Taq 2X PCR Master Mix

Catalog: RK20602
Size: 100 RXN / 500 RXN
Concentration: 2X
Components:

Taq 2X PCR Master Mix	RM20350

Product Description

Taq DNA Polymerase possesses a 5'→3' polymerase activity and 3' adenine (A) addition activity.

Taq 2X Master Mix is an optimized ready-to-use solution containing *Taq* DNA Polymerase, dNTPs, $MgCl_2$, KCI and stabilizers. It is ideally suited to routine PCR applications on various templates including pure DNA solutions, bacterial colonies, and cDNA products. It can amplify up to 4 kb from complex genomic DNA or up to 5 kb from lambda DNA. Applicable to PCR, colony PCR and primer extension.

Storage Temperature: -20 ℃

Heat Inactivation: No

5' - 3' Exonuclease: Yes

3' - 5' Exonuclease: No

Strand Displacement: +

Resulting Ends: Single-base 3 ´Overhangs

Error Rate: ~ 285x10^{-6} bases

1X Master Mix Composition:

10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 0.08% IPGAL 630, 0.05% Tween 20, pH8.6@25 ℃; 200 μM dNTPs, 5% Glycerol, 25 U/ml *Taq* DNA Polymerase.

Instructions

Reaction setup:

We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (95 ℃).

Take 25 μl /50 μl system as an example.

Composition	25 μl	50 μl	Final Conc.
Nuclease-free H_2O	to 25 μl	to 50 μl	
10 μM Forward Primer	0.5 μl	1 μl	0.2μM (0.05~1 μM)
10 μM Reverse Primer	0.5 μl	1 μl	0.2μM (0.05~1 μM)
Template DNA	variable	variable	<1 μg/50 μl
Taq 2x PCR Master Mix	12.5 μl	25 μl	1X

Incubated in a thermocycler as the below program:

Temperature	Time	Cycles
95 ℃	30s	1
95 ℃ 45-68 ℃ 68 ℃	15-30s 15-60s 1 kb/min	30
68 ℃ 4-10 ℃	5min ∞	1

General Guidelines:

1. Template:

Use of high quality, purified DNA templates greatly enhances the success of PCR. Recommended amounts of DNA template for a 50 μl reaction are as follows:

DNA	Amount
Genomic	1 ng–1 μg
Plasmid or viral	1 pg–1 ng

2. Primers:

Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as Primer3 (http://frodo.wi.mit.edu/primer3) can be used to design or analyze primers. The final concentration of each primer in a reaction may be 0.05–1 μM, typically 0.1–0.5 μM.

3. Mg++ and additives:

Mg++ concentration of 1.5–2.0 mM is optimal for most PCR products generated with *Taq* DNA Polymerase. The final Mg++ concentration in 1X *Taq* PCR Master Mix is 1.5 mM. This supports satisfactory amplification of most amplicons. However, Mg++ can be further optimized in 0.5 or 1.0 mM increments using $MgCl_2$.

Amplification of some difficult targets, like GC-rich sequences, may be improved with additives, such as DMSO or formamide.

4. Denaturation:

An initial denaturation of 30 seconds at 95 ℃ is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences, a longer denaturation of 2-4 minutes at 95 ℃ is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 5 minutes denaturation at 95 ℃ is recommended.

During thermocycling a 15–30 second denaturation at 95 ℃ is recommended.

5. Annealing:

The annealing step is typically 15–60 seconds. Annealing temperature is based on the T_m of the primer pair and is typically 45–68 ℃. Annealing temperatures can be optimized by doing a temperature gradient PCR starting 5 ℃ below the calculated T_m.

When primers with annealing temperatures above 65 ℃ are used, a 2-step PCR protocol is possible.

6. Extension:

The recommended extension temperature is 68 ℃. Extension times are generally 1 minute per kb. A final extension of 5 minutes at 68 ℃ is recommended.

7. Cycle number:

Generally, 25–35 cycles yields sufficient product. Up to 45 cycles may be required to detect low-copy-number targets.

8. 2-step PCR:

When primers with annealing temperatures above 65 ℃ are used, a 2-step thermocycling protocol is possible.

Thermocycling conditions for a routine 2-step PCR:

Temperature	Time	Cycles
95 ℃	30s	1
95 ℃ 65-68 ℃	15-30s 1kb/min	30
65-68 ℃ 4-10 ℃	5min ∞	1

9. PCR product:

The PCR products generated using *Taq* DNA Polymerase contain dA overhangs at the 3´-end; therefore the PCR products can be ligated to dT/dU-overhang vectors.