2× Phanta Max Master Mix (Dye Plus)

Catalog # P525

Version5.1 Vazyme Biotech Co. Ltd.

1 Introduction

Phanta Max Super-Fidelity DNA Polymerase is a new generation superior enzyme based on Phanta DNA Polymerase for robust PCR with higher fidelity. The unique extension factor, specificity-promoting factors and plateau-inhibiting factor newly added to Phanta Max greatly improve its long-fragment amplification ability, specificity, and PCR yield. Phanta Max is capable of amplifying long fragments such as 40 kb λ DNA, 40 kb plasmid DNA, 20 kb genomic DNA and 10 kb cDNA. The amplification error rate of Phanta Max is 53-fold lower than that of conventional Taq and 6-fold lower than that of Pfu. In addition, Phanta Max has a good resistance to PCR inhibitors and can be used for direct PCR amplifications of bacteria, fungi, plant tissues, animal tissues, and even whole blood samples. Phanta Max contains two monoclonal antibodies inhibiting the 5′→3′ polymerase activity and 3′→5′ exonuclease activity at room temperature, which enable Phanta Max to perform greatly-specific Hot-Start PCRs.

2× Phanta Max Master Mix (Dye Plus) contains Phanta Max Super-Fidelity DNA Polymerase, dNTP, an optimized buffer system, and loading dye. The amplification can start only with the addition of primer and template, thereby easing PCR setup and improving reproducibility. Protective agents in the 2× Phanta Max Master Mix enable the resistance to repeated freeze-thaw cycles. Amplification will generate blunt-ended products, which are compatible with ClonExpress II One Step Cloning Kit Series (Vazyme, Cat. No. #C112, #C113, #C114, #C115).

2 Package Information

Components	P525-01	P525-02	P525-03
2× Phanta Max Master Mix (Dye Plus)	1 ml	5 × 1 ml	15 × 1 ml

3 Storage

Store at -20℃; avoid repeated freezing and thawing.

4 Unit Definition

One unit (U) is defined as the amount of enzyme that incorporates 10 nmol of whole dNTPs into acid-insoluble products in 30 minutes at 74℃ with activated salmon sperm DNA as the template / primer.

5 Quality Control

Residual Endonulease Test: The product is tested in a reaction containing 25 μl of 2× lPhanta Max Master Mix and 0.3 μg of Supercoiled pBR322 DNA. After incubation at 37℃ for 4 hours, there is no visually discernible change in DNA bands determined by agarose gel electrophoresis.

Residual *E.Coli* gDNA Test: The residual nucleotide in 25 μl of 2× lPhanta Max Master Mix is tested by SYBR® Green qPCR using specific primers of *E.Coli* gDNA. The residual *E.Coli* gDNA is lower than 10 copies.

Functional Assay 1: In a 50μl PCR system with 1U of 2× Phanta Max Master Mix, 100 ng of human genomic DNA was used as template. After 35 cycles, 1/10 of PCR products were detected by 1% agarose gel electrophoresis. A single DNA band of 8.2 kb was detected after EB staining.

Functional Assay 2: In a 50μl PCR system with 1U of 2× Phanta Max Master Mix, 10 ng of λ DNA was used as template. After 30 cycles, 1/10 of PCR products were detected by 1% agarose gel electrophoresis. A single DNA band of 15 kb was detected after EB staining

6 Experimental Process

6.1 For Conventional PCR

Recommended PCR System

Keep all components on ice during the experiment. All components need to be mixed up thoroughly after thawing and put back to -20℃ immediately for storage after using.

ddH$_2$O	up to 50 μl
2 × Phanta Max Master Mix (Dye Plus)	25 μl
Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl
Template DNA[a]	x μl

The PCR Enhancer (Vazyme Cat. # P021) is recommended for unsuccessful amplification of fragments with GC content > 60%.
a. Optimal reaction concentration varies in different templates. In a 50 μl system, the recommended template usage is as follows:

Templates	Input Template DNA
Genomic DNA	50 - 400 ng
Plasmid or Virus DNA	10 pg- 30 ng
cDNA	1 - 5 μl (≤ 1/10 of the total volume of PCR system)

Recommended PCR Program

Steps	Temperature	Time	Cycles
Pre-denaturation[a]	95℃	30 sec / 3 min	1
Denaturation	95℃	15 sec	25 - 35
Annealing[b]	56℃ - 72℃	15 sec	
Extension[c]	72℃	30 - 60 sec / kb	
Final Extension	72℃	5 min	1

a. For pre-denaturation, the recommended temperature is 95℃, and the recommended time is 30 sec for plasmid / virus DNA and 3 min for genomic DNA / cDNA.

b. For annealing, the recommended temperature is the Tm of the primers. If the Tm of the primers is higher than 72℃, the annealing step can be removed (two-step PCR). If necessary, annealing temperature can be further optimized in a gradient. In addition, the amplification specificity depends directly on the annealing temperature. Raising annealing temperature is helpful to improve poor amplification specificity.

c. Longer extension time is helpful to increase the amplification yield.

