2 × Rapid Taq Master Mix

#Cat: NB-54-0145-01 Size: 5x1ml
#Cat: NB-54-0145-04 Size: 10×5ml
#Cat: NB-54-0145-02 Size: 15×1ml
#Cat: NB-54-0145-03 Size: 50×1ml

Product Description

2 × Rapid Taq Master Mix contains Taq DNA Polymerase, elongation promoting factor, dNTP and an optimized buffer system. The amplification speed of this product can reach 15 sec/kb, which is suitable for rapid PCR. The maximum amplification speed within 1 kb can reach 1 sec/kb, greatly saving reaction time. The pre-prepared 2 × Master Mix only needs to add primers and templates to perform amplification, which reduces pipetting operations and improves detection throughput and results reproducibility. The kit has excellent amplification performance and high storage stability. It is suitable for PCR amplification within 5 kb using genome as template and PCR amplification within 10 kb using plasmid and λDNA as template. 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 green loading dye, which can be directly electrophoresed after the reaction that is convenient to use. The PCR product has a poly(A) tail at the 3' end, which can be directly cloned into T vectors and is suitable for ClonExpress and TOPO cloning kits (Neo Biotech # NB-54-0002/ NB-54-0003/ NB-54-0004/ NB-54-0009).

Components

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2 × Rapid Taq Master Mix	5 × 1 ml	15 × 1 ml	50 × 1 ml	10 × 5 ml

Storage

Store at -30 ~ -15℃ and transport at ≤0℃. It can be stored at 4℃ for 3 months after thawing.

Applications

It is applicable for fast PCR method to amplify DNA.

Notes

For research use only. Not for use in diagnostic procedures.
Agarose gel electrophoresis: In 1% agarose gel electrophoresis, the blue dye and yellow dye are near the 4 kb and 50 bp positions, respectively.

Primer Design Guidance

1. It is recommended 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 50.0 µl
2 × Rapid Taq Master Mix	25.0 µl
Primer 1 (10 µM)	2.0 µl
Primer 2 (10 µM)	2.0 µl
Template DNA*	x µl

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

Genomic DNA of Animals and Plants	0.1 - 1 µg
Escherichia coli genomic DNA	10 - 100 ng
cDNA	1 - 5 µl (≤1/10 of the total volume of PCR system)
Plasmid DNA	0.1 - 10 ng
λDNA	0.5 - 10 ng

Reaction Program

95℃	3 min (Initial denaturation)[a]	
95℃	15 sec	30 - 35 cycles
60℃[b]	15 sec	
72℃	15 sec/kb[c]	
72℃	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℃ 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.
c. For higher yields, the extension time can be set to 2 - 5 sec for PCR reactions with products less than 1 kb; for PCR reactions with products more than 1 kb, the extension time can be extended to 20 - 30 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