Certificate of Analysis

Pfu DNA Polymerase:

Part No.	Size (units)
M774A	100
M774B	500

Part# 9PIM774

Revised 10/09

Description: *Pfu* DNA Polymerase is a thermostable enzyme that replicates DNA at 75°C. It catalyzes the polymerization of nucleotides into duplex DNA in the 5´→3´ direction in the presence of magnesium. The enzyme has a molecular weight of approximately 90,000 daltons as estimated from the predicted amino acid sequence and exhibits 3´→5´ exonuclease (proofreading) activity. *Pfu* DNA Polymerase is recommended for use in PCR and primer extension reactions that require high fidelity (1–4).

Enzyme Storage Buffer: *Pfu* DNA Polymerase is supplied in 50mM Tris-HCl (pH 8.2 at 25°C), 0.1mM EDTA, 1mM DTT, 0.05% CHAPS and 50% glycerol.

***Pfu* DNA Polymerase 10X Reaction Buffer with $MgSO_4$ (M776A):** 200mM Tris-HCl (pH 8.8 at 25°C), 100mM KCl, 100mM $(NH_4)_2SO_4$, 20mM $MgSO_4$, 1.0% Triton® X-100 and 1mg/ml nuclease-free BSA.

Source: Purified from *Pyrococcus furiosus* strain Vc1 DSM3638 (5).

Storage Temperature: Store at –20°C. Avoid multiple freeze-thaw cycles and exposure to frequent temperature changes. See the expiration date on the Product Information Label.

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nanomoles of dNTPs into acid insoluble material in 30 minutes at 75°C. The reaction conditions are specified below under Standard DNA Polymerase Assay Conditions. See the unit concentration on the Product Information Label.

Usage Note: Concentration gradients may form in frozen products and should be dispersed upon thawing. Mix well prior to use.

Quality Control Assays

Activity Assays

Functional Assay: *Pfu* DNA Polymerase is tested for performance in the polymerase chain reaction (PCR) using 1.25 units of enzyme to amplify a 1,200bp region of the α-1-antitrypsin gene from 100 molecules (0.33ng) of human genomic DNA. The resulting PCR product is visualized on an ethidium bromide-stained agarose gel.

Standard DNA Polymerase Assay Conditions (not PCR conditions): The polymerase activity is assayed in 20mM Tris-HCl (pH 9.0), 10mM KCl, 1mM $MgSO_4$, 6mM $(NH_4)_2SO_4$, 0.1% Triton® X-100, 0.1mg/ml BSA, 200µM each of dATP, dGTP, dCTP, dTTP (a mix of unlabeled and [^{3}H]dTTP) and 0.3mg/ml activated calf thymus DNA, in a final volume of 50µl. The test result is listed on the Product Information Label.

Contaminant Assays

Endonuclease Assay: To test for endonuclease activity, 1µg of lambda DNA is incubated with 12.5 units of *Pfu* DNA Polymerase for 8 hours at 45°C followed by 8 hours at 72°C in a 1X dilution of 10X nuclease testing buffer (100mM KCl, 200mM Tris-HCl (pH 8.0 at 25°C), 60mM $(NH_4)_2SO_4$, 20mM $MgCl_2$, 100µg/ml nuclease-free BSA and 1% Triton® X-100) with 400µM each of dATP, dCTP and dGTP. Following incubation, the DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible cutting. The test result is listed on the Product Information Label.

Exonuclease Assay: To test for contaminating exonucleases, 1µg of Lambda DNA/*Hin*d III Markers (Cat.# G1711) is incubated with 5 units of *Pfu* DNA Polymerase for 8 hours at 45°C followed by 8 hours at 72°C in a 1X dilution of 10X nuclease testing buffer (100mM KCl, 200mM Tris-HCl (pH 8.0 at 25°C), 60mM $(NH_4)_2SO_4$, 20mM $MgCl_2$, 100µg/ml nuclease-free BSA and 1% Triton® X-100) with 400µM each of dATP, dCTP and dGTP. Following incubation, the DNA is visualized on an ethidium bromide-stained agarose gel to verify the absence of visible smearing. The test result is listed on the Product Information Label.

PCR Satisfaction Guarantee

Promega's PCR Systems, enzymes and reagents are proven in PCR to ensure reliable, high performance results. Your success is important to us. Our products are backed by a worldwide team of Technical Support scientists. Please contact them for applications or technical assistance. If you are not completely satisfied with any Promega PCR product we will send a replacement or refund your account.

That's Our PCR Guarantee!

Product must be within expiration date and have been stored and used in accordance with product literature. See Promega Product Insert for specific tests performed.

Signed by:

H. Qin, Quality Assurance

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Part# 9PIM774
Printed in USA. Revised 10/09

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Usage Information

I. Description

Pfu DNA Polymerase is a thermostable enzyme of approximately 92kDa isolated from *Pyrococcus furiosus* DSM3638 (5). *Pfu* DNA Polymerase catalyzes the DNA-dependent polymerization of nucleotides into duplex DNA in the 5´→3´ direction in the presence of magnesium ions. The enzyme also exhibits 3´→5´ exonuclease (proofreading) activity. Base misinsertions that may occur infrequently during polymerization are rapidly excised by the proofreading activity of the polymerase. Consequently, *Pfu* DNA Polymerase is useful for polymerization reactions requiring high fidelity synthesis (1–4).

