Pfu-fast 2X PCR Master Mix

Catalog: RK20652
Size: 100 RXN/500 RXN
Concentration: 2X
Components:

Pfu-fast 2X PCR Master Mix	RM20450

Product Description

Pfu DNA Polymerase is a high-fidelity, thermostable enzyme isolated from *Pyrococcus furiosus*. *Pfu* DNA Polymerase possesses 3′→5′ exonuclease (proofreading) activity. Base misincorporations that may occur during polymerization are rapidly excised by this proofreading activity.

Pfu-fast DNA Polymerase has been genetically engineered to enhance the amplification efficiency by fusing DNA binding domains to the *Pfu* DNA polymerase. *Pfu*-fast DNA Polymerase has the characteristics of high amplification speed (extension ~2 kb/min), high fidelity and strong anti-interference ability.

Consequently, *Pfu*-fast DNA Polymerase is recommended for use in PCR and primer extension reactions that require high-fidelity synthesis. *Pfu*-fast DNA Polymerase-generated PCR fragments are blunt-ended.

Pfu-fast 2X PCR Master Mix is an optimized ready-to-use solution containing *Pfu*-fast DNA Polymerase, dNTPs, $MgCl_2$, KCI and stabilizers.

1X Master Mix Composition:

10 mM Tris-HCl, 1.5 mM $MgCl_2$, 50 mM KCl, 0.08% IPGAL630, 0.05% Tween 20, pH8.6 @25 ℃; 200 uM dNTPs, 5% Glycerol, 40 U/ml *Pfu*-fast DNA Polymerase.

Storage Temperature: -20 ℃

Heat Inactivation: No

5' - 3' Exonuclease: No

3' - 5' Exonuclease: Yes

Instructions

Reaction setup:

We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (98 ℃).

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
Pfu-fast 2X PCR Master Mix	12.5 µl	25 µl	1X

Notes: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Incubated in a thermocycler as the below program:

Temp	Plasmid /Genome DNA	cDNA	Cycles
95 ℃	2 min	1 min	1
95 ℃	20 s	20 s	Plasmid/genome: 30-35; cDNA: 40.
T_m-5 ℃	20 s	20 s	
72 ℃	15 s (≤1 kb) or 2-4 kb/min (>1 kb)	30 s (≤1 kb) or 2 kb/min (>1 kb)	
72 ℃	5 min	5 min	1
4-10 ℃	∞	∞	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 can be used to design or analyze primers. The final concentration of each primer in a PCR 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 *Pfu*-fast DNA Polymerase. The final Mg++concentration in 1X *Pfu*-fast 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 minute incubation at 95 ℃ is recommended to lyse cells.

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.

6. Extension:

The recommended extension temperature is 72 ℃. Extension times are generally 30S per kb. A final extension of 5 minutes at 72 ℃ is recommended.

7. Cycle number:

Generally, 30–35 cycles yield sufficient product. Up to 40 cycles may be required to detect low-copy-number targets.