6.2 For Long-fragment PCR

Phanta Max Super-Fidelity DNA Polymerase can extraordinarily perform a long-fragment amplification with high specificity and yields. If the recommended program is failure to work, the following Touch Down two-step PCR may be helpful:

Steps	Temperature	Time	Cycles
Pre-denaturation	95℃	3 min	1
Denaturation	92℃	15 sec	5
Extension	74℃	60 sec / kb	
Denaturation	95℃	15 sec	5
Extension	72℃	60 sec / kb	
Denaturation	95℃	15 sec	5
Extension	70℃	60 sec / kb	
Denaturation	95℃	15 sec	25
Extension	68℃	60 sec / kb	
Final Extension	68℃	5 min	1

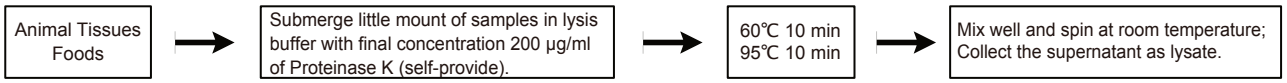
It is recommended to use high-quality templates and long primers . Increasing the input of template DNA may be helpful to improve the amplification yield.

6.3 For PCR Using Crude Material as Template

Phanta Max Master Mix have a good resistance to PCR inhibitors and can be used for direct PCR amplifications of bacteria, fungi, plant tissues, animal tissues, and even whole blood samples. Crude materials that have been successfully amplified with Phanta Max Master Mix are as follows:

Sample Type	Amplification Method	Template Recommendation (for a 50 μl PCR system)
Whole Blood	Direct PCR	1 - 5 μl
Filter Paper Dry Blood	Direct PCR	1 - 2 mm^2 filter paper
Cultured Cells	Direct PCR	Little amount of cells
Yeast	Direct PCR	A monoclone or 1 μl suspension
Bacteria	Direct PCR	A monoclone or 1 μl suspension
Mold	Direct PCR	Little amount of sample
Sperm	Direct PCR	Little amount of sample
Plankton	Direct PCR	Little amount of sample
Plant Tissue	Direct PCR	1 - 2 mm^2 tissue
Mouse Tail	PCR with lysate	1 - 5 μl lysate*
Food	PCR with lysate	1 - 5 μl lysate*

* Lysate Preparation:



Lysis Buffer: 20 mM of Tris-HCl, 100 mM of EDTA, 0.1% SDS, pH 8.0 (not included in this kit).

7 Attentions

1. Use high-quality templates.
2. DO NOT use dUTP or any primers or templates that contain uracil.
3. The Phanta Max Super-Fidelity DNA Polymerase has strong proofreading activity. Therefore, the PCR products must be purified before adding A-Tailing when TA cloning.
4. Primers design notes:
   - Choose C or G as the last base of the 3'-end of the primer.
   - Avoid continuous mismatching at the last 8 bases of the 3'-end of the primer.
   - Avoid hairpin structure at the 3'-end of the primer.
   - Tm of the primers should be within the range of 55℃ - 65℃ (recommend to calculate in Primer Premier 5), and the Tm difference between F and R primers should be less than 1℃.
   - Additional sequence should not be included when calculating Tm of the primers.
   - GC content of the primers should be within the range of 40% - 60%.
   - The general distribution of A, G, T, C in the primers should be uniform, and avoid using regions with rich GC and rich AT.
   - Keep complementary sequence less than 5 bases within the primers or between two primers, and complementary sequence less than 3 bases at the 3'-end of the primers.
   - Please search the specificity of the designed primers by NCBI BLAST to avoid non-specific amplification.

8 Troubleshooting

No or Low Yield of PCR Products

Primers	Optimize primer design
Annealing Temperature	Set gradient annealing temperature to find out the optimal one
Concentration of Primers	Appropriately improve the concentration of primers
Extension Time	Appropriately increase the extension time to 30 sec/kb-1 min/kb
Cycle Numbers	Increase cycle numbers to 35 - 40
Purity of Templates	Use high - purity templates
Template Input	Refer to the recommended reaction system and increase the input properly

Unspecific or Smear Bands in Electrophoresis

Primers	Optimize primer design
Annealing Temperature	Try to improve annealing temperature and set gradient annealing temperature to optimize
Concentration of Primers	Decrease the concentration of primers to final concentration as 0.2 μM
Extension Time	Appropriately decrease the extension time when blend bands longer than target bands appears
Cycle Numbers	Decrease cycle numbers to 25 - 30
PCR Programs	Use Two-Step PCR or Tough down PCR
Purity of Templates	Use high purity templates
Template Input	Modify or decrease templates input refering to the recommended reaction system