PRODUCT INFORMATION

Pfu DNA Polymerase

#EP0572 500 U

Concentration: 2.5 U/µL
Lot: —
Expiry Date: —

Store at -20°C

www.thermoscientific.com/onebio

Ordering Information

***Pfu* DNA polymerase** (native)

Component	#EP0571	#EP0572
Pfu DNA polymerase, native, 2.5 U/µL	100 U	500 U
10X *Pfu* Buffer with MgSO$_4$	0.6 mL	2x1.25 mL
10X *Pfu* Buffer	0.6 mL	2x1.25 mL
25 mM MgSO$_4$	0.6 mL	2x1.25 mL

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Description

Pfu DNA Polymerase is a highly thermostable DNA polymerase from the hyperthermophilic archaeum *Pyrococcus furiosus*. The enzyme catalyzes the template-dependent polymerization of nucleotides into duplex DNA in the 5'→3' direction. *Pfu* DNA Polymerase also exhibits 3'→5' exonuclease (proofreading) activity, that enables the polymerase to correct nucleotide incorporation errors. It has no 5'→3' exonuclease activity and no detectable reverse transcriptase activity.

The error rate of *Pfu* DNA Polymerase in PCR is 2.6×10^{-6} errors per nt per cycle, as determined by a modified method described in (2).

Note. dUTP, dITP and primers containing these nucleotides should not be used in PCR with *Pfu* DNA Polymerase because the binding of this enzyme to DNA templates with uracil and hypoxanthine stalls DNA synthesis (3, 4).

Applications

- High fidelity PCR.
- Generation of PCR products for cloning and expression.
- RT-PCR for cDNA cloning and expression.
- Generation of PCR product for blunt-end cloning (1).
- Site-directed mutagenesis.

Source

Pyrococcus furiosus

Definition of Activity Unit

One unit of the enzyme catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction (adsorbed on DE-81) in 30 min at 72°C.

Enzyme activity is assayed in the following mixture: 20 mM Tris-HCl (pH 8.8 at 25°C), 2 mM MgSO$_4$, 10 mM (NH$_4$)$_2$SO$_4$, 10 mM KCl, 0.1 mg/mL BSA, 0.1% (v/v) Triton X-100, 0.75 mM activated calf thymus DNA, 0.2 mM of each dNTP, 0.4 MBq/mL [^{3}H]-dTTP.

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 8.2), 1 mM DTT, 0.1 mM EDTA, 100 mM KCl, 0.1% (v/v) Nonidet P40, 0.1% (v/v) Tween 20 and 50% (v/v) glycerol.

10X *Pfu* Buffer with 20 mM MgSO$_4$

200 mM Tris-HCl (pH 8.8 at 25°C), 100 mM (NH$_4$)$_2$SO$_4$, 100 mM KCl, 1 mg/mL BSA, 1% (v/v) Triton X-100, 20 mM MgSO$_4$.

10X *Pfu* Buffer

200 mM Tris-HCl (pH 8.8 at 25°C), 100 mM (NH$_4$)$_2$SO$_4$, 100 mM KCl, 1% (v/v) Triton X-100, 1 mg/mL BSA.

Inhibition and Inactivation

Inactivated by phenol/chloroform extraction.

PROTOCOL

To prepare several parallel reactions and to minimize the possibility of pipetting errors, prepare a PCR master mix by mixing water, buffer, dNTPs, primers and template DNA. *Pfu* DNA Polymerase should be the last component added. Prepare sufficient master mix for the number of reactions plus one extra to allow for pipeting error.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 50 µL reaction:

Water, nuclease-free (#R0581)	variable
10X *Pfu* Buffer with MgSO$_4$*	5 µL
dNTP Mix, 2 mM each (#R0241)	5 µL (0.2 mM of each)
Forward primer	0.1-1.0 µM
Reverse primer	0.1-1.0 µM
Template DNA	50 pg - 1 µg
***Pfu* DNA Polymerase**	1.25-2.5 U
Total volume	50 µL

3. Gently vortex the samples and spin down.
4. If using a thermal cycler that does not use a heated lid, overlay the reaction mixture with 25 µL of mineral oil.
5. Perform PCR using the following thermal cycling conditions:

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	95	1-3 min	1
Denaturation	95	30 s	25-35
Annealing	Tm-5	30 s	
Extension	72	2 min/kb	
Final extension	72	5-15 min	1

*If using 10X *Pfu* Buffer without MgSO$_4$, a 25 mM MgSO$_4$ solution should be added to 50 µL of the master mix:

Final concentration of MgSO$_4$, mM	1	1.25	1.5	1.75	2	2.5	3	4
Volume of 25 mM MgSO$_4$, µL	2	2.5	3	3.5	4	5	6	8

GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers, dedicated for PCR. Use positive displacement pipettes, or pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Use PCR-certified reagents, including high quality water (e.g., Water, nuclease-free, #R0581).
- Always perform “no template control” (NTC) reactions to check for contamination.
- Carryover contamination control in conjunction with UDG is not applicable using *Pfu* DNA Polymerase.

GUIDELINES FOR PRIMER DESIGN

Use the Thermo Scientific REviewer primer design software at www.thermoscientific.com/reviewer or follow general recommendations for PCR primer design as outlined below:

- PCR primers are generally 20-30 nucleotides long.
- Optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.
- Avoid placing more than three G or C nucleotides at the 3'-end to lower the risk of non-specific priming.
- If possible, the primer should terminate with a G or C at the 3'-end.
- Avoid self-complementary primer regions, complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.

