

GenScript *Pfu* DNA Polymerase Manual



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I	Introduction	1
II	Application	1
III	Key Features	1
IV	Ship and Storage	1
V	General PCR Protocol Using <i>Pfu</i> DNA Polymerase	2
VI	Troubleshooting	3
VII	Order Information	3

I. INTRODUCTION

Pfu DNA polymerase is a thermostable DNA Polymerase isolated from an *E.coli* strain that carries the pol gene from *Pyrococcus furiosus*. As one of the most thermostable DNA polymerase known with 3' to 5' proofreading activity, the enzyme catalyze DNA sythesis at optimal temperature near 75°C with very low error rate (about eight times more accurate than Taq DNA polymerase). *Pfu* DNA polymerase generates blunt-ended PCR fragments that are required for blunt-end PCR cloning.

II. APPLICATIONS

Pfu DNA Polymerase can be used in most PCR applications including the following:

- High fidelity PCR amplification.
- High fidelity blunt-end cloning.
- Site-directed mutagenesis.

III. KEY FEATURES

Key features of *Pfu* DNA Polymerase:

- Eight times more accurate than Taq DNA polymerase.
- Highly thermostable, remaining 95% active after 2 hours incubation at 95 °C.
- Generating blunt-ended PCR fragments. fidelity blunt-end cloning.
- Incorporating modified nucleotides (e.g. biotin-, fluorescent-labeled nucleotides).

IV. SHIPPING AND STORAGE

This product is shipped on blue ice. Store the product at -20 °C.



V. GENERAL PCR PROTOCOL USING *Pfu* DNA POLYMERASE

This is a general PCR amplification protocol, optimization may be needed to get satisfactory results.

1. Set up 50 μ l PCR reaction by adding the following reagents to a thin-walled PCR microcentrifuge tube or plate and mixing gently.

Reagent	Volume	Final Concentration
Water, PCR grade	40 μ l	
10X Buffer	5 μ l	1X
10 mM dNTP	1 μ l	200 nM each
20 μ M Forward primer	1 μ l	400 nM
20 μ M Reverse primer	1 μ l	400 nM
Template DNA	1 μ l	1 – 100 ng per reaction
2.5 U/ μ l <i>Pfu</i> Polymerase	1 μ l	0.05 U/ μ l
Total	50 μ l	

2. Suggested PCR profile:

Initial Denaturation: 94-96 °C for 2 min

25-30 cycles of Denaturation: 94-96 °C for 40 sec
Annealing: 50 – 60 °C for 1 min (primer T_m - 5 °C)
Extension: 72 °C for 30 sec to 5 min (0.5 ~ 1 kb/min)

Final extension: 72 °C for 7 min.



VI. TROUBLESHOOTING

The table below is guideline for troubleshooting.

Problem	Probable Cause	Solution
No PCR DNA	Extension time is too short	<i>Pfu</i> DNA Polymerase require a minimum extension time of 1-2 minutes for 1 kb template.
	One or more PCR components may be missing.	Run a positive control to make sure the PCR reaction is properly set-up.
	PCR conditions are not optimized. The annealing temperature may be too high; More cycles may be needed; The denaturation time may be too short;	Optimize the PCR conditions by decreasing annealing temperature in 2-4 °C increments, or increasing the number of cycles, or increasing the denaturation time in 10 second increments. It is recommended to change only one parameter each time.
	The primers may not be designed optimally.	The primer designing is critical for high quality PCR. Longer primers of 25-30 nucleotides with a GC content of 45-60% and with a more stable 5'-end than 3'-end usually make good primers.
	Target template is highly GC-rich.	The target will be difficult to denature even with a longer denaturation step. Betaine, DMSO and formamide can help amplification of high GC-rich template.
Non-specific DNA products	The primers may not be designed optimally.	See above.
	Annealing temperature is too low.	Optimize the PCR conditions by increasing annealing temperature in 2-4 °C increments, or decreasing the number of cycles.
False positive	Reagents are contaminated.	It is recommended that a negative control without using genomic DNA be run to make sure no contamination occurs.

VII. ORDER INFORMATION

Pfu DNA Polymerase, Cat. No. E00021

Telephone: 732-885-9188, 732-357-3839
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- The PCR process is covered by US. Patent numbers 4683195 and 4683202 issued to Cetus and owned by Hoffman-La Roche Inc. Genscript does not encourage or support the unauthorized use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

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