2 × Rapid Taq Plus Master Mix (Dye Plus)

P223

Version 24.1

Product Description

Rapid Taq Plus DNA Polymerase is a new generation *Taq* DNA polymerase with a high amplification success rate. This product combines high blocking rate dual-species antibodies (binding different antigenic epitopes) and an optimized buffer system, demonstrating excellent success rate and specificity at an extension rate of 1 - 10 sec/kb. The pre-prepared 2 × Master Mix only requires the addition of primers and templates for PCR amplification, reducing pipetting operations and increasing detection throughput and result reproducibility. This product has robust amplification performance and high storage stability. It supports PCR amplification of genomic, plasmid, and λDNA templates up to 15 kb, and cDNA templates up to 10 kb. The unique protectant added to the product ensures that 2 × Master Mix does not freeze when stored at -20°C, allowing for convenient use. Additionally, this product contains tracking dyes, enabling direct electrophoresis analysis after the reaction. The amplification products are applicable for ClonExpress Ultra One Step Cloning Kit V3 (Vazyme #C117) and Ultra-Universal TOPO Cloning Kit (Vazyme #C603).

Components

Components	P223-01	P223-02	P223-03	P223-04
2 × Rapid Taq Plus Master Mix (Dye Plus)	5 × 1 ml	15 × 1 ml	50 × 1 ml	10 × 5 ml

Storage

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

Applications

It is applicable for amplifying DNA by rapid PCR.

Notes

Primer Design Guidance

1. It is recommended that the last base at the 3' end of the primer should be G or C。
2. 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.
3. Differences in the Tm value of the forward primer and the reverse primer should be no more than 1°C. The Tm value should be adjusted to 55 ~ 65°C (Primer Premier 5 is recommended to calculate the Tm value).
4. The Tm differences between the primers in multiple PCR should be as small as possible, while minimizing the complementary pairing between the primer pairs.
5. Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer Tm value. The recommended GC content of primers is 40% - 60%.
6. The overall distribution of Primer A, G, C, and T in the primer should be as uniform as possible. Avoid using regions with high GC or AT contents.
7. Avoid the presence of complementary sequences of 5 or more bases either within the primer or between two primers and avoid the presence of complementary sequences of 3 or more bases at the 3' end of two primers。
8. When amplifying long fragments (≥5 kb), the length of the primer should be 25 - 35 nt and the Tm value should be >62°C.
9. Use the NCBI BLAST function to check the specificity of the primer to prevent nonspecific amplification.

Experiment Process

Reaction system

Components	Volume
ddH_2O	up to 50 μl
2 × Rapid Taq Plus Master Mix (Dye Plus)	25 μl
Forward primer (10 μM)	1.5 μl
Reverse primer (10 μM)	1.5 μl
Template[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	10 - 200 ng
Plasmid or Virus DNA	50 pg - 50 ng
cDNA or Crude samples	1 - 5 μl (≤1/10 of the total volume of PCR system)

Reaction program

Steps	Temperature	Time	cycles
Initial Denaturation	95℃	2 min	
Denaturation	98℃	10 sec	28 - 35
Annealing[a]	Tm	30 sec	
Extension[b]	68℃	1 - 10 sec/kb	
Final Extension	68℃	5 min	

a. Please set the annealing temperature according to the Tm value of the primers. If necessary, the annealing temperature can be further optimized through setting the temperature gradient. In addition, the amplification specificity depends directly on the annealing temperature. Increasing the annealing temperature can improve the specificity of amplification.

b. Please refer to the following table to set the extension time based on the length of the target fragment:

PCR Types	Size	Recommended Extension Time
Singleplex PCR	<2 kb	1 sec/kb
	<5 kb	5 sec/kb
	≤10 kb	10 sec/kb

To increase the yield of PCR products, please extend the extension time appropriately.

This product is compatible with 7-plex PCR amplification (≤10 kb fragment), and the recommended extension time for multiple amplifications is 15 sec/kb.

FAQ & Troubleshooting

	No amplification products or low yield	Nonspecific bands or smear bands
Primer	Optimize primer design	Optimize primer design
Annealing temperature	Set temperature gradient and find the optimal annealing temperature	Try to increase the annealing temperature to 65℃ at 2℃ intervally
Primer concentration	Increase the concentration of primers properly	Decrease the final concentration of primer to 0.2 μM
Extension time	Increase the extension time properly	Reduces the extension time when there are nonspecific bands larger than the target bands
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
Input amounts of template	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	Adjust the dosage according to the recommended amount of the reaction system

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