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- The 3'→5' exonuclease activity associated with *Pfu* DNA Polymerase may degrade the primers. It is therefore important that *Pfu* DNA Polymerase be added last to the reaction mixture. Use the longer primers (20-30 bp) with higher CG content.
- Check for possible sites of undesired complementary between primers and template DNA.
- When designing degenerate primers, place at least 3 conserved nucleotides at the 3'-end.
- When introducing restriction enzyme sites into primers, refer to the table "Cleavage efficiency close to the termini of PCR fragments" located on www.thermoscientific.com/onebio to determine the number of extra bases required for efficient cleavage.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.

Estimation of primer melting temperature

For primers containing less than 25 nucleotides, the approx. melting temperature (Tm) can be calculated using the following equation:

$$Tm = 4\,(G + C) + 2\,(A + T),$$

where G, C, A, T represent the number of respective nucleotides in the primer.

If the primer contains more than 25 nucleotides specialized computer programs e.g., REviewer™ (www.thermoscientific.com/reviewer) are recommended to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA

Optimal amounts of template DNA in the 50 µL reaction volume are 0.05-1 ng for both plasmid and phage DNA, and 0.1-1 µg for genomic DNA. Higher amounts of template increase the risk of generating of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.
All routine DNA purification methods are suitable for template preparation e.g., Genomic DNA Purification Kit (#K0512) or Thermo Scientific GeneJET Plasmid Miniprep Kit (#K0502). Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, can inhibit DNA polymerase. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol normally remove trace contaminants from DNA samples.

$MgSO_4$ concentration

Pfu DNA Polymerase is provided with an optimized 10X *Pfu* Buffer which includes $MgSO_4$ at a concentration of 20 mM. A final $MgSO_4$ concentration of 2 mM is generally ideal for PCR. The $MgSO_4$ concentration can be optimised by using 10X *Pfu* Buffer and a $MgSO_4$ solution provided in a separate vial. If the DNA samples contain EDTA or other metal chelators, the Mg^{2+} ion concentration in the PCR mixture should be increased accordingly (1 molecule of EDTA binds one Mg^{2+}).

dNTPs

The recommended final concentration of each dNTP is 0.2 mM. In certain PCR applications, higher dNTP concentrations may be necessary. Due to the binding of Mg^{2+} to dNTPs, the $MgSO_4$ concentration needs to be adjusted accordingly. It is essential to have equal concentrations of all four nucleotides (dATP, dCTP, dGTP and dTTP) present in the reaction mixture.
To achieve 0.2 mM concentration of each dNTP in the PCR mixture, use the following volumes of dNTP mixes:

Volume of PCR mixture	dNTP Mix, 2 mM each (#R0241)	dNTP Mix, 10 mM each (#R0191)	dNTP Mix, 25 mM each (#R1121)
50 µL	5 µL	1 µL	0.4 µL
25 µL	2.5 µL	0.5 µL	0.2 µL
20 µL	2 µL	0.4 µL	0.16 µL

Primers

The recommended concentration range of the PCR primers is 0.1-1 µM. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products. For degenerate primers and primers used for long PCR higher primer concentrations in the range of 0.3-1 µM are often favorable.

CYCLING PARAMETERS

Initial DNA denaturation

It is essential to completely denature the template DNA at the beginning of PCR to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 95°C is sufficient. For GC-rich templates this step should be prolonged up to 10 min.

Denaturation

A DNA denaturation time of 30 seconds per cycle at 95°C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3-4 min.

Primer annealing

The annealing temperature should be 5°C lower than the melting temperature (Tm) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments.

Extension

The optimal extension temperature for *Pfu* DNA Polymerase is 70-75°C. The recommended extension step is 2 min/kb at 72°C for PCR products up to 2 kb. For larger products, the extension time should be prolonged by 1 min/kb.

Number of cycles

The number of cycles may vary depending on the amount of template DNA in the PCR mixture and the expected PCR product yield.
In most cases, 25-35 cycles are sufficient.

Final extension

After the last cycle, it is recommended to incubate the PCR mixture at 72°C for additional 5-15 min to fill-in any possible incomplete reaction products.

Troubleshooting

For troubleshooting please visit www.thermoscientific.com/onebio

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable degradation of DNA was observed after incubation of 1 µg lambda DNA with 10 U of *Pfu* DNA Polymerase for 4 hours at 72°C.

Exodeoxyribonuclease Assay

No detectable degradation was observed after incubation of 1 µg of lambda DNA/HindIII fragments with 10 U of *Pfu* DNA Polymerase for 4 hours at 72°C.

Functional Assay

Pfu DNA Polymerase was tested for amplification of 950 bp single copy gene from human genomic DNA.

Quality authorized by: Jurgita Zilinskiene

References

1. Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
2. Lundberg, K.S., et al., High-fidelity amplification using a thermostable DNA polymerase isolated from *Pyrococcus furiosus*, Gene, 108, 1-6, 1991.
3. Shuttleworth, G., et al., Recognition of the pro-mutagenic base uracil by family B DNA polymerases from Archaea, J.Mol. Biol., 337, 621-634, 2004.
4. Gruz, P., et al., Processing of DNA lesions by archaeal DNA polymerases from *Sulpholobus solfataricus*, Nucleic Acids Res., 31, 4024-4030, 2003.

PRODUCT USE LIMITATION