Pfu DNA Polymerase

with 10X Reaction Buffer

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Product Information

Catalogue numbers: PCR003 | PCR004 | SP-PCR003
Enzyme concentration: 5 U/µL
Use: High-fidelity PCR, primer extension and blunt-end cloning

Storage & Handling

Store at -20°C; avoid repeated freeze-thaw cycles.
Thaw Reaction Buffer on ice and mix well before use.
Keep polymerase cold during reaction setup.

Principle and Applications

Pfu DNA Polymerase is a thermostable DNA polymerase originating from Pyrococcus furiosus. It extends primers in the 5′→3′ direction and possesses 3′→5′ exonuclease (proofreading) activity that removes some misincorporated nucleotides during synthesis. This property makes Pfu useful for PCR applications requiring enhanced sequence accuracy. Amplification generally produces blunt-ended DNA fragments suitable for blunt-end cloning and other downstream molecular biology workflows.

Ordering Information and Kit Contents

Component / pack	PCR003	PCR004	Sample pack
Catalogue number	PCR003	PCR004	SP-PCR003
Pack size	200 U	1,000 U	100 U
Pfu DNA Polymerase (5 U/µL)	40 µL	200 µL	20 µL
10X Reaction Buffer	1 mL	3 mL	1 mL
50 mM $MgCl_2$ solution	100 µL	400 µL	50 µL

Standard PCR Reaction Setup

Prepare reactions on ice using nuclease-free tubes and pipette tips. The example below provides a starting point for high-fidelity amplification; optimize according to target sequence and sample quality.

Component	25 µL PCR	50 µL PCR	Final concentration
DNA template	Variable	Variable	According to sample
Forward primer (10 µM)	0.5 µL	1.0 µL	0.2 µM
Reverse primer (10 µM)	0.5 µL	1.0 µL	0.2 µM
dNTP mix (10 mM each)	0.5 µL	1.0 µL	200 µM each
10X Reaction Buffer	2.5 µL	5.0 µL	1X
Pfu DNA Polymerase (5 U/µL)	0.25 µL	0.5 µL	1.25 U / 25 µL; 2.5 U / 50 µL
Nuclease-free water	To 25 µL	To 50 µL	Final volume

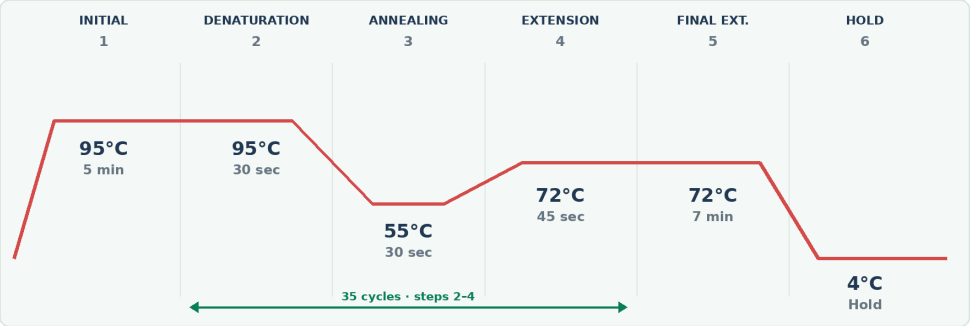
$MgCl_2$: The original G2P formulation specifies 20 mM $MgCl_2$ in the 10X Reaction Buffer (2 mM at 1X). The separate 50 mM $MgCl_2$ solution is provided for reactions that require further optimization; avoid routine addition without establishing the desired final Mg^{2+} concentration.

Recommended Thermal Cycling Programme

Example thermal programme for amplification of an approximately 1 kb DNA fragment with Pfu DNA Polymerase. Annealing temperature depends on primer Tm; extension duration varies with amplicon length and template complexity.

Step	Temperature	Duration	Cycles
Initial denaturation	95°C	5 min	1 cycle
Denaturation	95°C	30 s	**35 cycles**
Annealing	55°C	30 s	
Extension	72°C	45 s	
Final extension	72°C	7 min	1 cycle
Hold	4°C	Until recovery	—

Figure 1. Recommended 35-cycle Pfu PCR thermal profile.



Optimization note. Select an annealing temperature suited to primer Tm and the template. As a starting guideline, allow approximately 1 minute of extension at 72°C per kilobase of target sequence; adjust based on assay performance.

