below:

Standard condition:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	94˚C	5 min	1 cycle
Denaturation	94˚C	30 sec	25-35 cycles
Annealing	58˚C	30 sec	
Extension	72˚C	1 min	
Final Extension	72˚C	7 min	1 cycle
Soak	4˚C	hold	1 cycle

Fast Condition (recommended for PCR products up to 1kb):

Step	Temperature	Time	Cycles
Initial Denaturation	94˚C	1 min	1 cycle
Denaturation	94˚C	15 sec	25-35 cycles
Annealing	56˚C	15 sec	
Extension	72˚C	10 sec	
Final Extension	72˚C	5 min	1 cycle
Soak	4˚C	hold	1 cycle

4. Result detection：Load 5μL of the PCR product to agarose gel for detection.

Note: The PCR product can be directly loaded to a 1% agarose gel for electrophoresis. In the result, the band of blue dye should be at approximately 4 kb, while the yellow dye should be at <50 bp.

This product is for research only, not for diagnostic or clinical use.