

2 × Phanta Ultra Master Mix (Dye Plus)

P528

Version 25.1



Product Description

2 × Phanta Ultra Master Mix (Dye Plus) is a new generation high-fidelity DNA polymerase carefully selected based on the BioSmart platform, featuring ultra-high fidelity, 5 sec/kb rapid amplification, and broad T_m value compatibility. Combined with the latest hot-start technology and superior buffer system, this product demonstrates high success rate and specificity at universal annealing temperature, enabling amplification of fragments up to 40 kb in length. Its fidelity is up to 200-fold higher than that of *Taq* polymerase, providing an efficient and convenient solution for PCR experiments requiring highly accurate amplification, such as molecular cloning, sequencing, and site-directed mutagenesis. 2 × Phanta Ultra Master Mix (Dye Plus) is a premix containing Phanta Ultra DNA Polymerase, dNTPs, and Mg^{2+} as essential components for PCR amplification. It only requires the addition of primers and templates for amplification, thereby reducing pipetting operations and improving detection throughput and result stability. This product is applicable for standard templates, crude samples, and GC-rich systems (including primers and templates). In addition, it contains a tracking dye, allowing direct gel electrophoresis analysis after PCR amplification. The amplification products are blunt-ended, subsequently applicable for ClonExpress Ultra One Step Cloning Kit V3 (Vazyme #C117).

Components

Components	P528-01	P528-02	P528-03
<input type="checkbox"/> 2 × Phanta Ultra Master Mix (Dye Plus)	1 ml	5 × 1 ml	15 × 1 ml

Storage

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

Applications

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

Notes

1. Please use high-quality templates to increase the success rate and yield of amplification.
2. If TA cloning needs to be performed, it is recommended to purify the DNA before adding the adenine.
3. Primer Design Guidance:
 - a. It is recommended that the primer length be 21 - 25 nt, and 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, and also avoid the formation of hairpin structures.
 - c. Differences in the T_m value of the forward primer and the reverse primer should be no more than 1°C. The T_m value should be adjusted to 55 ~ 65°C (Primer Premier 5 is recommended to calculate the T_m value).
 - d. The T_m value difference between multiple PCR primers should be as small as possible, while minimizing the complementary base pairing between primer pairs.
 - e. Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer T_m value; it is recommended that the GC content of the primer be 40% - 60%.
 - f. The overall distribution of Primer A, G, C, and T should be as uniform as possible, avoiding regions with high GC or AT content.
 - g. 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.
 - h. When amplifying long fragments (≥5 kb), the length of the primer should be 25 - 35 nt and the T_m value should be >62°C.
 - i. Primer cannot contain inosine (I) or uracil (U).
 - j. After the primer design, please use NCBI BLAST function to check primer specificity to avoid nonspecific amplification.

Experiment Process

Reaction System

Thaw and mix each component thoroughly before use. Please return components to -20°C in time for storage.

Components	Volume
Nuclease-free ddH ₂ O	up to 50 µl
2 × Phanta Ultra Master Mix (Dye Plus)	25 µl <input type="checkbox"/>
Primer 1 (10 µM)	1.5 µl
Primer 2 (10 µM)	1.5 µl
Template DNA ^a	x µl

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

Template Types	Amount
Genomic DNA	5 - 200 ng (≥10 kb fragments, 100 ng input is recommended)
Plasmid or Virus DNA	100 pg - 50 ng (For plasmid, 1 ng input is recommended)
cDNA	1 - 5 µl (≤1/10 of the total volume of PCR system)
Crude samples	1 - 5 µl (≤1/10 of the total volume of PCR system)

Reaction Program

Steps	Temperature	Time	Cycles
Denaturation	98°C	10 sec	28 - 35 ^c
Annealing ^a	T _m °C	5 sec	
Extension ^b	68°C	5 - 10 sec/kb	

a. ① When the T_m values of both forward and reverse primers are >50°C, the annealing temperature should be 60°C; ② When the T_m value of one of the forward or reverse primer is ≤50°C or both primers have T_m values ≤50°C, the annealing temperature should be 50°C; ③ It can also set the annealing temperature according to the T_m value of the primer itself.

b. Please set the extension time according to the length of the target fragment using the following methods:

PCR Types	Target Fragment Size	Extension Time
Singleplex PCR	<10 kb	5 sec/kb
	≥10 kb	10 sec/kb
Multiplex PCR	≤10 kb (Maximum Length)	10 sec/kb

c. For multiplex PCR, it is recommended to use 30 cycles. Excessive cycle numbers may affect amplification uniformity.

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 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 Template purity: Use templates with high purity.
- ⑥ Template amount: Adjust the template amount according to the recommended amount and decrease it properly.

◇ Products plugged agarose wells

- ① Experimental environment: To avoid aerosol pollution, the experimental environment needs to be thoroughly cleaned, or the operating environment, experimental reagents and consumables should be replaced before re-amplification.
- ② Template amount: Decrease the amount of templates.
- ③ Cycles: Decrease the number of cycles to 25 - 30 cycles.
- ④ Annealing temperature: Set temperature gradient and find the optimal annealing temperature.

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