Pfu DNA Polymerase

User Manual | Recombinant, Research Grade

Cat. No. EV-MOL-006 | Version 1.0 | April 2026

Cat. No.	EV-MOL-006	**Size**	500 U / 2,500 U	**Storage**	-20°C

1. Overview

Pfu DNA Polymerase (EV-MOL-006) is a recombinant, thermostable, high-fidelity DNA polymerase produced in E. coli Rosetta(DE3) from the cloned gene of the hyperthermophilic archaeon Pyrococcus furiosus. It is a 775 amino acid, ~92 kDa enzyme with a robust 3'→5' proofreading exonuclease that yields an error rate of approximately 1.3 × 10□⁶ per base pair — roughly 18-fold lower than Taq DNA Polymerase. Pfu generates blunt-ended PCR products free of non-templated 3'-A overhangs.

EV-MOL-006 is optimal at 72–75°C and is fully stable through standard PCR cycling conditions including denaturation at 98°C. Each lot is tested for thermostable polymerase activity, proofreading fidelity, and nuclease contamination before release.

2. Catalytic Mechanism

Pfu DNA Polymerase catalyzes template-directed DNA synthesis in the 5'→3' direction at temperatures up to 75°C. Its 3'→5' proofreading exonuclease excises misincorporated nucleotides, yielding an error rate comparable to other high-fidelity thermostable polymerases. Unlike Taq, Pfu does not add non-templated 3'-A nucleotides, resulting in blunt-ended products. No 5'→3' exonuclease or strand displacement activity is present.

3. Substrate Specificity

Substrate Type	Efficiency	Conditions
Genomic DNA (PCR)	High	Standard cycling; 72°C extension at 1 kb/min
Plasmid DNA (colony/inverse PCR)	High	Standard cycling; optimize annealing temp
GC-rich templates	Moderate	Add DMSO (2–5%) or use GC-enhancer additive
Long-range PCR (>5 kb)	Moderate	Extend elongation time to 2 min/kb; lower Tm

4. Reaction Conditions & Protocol

4.1 Recommended Reaction Setup

Component	Volume
Template DNA (1 pg – 100 ng)	x µL
5× Pfu HF Buffer (EV-MOL-006-RB)	4 µL
dNTPs (10 mM each)	0.4 µL
Forward primer (10 µM)	1 µL
Reverse primer (10 µM)	1 µL
Pfu DNA Polymerase (EV-MOL-006)	0.5 µL (1.25 units)
Nuclease-free water	to 20 µL

- Initial denaturation: 95°C for 2 min
- Cycling (30–35 cycles): 95°C 30s | Tm-5°C 30s | 72°C 1 min/kb
- Final extension: 72°C for 5 min
- Products are blunt-ended; not compatible with TA cloning without A-tailing

5. Unit Definition

One unit (U) is defined as the amount of Pfu DNA Polymerase required to incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 72°C in a 50 µL reaction containing 10 µg activated calf thymus DNA and 200 µM each dNTP.

6. Quality Control

Test	Specification
Purity (SDS-PAGE)	>95% (single band at ~92 kDa)
Molecular Weight	~92 kDa (SDS-PAGE)
Concentration	2.5 U/µL
PCR Activity	Specific amplification of 1 kb product from human genomic DNA (30 cycles)
Error Rate	<2 × 10■■ per bp (fidelity assay)
Blunt-end Fidelity	No non-templated 3'-A addition detected
Thermostability	>95% activity retained after 95°C, 30 cycles
Exonuclease Contamination	No detectable degradation (λDNA, 200 U, 4 h, 37°C)
Endonuclease Activity	No nicking of supercoiled pUC19 (200 U, 4 h, 37°C)
RNase Activity	No degradation of 5 µg RNA (200 U, 2 h, 37°C)

pH (formulation buffer)	7.6–8.2
Sterility	No microbial growth (7-day incubation)

7. Storage & Stability

- **Storage temperature:** -20°C (avoid repeated freeze-thaw; aliquot upon receipt)
- **Supplied in:** 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% glycerol
- **Stability:** 24 months from date of manufacture when stored correctly
- **Shipping:** On dry ice

8. Applications

- High-fidelity PCR for cloning applications where insert sequence accuracy is critical
- Amplification of expression constructs for subcloning into pET and related vectors
- Site-directed mutagenesis by overlap extension PCR
- Blunt-end PCR product generation for ligation into blunt-cloning vectors
- PCR for diagnostic and genotyping applications requiring low error rates

9. Troubleshooting

Problem	Possible Cause	Suggested Action
No PCR product	Annealing temp too high or Mg²■ suboptimal	Lower annealing temp by 5°C; optimize MgCl■ from 1.5 to 3 mM
Multiple bands	Non-specific priming	Raise annealing temp; use hot-start approach or touchdown PCR
Low yield from GC-rich template	Secondary structure	Add 5% DMSO or betaine; increase denaturation to 98°C
Blunt product fails TA cloning	Pfu is A-tail negative	A-tail with Taq for 15 min at 72°C; or use blunt-cloning vector

10. Safety Information

This product is intended for research use only. Handle in accordance with standard laboratory safety guidelines. Refer to the accompanying Safety Data Sheet (SDS) for full hazard information. Avoid ingestion, inhalation, or contact with eyes and skin. Dispose of in accordance with local, state, and federal regulations.

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