2 × Taq Plus Master Mix

P211/P212

Version 21.1

Product Description

2 × Taq Plus Master Mix contains Taq Plus DNA Polymerase, dNTP and an optimized buffer system. Compared with Taq DNA Polymerase, it has higher fidelity, stronger amplification performance and yield. It can be used for PCR amplification within 10 kb using genome as template and PCR amplification within 15 kb using plasmid and λDNA as template. The pre-prepared 2 × Master Mix only needs to add primers and templates to perform amplification when used in PCR reactions, which reduces pipetting operations and improves detection throughput and results reproducibility. The protective agent added to the system allows 2 × Master Mix to maintain stable activity after repeated freezing and thawing. The kit provides a version containing electrophoresis buffer and dyes, which can be directly electrophoresed after the reaction that is convenient to use. The PCR product has A-tailing at the 3' end, which can be cloned into T vector and is suitable for ClonExpress and TOPO cloning kits (Vazyme #C112/C113/C115/C601).

Components

Components	P211-01	P211-02	P211-03
2 × Taq Plus Master Mix	5 × 1 ml	15 × 1 ml	50 × 1 ml

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

Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

Applications

It is applicable for amplification reaction of animal, plant and microbial DNA.

Notes

Primer Design Guidance

1. It is recommend that the last base at the 3' end of primer should be G or C.
2. Consecutive mismatches should be avoided in the last 8 bases at the 3' end of the primer.
3. Avoid hairpin structures at the 3' end of the primer.
4. Differences in the Tm value of the forward primer and the reverse primer should be no more than 1℃ and the Tm value should be adjusted to 55 ~ 65℃ (Primer Premier 5 is recommended to calculate the Tm value).
5. Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer Tm value.
6. Control the GC content of the primer to be 40% - 60%.
7. 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.
8. 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.
9. Use the NCBI BLAST function to check the specificity of the primer to prevent non-specific amplification.

Experiment Process

Reaction System

ddH_2O	To 50 μl
2 × Taq Plus Master Mix	25 μl
Primer 1 (10 μM)	2 μl
Primer 2 (10 μM)	2 μl
Template DNA*	x μl

* The optimal reaction concentration is different for different templates. The following table shows the recommended template usage for 50 μl reaction system:

Genomic DNA of Animals and Plants	0.1 - 1 μg
Escherichia coli genomic DNA	10 - 100 ng
cDNA	1 - 5 μl (not exceed 1/10 of the total volume of the PCR reaction)
Plasmid DNA	0.1 - 10 ng
λDNA	0.5 - 10 ng

Reaction Program

95˚C	3 min (Initial denaturation)[a]	
95˚C	15 sec	30 - 35 cycles
60˚C[b]	15 sec	
72˚C	60 sec/kb	
72˚C	5 min (Final extension)	

a. The initial denaturation conditions are suitable for most amplification reactions and can be adjusted according to the complexity of the template structure. If the template structure is complex, the initial denaturation time can be extended to 5 - 10 min to improve the initial denaturation effect.

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

FAQ & Troubleshooting

	No amplification products or low yield	Unspecific products 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˚C at 2˚C 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 unspecific 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