HotStart *Taq* 2X PCR Master Mix

Catalog: RK20603
Size: 100 RXN / 500 RXN
Concentration: 2X
Components:

HotStart *Taq* 2X PCR Master Mix	RM20351

Product Description

HotStart *Taq* DNA polymerase is an innovative antibody-modified thermostable enzyme. The activity of the enzyme is inhibited at room temperature, avoiding non-specific amplification and primer dimer formation during preparation of the sample and the low initial first cycle temperature during PCR cycling, increasing the specificity of DNA amplification.

HotStart *Taq* 2X PCR Master Mix is an optimized, ready-to-use solution containing HotStart *Taq* DNA Polymerase, dNTPs, $MgCl_2$, KCl, and stabilizers. It is ideally suited to routine PCR applications from templates such as 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°C

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°C; 200 μM dNTPs, 5% Glycerol, 25 U/ml HotStart *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°C).

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	0.2 μM (0.05~1 μM)
10 μM Reverse Primer	0.5 μl	1 μl	0.2 μM (0.05~1 μM)
Template DNA	variable	variable	<1 μg/50 μl
HotStart *Taq* 2X PCR Master Mix	12.5 μl	25 μl	1X

Incubated in a thermocycler as the below program:

Temperature	Time	Cycles
95°C	5 min	1
95°C 45-68°C 68°C	15-30 s 15-60 s 1 kb/min	30
68°C 4-10°C	5 min ∞	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 HotStart *Taq* PCR Master Mix is 1.5 mM. This supports the 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°C 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°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 5 minutes denaturation at 95°C is recommended.

During thermocycling, a 15–30 second denaturation at 95°C 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°C. Annealing temperatures can be optimized by doing a temperature gradient PCR starting 5°C below the calculated T_m.

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

6. **Extension:**

The recommended extension temperature is 68°C. Extension times are generally 1 minute per kb. A final extension of 5 minutes at 68°C 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°C are used, a 2-step thermocycling protocol is possible.

Thermocycling conditions for a routine 2-step PCR:

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

9. **PCR product:**

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