# 2 × Taq Plus Master Mix II (Dye Plus)

**P213-01/02/03**

Version 8.1

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## Introduction

This product contains Taq Plus DNA Polymerase, dNTP and an optimized buffer system for high yield PCR reactions. Compared with common PCR, it has higher fidelity, greater amplification performance and yield, and can be used for PCR amplification of genome-based template within 10 kb and plasmid-based or λDNA-based template within 15 kb. The pre-formulated 2 × Taq Plus Master Mix II can be used for PCR reactions by simply adding primers and templates, reducing pipetting and improving the throughput and reproducibility of results. The addition of a protective agent to the amplification system makes 2 × Taq Plus Master Mix II maintain stable activity after repeated freeze-thaw cycles. This product contains blue double dye, which can be directly electrophoresed after the reaction. The PCR products contain A at the 3'-end and can be directly cloned into T-Vectors, compatible with ClonExpress II One Step Cloning Kit series (Vazyme, #C112, #C113).

## Components

| Components | P212-01 | P212-02 | P212-03 |
|---|---|---|---|
| 2 × Taq Plus Master Mix II(Dye Plus) | 5 × 1 ml | 15 × 1 ml | 50 × 1 ml |

## Storage

Store at -20℃.

## Quality Control

Exonuclease residue detection: 20 μl of this product was incubated with 50 pmol of single-stranded DNA substrate and double-stranded DNA substrate for 16 h at 37℃. After denaturing PAGE electrophoresis, the electrophoresis bands of DNA did not change.

Endonuclease residue detection: 20 μl of this product and 0.3 μg of pBR322 DNA were incubated at 37℃ for 4 h. After electrophoresis on agarose gel, the electrophoresis bands of the plasmid did not change.

E. coli DNA residue detection: 20 μl of the remaining nucleic acid in this product was detected by E.coli gDNA-specific TaqMan qPCR, and the E. coli genome residue was less than 10 copies.

Functional detection: In the 50 μl PCR system, 100 ng mouse genomic DNA, 100 ng wheat genomic DNA, 10 ng λDNA, and 1 μl HeLa cell cDNA were used as templates. Five different fragments of interest ranging from 0.5 kb to 10 kb were amplified. After 35 cycles, 1/10 PCR products were subjected to 1% agarose gel electrophoresis, and EB staining showed a single corresponding band.

## Protocol

**1. General reaction mixture for PCR:**

| | |
|---|---|
| $ddH_2O$ | to 50 μl |
| 2 × Taq Plus Master Mix II (Dye Plus) | 25 μl |
| Primer 1 (10 μM) | 2 μl |
| Primer 2 (10 μM) | 2 μl |
| Template DNA* | x μl |

*The recommended amount of DNA template for a 50 μl reaction system is as follows:

| | |
|---|---|
| Animal and plant Genomic DNA | 0.1 - 1 μg |
| Bacterial Genomic DNA | 10 - 100 ng |
| cDNA | 1 - 5 μl (No more than 1/10 of the total volume of PCR system) |
| Plasmid DNA | 0.1 - 10 ng |
| λDNA | 0.5 - 10 ng |

**2. Thermocycling conditions for a routine PCR:**

| | | | |
|---|---|---|---|
| 95℃ | 3 min (Pre-denaturation)[a] | | |
| 95℃ | 15 sec | } | 30 - 35 cycles |
| 60℃[b] | 20 sec | | |
| 72℃ | 60 sec/kb | | |
| 72℃ | 5-10 min (Final extension) | | |

a. This pre-denaturing condition is suitable for most amplification reactions and can be adjusted according to the complexity of the template structure. If the template structure is complex, the pre-denaturation time can be extended to 5-10 min to improve the pre-denaturation effect.

b. The annealing temperature needs to be adjusted according to the Tm value of the primer, and is generally set to be lower than the primer Tm value of 3-5 ° C; for complex templates, it is necessary to adjust the annealing temperature and extend the extension time to achieve high efficiency amplification.

## Notes

**Primers Designing**

1. Choose C or G as the last base of the 3'-end of the primer;
2. Avoid continuous mismatching at the last 8 bases of the 3'-end of the primer;
3. Avoid hairpin structure at the 3'-end of the primer;
4. It is better that the Tm values of the forward and reverse primers differ by no more than 1℃. Tm of the primers should be within the range of 55℃- 65℃ (Primer Tm values are recommended for calculation using Primer Premier 5);
5. Additional sequence should not be included when calculating Tm of the primers;
6. GC content of the primers should be within the range of 40% - 60%;
7. The overall distribution of primers A, G, C, and T should be as uniform as possible to avoid the use of high GC or AT content;
8. Avoid complementary sequences of more than 5 bases in the primer or between the two primers. The 3' ends of the two primers avoid complementary sequences of more than 3 bases;
9. Use the NCBI BLAST function to search for primer specificity to avoid non-specific amplification after primer design.

## Common problems and solutions

| | No product or insufficient products | Miscellaneous or diffuse bands |
|---|---|---|
| Primers | Optimize primer design | Optimize primer design |
| Asnnealing temperature | Set the annealing temperature gradient to find the proper annealing temperature | Try to increase the annealing temperature to 65℃ at 2℃ intervally. |
| Primer concentration | Increase the concentration of primers properly. | Reduce the primer concentration to a final concentration of 0.2 μM. |
| Extension time | Increase the extension time properly. | Reduces the extension time when there are bands larger than the target band. |
| Number of cycles | Increase the number of cycles to 35 - 40 cycles. | Reduce the number of cycles to 25 - 30 cycles. |
| Template purity | Use templates with high purity. | Use templates with high purity. |
| Template input | Crude samples may need to be reduced in usage; other sample usage refers to the recommended amount of the reaction system and increases in moderation. | The amount of use is adjusted referring to the recommended amount of the reaction system. |

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