

Super PCR MasterMix 2X

<< For Research Use Only >>

Cat No :YT1553 Size: 1ml**Storage:** Store at -20°C.**Introduction**

The Enzyme used in this mastermix is *Taq*plus. *Taq* Plus DNA polymerase is a mixture of *Taq* DNA polymerase and an enzyme containing 3'→5' exonuclease activity.

Its fidelity is 6 times greater than that of *Taq* DNA Polymerase. Compared with *Taq* DNA Polymerase, *Taq* Plus DNA polymerase has stronger amplification performance, higher sensitivity, and is more tolerant of impurities within 5kb amplifying range.

2X super PCR MasterMix contains everything required for PCR, except primers and template, thereby easing PCR setup and improving reproducibility. It can amplify up to **10 kb** from human genomic DNA or up to **15 kb** from λ DNA. Protective agents in the 2X super PCR MAsterMix enable the resistance to repeated freeze-thaw cycles.

Attention: if you have more than 20 freeze-thaw cycles, then first aliquot the mastermix.

Dyes contained in 2X super PCR MAsterMix enable direct loading PCR products onto agarose gels. The obtained PCR

PCR products contain A at the 3'ends and can be directly cloned into T-Vectors.

Package Information**Components**

2X super PCR MAsterMix (Dye Plus) Cat No: YT1553 Size:1ml

Unit Definition

One unit (U) is defined as the amount of enzyme that incorporates 10 nmol of dNTPs into acid-insoluble products in 30

minutes at 74°C with activated salmon sperm DNA as the template/primer.

Protocol

General reaction Mixture for PCR	50 μ l reaction	25 μ l reaction
ddH ₂ O	To 50 μ l	To 25 μ l
Super PCR mastermix 2X	25 μ l	12.5 μ l
Template DNA*	Optional	Optional
Primer1 (10 μ M)	2 μ l	1 μ l
Primer 2 (10 μ M)	2 μ l	1 μ l

***Recommended amount of DNA template for a 50 μ l reaction system is as follow:**

Human genomic DNA	0.1~1 μ g
Bacterial Genomic DNA	10~100ng
λ DNA	0.5~5ng
Plasmid DNA	0.1~10ng

Thermocycling Condition for a routine PCR

94°C	5 min (pre-denaturation)	
94°C	30 sec	} 35 Cycles
55°C *	30 sec	
72°C	60 sec/kb	
72°C	7 min (final extention)	
4°C	Hold	

***The optional annealing temperature should be 1-2°C lower than the T_m of primers used.**

Primers Designing Notes

1. Choose C or G as the last base of the 3' end of the primer;
2. Avoid continuous mismatching at the last 8 bases of the 3' end of the primer;
3. Avoid hairpin structure at the 3' end of the primer;
4. T_m of the primers should be within the range of 55°C~65°C;
5. Additional sequence should not be included when calculating T_m of the primers;
6. GC content of the primers should be within the range of 40%~60%;
7. T_m and GC content of forward and reverse primers should be as similar as possible.