II. Standard Application

A. PCR Amplification

Reagents to Be Supplied by the User

(Solution compositions are provided in Section IV.)

- dNTP mix (Cat.# C1141 or C1145)
- downstream primer
- upstream primer
- Nuclease-Free Water (Cat.# P1193)
- mineral oil

1. In a sterile, nuclease-free microcentrifuge tube, combine the following components:

		Final Concentration
Pfu DNA Polymerase 10X Buffer with $MgSO_4$	5µl	1X
dNTP mix, 10mM each	1µl	200µM each
upstream primer	5–50pmol	0.1–1.0µM
downstream primer	5–50pmol	0.1–1.0µM
DNA template	variable	<0.5µg/50µl
Pfu DNA Polymerase (2–3u/µl)	variable	1.25u/50µl
Nuclease-Free Water to final volume of	50µl	

Note: It is critical to withhold *Pfu* DNA Polymerase until after the addition of dNTPs; otherwise, the proofreading activity of the polymerase may degrade the primers, resulting in nonspecific amplification and reduced product yield. Assemble on ice.

2. If using a thermal cycler without a heated lid, overlay the reaction mix with 1–2 drops (approximately 50µl) of mineral oil to prevent evaporation during thermal cycling. Centrifuge the reaction mix in a microcentrifuge for 5 seconds.
3. Immediately place the reactions in a thermal cycler that has been preheated to 95°C. We recommend heating the samples at 95°C for 1–2 minutes to ensure that the target DNA is completely denatured. Incubation for longer than 2 minutes at 95°C is unnecessary and may reduce the yield due to DNA damage.
4. Start the thermal cycling program. The cycling profile given in Table 1 may be used as a guideline. Optimize the amplification profile for each primer/target combination.

Table 1. Recommended thermal cycling conditions for *Pfu* DNA Polymerase-mediated PCR amplification. These guidelines apply to target sequences between 200 and 2,000bp and are optimal for the Perkin-Elmer Thermal Cycler Model 480 or comparable thermal cyclers.

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	1–2 minutes	1 cycle
Denaturation	95°C	0.5–1 minute	25–35 cycles
Annealing*	42–65°C	30 seconds	
Extension**	72–74°C	2–4 minutes	
Final Extension	72–74°C	5 minutes	1 cycle
Soak	4°C	Indefinite	1 cycle

*The annealing temperature for a specific amplification reaction will depend upon the sequences of the two primers. See Section III for discussions on determining optimal annealing temperatures for PCR amplification.

**Allow approximately 2 minutes for every 1kb to be amplified.

III. General Considerations

A. Enzyme Concentration

We recommend that 1.25 units of *Pfu* DNA Polymerase be used per 50µl amplification reaction. The inclusion of more enzyme will increase the likelihood of primer degradation due to the intrinsic 3´→5´ exonuclease (proofreading) activity. It is essential to withhold *Pfu* DNA Polymerase from the reaction until after the addition of the dNTP mix and to assemble components on ice.

B. Primer Design

The sequences of the primers are a major consideration in determining the optimal temperature of the PCR amplification cycles. For primers with a high T_m, it may be advantageous to increase the annealing temperature. Higher temperatures minimize nonspecific primer annealing, increase the amount of specific product produced and reduce the amount of primer-dimer formation.

The 3´→5´ exonuclease activity may degrade primers. To overcome the degradation, longer primers with maximized GC content could be used. Primers can also be protected by introducing phosphorothioate bonds at their 3´ termini (6).

C. Extension Time

The extension rate of *Pfu* DNA Polymerase is lower than that of *Taq* DNA Polymerase. Therefore, during the extension step, allow approximately 2 minutes for every 1kb to be amplified (minimum extension time of 1 minute). For most reactions, 25–35 cycles are sufficient.

IV. Composition of Buffers and Solutions

Pfu DNA Polymerase 10X Reaction Buffer with $MgSO_4$ (provided)

200mM	Tris-HCl (pH 8.8 at 25°C)
100mM	KCl
100mM	$(NH_4)_2SO_4$
20mM	$MgSO_4$
1mg/ml	nuclease-free BSA
1%	Triton® X-100

dNTP mix

10mM	each of dATP, dCTP, dGTP and dTTP in water

PCR-tested dNTPs are available: PCR Nucleotide Mix (Cat.# C1141 or C1145) and dNTPs (Cat.# U1240).

V. References

1. Lundberg, K.S. *et al.* (1991) High-fidelity amplification using a thermostable DNA polymerase isolated from *Pyrococcus furiosus. Gene* **108**, 1–6.
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4. Andre, P. *et al.* (1997) Fidelity and mutational spectrum of *Pfu* DNA polymerase on a human mitochondrial DNA sequence. *Genome Res.* **7**, 843–52.
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Part#9PIM774
Printed in USA. Revised 10/09