Representative Pfu PCR Amplification Result

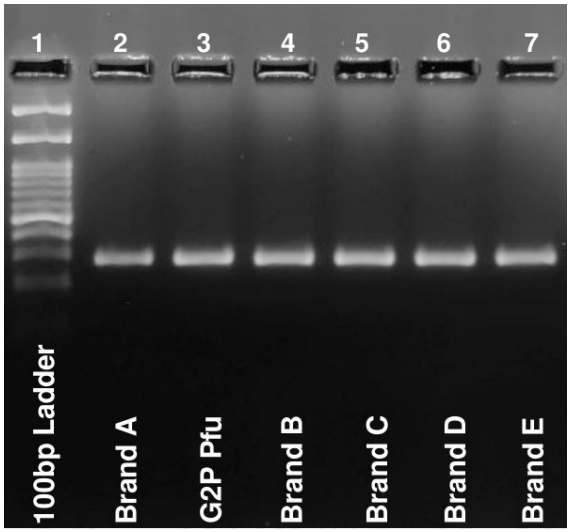


Figure 2 | Comparative PCR amplification

Representative PCR amplicons separated by electrophoresis on a 2% agarose gel. Lane 1: 100 bp DNA ladder; lane 2: Brand A; lane 3: G2P Pfu DNA Polymerase; lanes 4–7: Brands B, C, D and E, respectively.

Reaction Component Guidance

Component	Practical guidance
DNA template	Use clean DNA of appropriate concentration; excess template or residual purification reagents may inhibit PCR or increase nonspecific products.
Primers	Start near 0.2 µM each forward and reverse primer. Adjust primer concentrations and annealing temperature based on specificity.
dNTPs	Use approximately 200 µM each dNTP as a starting concentration; all four nucleotides should be present at equivalent concentrations.
$MgCl_2$	The 10X Reaction Buffer provides $MgCl_2$ according to the original formulation. Use the separate 50 mM stock only when further magnesium optimization is required.

Troubleshooting Guide

If the expected DNA product is not obtained, review template quality, primer design, reaction composition and cycling conditions. Maintain a positive control and a no-template control when optimizing.

Observation	Possible cause	Recommended action
Little or no PCR product	Poor-quality or insufficient template	Check DNA concentration and integrity; remove carry-over inhibitors from the template preparation.
Little or no PCR product	Primer mismatch or inappropriate annealing	Confirm primer sequences and use an annealing-temperature gradient if necessary.
Little or no PCR product	Incorrect enzyme or cycling conditions	Confirm the amount of Pfu polymerase, initial denaturation, cycle number and extension time.
Multiple bands	Annealing temperature too low	Increase annealing temperature or redesign primers to improve specificity.
Smearing or weak bands	Excess template, salts or Mg^{2+}	Reduce template input and assess buffer/$MgCl_2$ concentrations; avoid excessive Mg^{2+}.
Primer-dimer	Primer complementarity or concentration	Review primer design and titrate primer concentration.
Poor amplification of GC-rich templates	Secondary structure or incomplete denaturation	Consider a validated GC-rich PCR approach and optimize denaturation/annealing without compromising enzyme activity.
Band in negative control	DNA or amplicon contamination	Replace suspect reagents, use filtered tips and separate pre-PCR and post-PCR areas.
Difficulty with TA cloning	Pfu products are usually blunt ended	Use a blunt-end cloning strategy or add A-overhangs using an appropriate downstream treatment.

Additional Information

Enzyme unit definition. One unit of Pfu DNA Polymerase catalyzes the incorporation of 10 nmol of deoxyribonucleotides into polynucleotide material in 30 minutes at 72°C.

Storage. Store the enzyme at −20°C and avoid repeated freeze–thaw cycles. Thaw and mix the Reaction Buffer before use; keep the enzyme chilled during PCR setup.

Reaction Buffer. The 10X buffer contains Tris-HCl, KCl and $MgCl_2$; the original formulation specifies 20 mM $MgCl_2$ in the concentrated buffer. A separate 50 mM $MgCl_2$ stock is included for optimization.

Application scope. Pfu polymerase has 3′→5′ proofreading activity and typically produces blunt-ended products. A-tailing is required before conventional TA cloning. For research use only.

Ordering. PCR003: 200 U; PCR004: 1,000 U and SP-PCR003 100 U sample packs. Refer to the current catalogue for ordering information.