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A Geno Technology, Inc. (USA) brand name

Pfu DNA Polymerase

(Cat. # 786-816)

INTRODUCTION

Pfu DNA polymerase, derived from the hyperthermophilic archae *Pyrococcus furiosus*, has superior thermostability and proofreading properties compared to the other thermostable polymerase. Its molecular weight is 90 kD. It can amplify DNA target up to 2kb. The elongation velocity is 0.2~0.4kb/min (70~75°C). Pfu DNA polymerase possesses 3' to 5' exonuclease proofreading activity that enables the polymerase to correct nucleotide-misincorporation errors. This means that Pfu DNA polymerase-generated PCR fragments will have fewer errors than Taq-generated PCR inserts. Using, Pfu DNA polymerase in your PCR reaction results in a blunt-ended PCR products, which are ideal for cloning into blunt-ended vectors. The Pfu DNA polymerase is superior for techniques that require high-fidelity DNA synthesis.

ITEM(S) SUPPLIED (CAT. # 786-816)

Description	**Size**
Pfu DNA Polymerase (5U/µl)	500U
10X Pfu Buffer (Mg^{2+} plus)	1.25ml

STORAGE CONDITIONS

The kit is shipped in Blue Ice. Upon arrival, store at -20°C. Storage buffer is 20mM Tris.HCl (pH8.0), 100mM KCl, 3mM $MgCl_2$, 1mM DTT, 0.1% Nonidet® P-40, 0.1% Tween® 20, 0.2mg/ml BSA, 50% (v/v) glycerol.

10X PFU BUFFER

200mM Tris-HCl (pH8.8), 100mM KCl, 100mM $(NH_4)_2SO_4$, 20mM $MgSO_4$, 1% Triton® X-100, and 1mg/ml BSA

UNIT DEFINITION

One unit (U) of Pfu polymerase is defined as the amount of enzyme needed to catalyze the incorporation of 10 nanomoles of deoxyribonucleotides into acid-insoluble material in 30 minutes at 70°C using herring sperm DNA as a substrate.

GENERAL PCR GUIDELINES

The following protocol is a general guideline and starting point for PCR amplification, however, as with all polymerases, optimal reaction conditions for incubation times, temperatures and reagent concentrations of all components vary and require precise optimization.

Primers

- Normally 15-30 nucleotides long.
- G-C content of 40-60%.
- Not self-complementary or complementary to other primers in reaction.
- The melting temperature of primer pairs should not differ by >5°C.

Polymerase

- Recommend 1-2U polymerase/50µl reaction. 1U for DNA template less than 10kb and 2U for DNA template greater than 10kb. Higher polymerase concentrations may result in amplification of non-specific products.

PCR Program

- *Denaturation*
    - 0.5- 2min at 94-98°C is normally sufficient.
- *Annealing*
    - Optimal annealing temperature is ~5°C lower than the melting temperature of primer-template DNA duplex
- *Extension*
    - Normally performed at 72-75°C.
    - Extension time is 1 minute for <2kb fragments.
    - For larger DNA fragments, increase extension time by 1 minute/kb
- *Cycle Number*
    - For less than 10 copies of template DNA use 40 cycles
    - If >10 copies, use 25-35 cycles

IMPORTANT INFORMATION

- The optimal reaction conditions (incubation time and temperature, concentration of Pfu DNA Polymerase, template DNA, $MgSO_4$) depend on the template-primer pair and must be determined individually. It is especially important to titrate the $MgSO_4$ concentration and the amount of enzyme required per assay. The standard concentration of $MgSO_4$ is 2mM and the amount of Pfu DNA Polymerase is 1.25U per 50µl of reaction mixture.
- Pfu DNA Polymerase remains 95% active after 2 hours incubation at 95°C.
- The error rate of Pfu DNA Polymerase in PCR is $2.6x10^{-6}$ errors per nt per cycle; the accuracy (an inverse of error rate) an average number of correct nucleotides incorporated before making an error is $3.8x10^{-5}$
- Pfu DNA Polymerase accepts modified nucleotides (e.g. Biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.
- The enzyme has no detectable reverse transcriptase activity.
- Do not use dUTP in PCR.
- The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. 25-35 cycles are usually sufficient for the majority PCR reaction. Low amounts of starting template may require 40 cycles.

The PCR reactions should be assembled in a DNA-free environment. We recommend setting up a control reaction that is performed in the absence of DNA to ensure no DNA contamination. Briefly centrifuge the Pfu polymerase tubes to collect all the enzyme at the tube bottom.

PROTOCOL

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation time and temperature, concentration of Pfu DNA Polymerase, primers, Mg^{2+}, and template DNA) vary and need to be optimized.

1. Add the following components to a sterile Microcentrifuge tube sitting on ice:

Reagent	**Quantity, for 50µl of reaction mixture**	**Final concentration**
Sterile deionized water	Variable	-
Pfu DNA Polymerase buffer (Mg^{2+} plus)	5µl	1X
dNTPs (10mM each)	1µl	0.2mM each
Primer I	Variable	0.4-1µM
Primer II	Variable	0.4-1µM
Pfu DNA Polymerase (5U/µl)	0. 25-0.5µl	1.25-2.5U/50µl
Template DNA	Variable	10pg-1µg
Total		50µl

Recommendations with Template DNA in a 50µl reaction volume

Human genomic DNA	0.1µg-1µg
Plasmid DNA	0.5ng-5ng
Phage DNA	0.1ng-10ng
E. coli genomic DNA	10ng-100ng

2. Mix the contents of the tube. Cap the tubes and centrifuge briefly to collect the contents to the bottom. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25µl mineral oil.
3. Perform 25-35 cycles of PCR amplification as follows:

Initial Denaturation	94°C	3 minutes
25-35 Cycles	94°C 55-68°C 72°C	30 seconds 30 seconds 1 min
Final Extension	72°C	10 minutes

4. Incubate for an additional 10 min at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.
5. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide or any other suitable staining. Use appropriate molecular weight standards.

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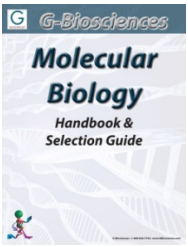

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