# 2 × Phanta Flash Master Mix

**P510**

Version 23.1

## Product Description

2 × Phanta Flash Master Mix is a new generation superior enzyme based on Phanta Flash Super-Fidelity DNA Polymerase. Through directed optimization of Phanta DNA Polymerase, Phanta Flash Super-Fidelity DNA Polymerase has the characteristics of rapid amplification (4 - 5 sec/kb) while maintaining high fidelity and yield. The amplification error rate of Phanta Flash Super-Fidelity DNA Polymerase is 81-fold lower than that of conventional Taq DNA Polymerase. Matched with optimized buffer system, this kit can achieve high amplification specificity. And it has excellent compatibility with crude samples, templates with uracil and GC-rich system (primer/template). This kit contains two types of monoclonal antibodies that inhibit the 5'→3' polymerase activity and 3'→5' exonuclease activity at room temperature, enabling it to perform hot start PCR with great specificity. It contains all required reaction components (Phanta Flash Super-Fidelity DNA Polymerase, dNTP and optimized buffer), except primers and templates, thereby simplifying the operation process and improving the detection throughput and repeatability. Amplification will generate blunt-ended products, which are compatible with ClonExpress kits (Vazyme #C112/C113/C116) and TOPO cloning kit (Vazyme #C603).

## Components

| Components | P510-01 | P510-02 | P510-03 |
|---|---|---|---|
| 2 × Phanta Flash Master Mix | 1 ml | 5 × 1 ml | 15 × 1 ml |

## Storage

Store at -30 ~ -15°C and transport at ≤0°C.

## Applications

It is applicable for amplification reaction of genomic DNA, cDNA, dU-containing DNA and crude samples as templates.

## Notes

For research use only. Not for use in diagnostic procedures.

1. For fragments ≤10 kb, the recommended extension time is 4 - 5 sec/kb. For fragments >10 kb, the recommended extension time is 10 sec/kb.
2. Please use high-quality templates to improve amplification success rate and yield.
3. Phanta Flash Super-Fidelity DNA Polymerase has strong proof-reading activity. If the DNA product needs to perform TA cloning, it is recommended to purify it before adding an A-tail.
4. Primer Design Guidance
   a. It is recommended that the last base at the 3' end of the primer should be G or C.
   b. Consecutive mismatches should be avoided in the last 8 bases at the 3' end of the primer.
   c. Avoid hairpin structures at the 3' end of the primer.
   d. Differences in the Tm value of the forward primer and the reverse primer should be no more than 1°C and the Tm value should be adjusted to 55 ~ 65°C (Primer Premier 5 is recommended to calculate the Tm value).
   e. Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer Tm value.
   f. It is recommended that the GC content of the primer to be 40% - 60%.
   g. The overall distribution of A, G, C, and T in the primer should be as even as possible. Avoid using regions with high GC or AT contents.
   h. Avoid the presence of complementary sequences of 5 or more bases either within the primer or between two primers. Avoid the presence of complementary sequences of 3 or more bases at the 3' end of two primers.
   i. Use the NCBI BLAST function to check the specificity of the primer to prevent nonspecific amplification.

## Experiment Process

### Reaction System

Keep all components on ice during the experiment. Thaw, mix, and briefly centrifuge each fraction before use. After use, please return it to -20°C in time for storage.

| Components | Volume |
|---|---|
| $ddH_2O$ | up to 50 μl |
| 2 × Phanta Flash Master Mix | 25 μl |
| Primer 1 (10 μM) | 2 μl |
| Primer 2 (10 μM) | 2 μl |
| Template DNA* | x μl |

* Optimal reaction concentration varies in different templates. In a 50 μl system, the recommended template usage is as follows:

| Template Types | Amount |
|---|---|
| Genomic DNA | 10 - 500 ng |
| Plasmid or Virus DNA | 5 pg - 20 ng |
| cDNA | 1 - 5 μl (≤1/10 of the total volume of PCR system) |

### Reaction Program

Standard program

| Steps | Temperature | Time | Cycles |
|---|---|---|---|
| Initial Denaturation | 98°C | 30 sec | |
| Denaturation | 98°C | 10 sec | |
| Annealing[a] | Tm | 5 sec | 28 - 35 |
| Extension[b] | 72°C | 4 - 5 sec/kb | |
| Final Extension | 72°C | 1 min | |

Fast program[c]

| Steps | Temperature | Time | Cycles |
|---|---|---|---|
| Denaturation | 98°C | 10 sec | |
| Annealing[a] | Tm | 5 sec | 28 - 35 |
| Extension[b] | 72°C | 4 - 5 sec/kb | |

a. Please set the annealing temperature according to the Tm value of the primers. When the Tm value of the primers is higher than 72°C, the annealing step can be removed (Two-Step PCR). If necessary, annealing temperature can be further optimized through setting temperature gradient. In addition, the amplification specificity depends directly on the annealing temperature. Raising annealing temperature is helpful to improve amplification specificity.

b. Set the extension time according to the following table:

| Target fragment size | Extension time |
|---|---|
| ≤10 kb | 4 - 5 sec/kb |
| >10 kb | 10 sec/kb |

c. Through experimental verification, there is no significant difference in performance when adopting either standard program or fast program. You can choose according to your operating habits.

## FAQ & Troubleshooting

### ◇ No amplification products or low yield

① Primer: Optimize primer design.
② Annealing temperature: Set temperature gradient and find the optimal annealing temperature.
③ Primer concentration: Increase the concentration of primers properly.
④ Extension time: Increase the extension time to 10 - 15 sec/kb properly.
⑤ Cycles: Increase the number of cycles to 36 - 40 cycles.
⑥ Template purity: Use templates with high purity.
⑦ Template amount: Adjust the template amount according to the recommended amount and increase it properly.

### ◇ Nonspecific products or smeared bands

① Primer: Optimize primer design.
② Annealing temperature: Try to increase the annealing temperature and set temperature gradient.
③ Primer concentration: Decrease the concentration of primers properly.
④ Cycles: Decrease the number of cycles to 25 - 30 cycles.
⑤ PCR program: Use Two-Step method or Touchdown PCR program.
⑥ Template purity: Use templates with high purity.
⑦ Template amount: Adjust the template amount according to the recommended amount and decrease it properly.