Pfu-fast DNA Polymerase (Mg^{2+} Plus Buffer)

Catalog: RK20650
Size: 1,000 U / 5,000 U
Concentration: 2,000 U/ml
Components:

Pfu-fast DNA Polymerase (2,000 U/ml)	RM20400
10X PCR Reaction Buffer, Mg^{2+} plus	RM20101

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.

Product Source:
An *E.coli* strain that carries the *Pfu* DNA Polymerase gene from *Pyrococcus furiosus*.

Unit Definition:
One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 72 ℃.

Reaction Conditions:
1X PCR Reaction Buffer, Mg^{2+} plus

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20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100, pH 8.8 @ 25 ℃
Storage Temperature: -20 ℃

Storage Conditions:
10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5% Tween 20, 0.5% NP-40, 50% Glycerol, pH 7.4 @ 25 ℃

Heat Inactivation: No

5' - 3' Exonuclease: No

3' - 5' Exonuclease: Yes

Resulting Ends: Blunt Ends

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 50 µl system as an example.

Composition	Amount	Final Conc.
ddH_2O	up to 50 µl	
10x PCR Reaction Buffer, Mg^{2+} plus	5 µl	1x (Final 2 mM Mg^{2+})
10 mM dNTP	1 µl	200 uM
50 mM $MgSO_4$ (Optimal)	0-2 µl	0-2 mM
Primer F (10 µM)	1 µl	0.2 µM (0.05-1 µM)
Primer R (10 µM)	1 µl	0.2 µM (0.05-1 µM)
Template DNA	1 µl	<1 µg/50 µl
Pfu-fast DNA Polymerase	1 µl	2 U/50 µl (0.5–4 U/50 µl)

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.
Tm-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.

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

4. Deoxynucleotides:

The final concentration of dNTPs is typically 200 µM of each deoxynucleotide.

5. *Pfu*-fast DNA Polymerase Concentration:

We generally recommend using *Pfu*-fast DNA Polymerase at a concentration of 2 units/50 µl reaction. However, the optimal concentration of *Pfu*-fast DNA Polymerase may range from 30–50 units/ml (1.5–2.5 units/50 µl reaction) in specialized applications.

6. 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.

7. 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 Tm.

8. Extension:

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

9. Cycle number:

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