Taq 2X PCR Master Mix with Dye

Catalog: RK20604
Size: 100 RXN / 500 RXN
Concentration: 2X
Components:

Taq 2X PCR Master Mix with Dye	RM20375

Product Description

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

Taq 2x Master Mix with Dye is an optimized ready-to-use solution containing *Taq* DNA Polymerase, dNTPs, $MgCl_2$, KCI and stabilizers, as well as two commonly used tracking dyes for DNA gels. On a 1% agarose gel in 1X TBE, Xylene Cyanol FF migrates at ~4 kb and Tartrazine migrates at ~10 bp. Both dyes are present in concentrations that do not mask comigrating DNA bands. It is ideally suited to routine PCR applications from 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 the PCR reaction, colony PCR, primer extension, *etc*.

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, 1X Xylene Cyanol, 1X Tartrazine.

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 water	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 with Dye	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 1kb/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 with Dye 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 Tm 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 1 kb/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.