EV5™ High-Fidelity DNA Polymerase

User Manual | Recombinant, Research Grade

Cat. No. EV-MOL-007 | Version 1.0 | April 2026

Cat. No.	EV-MOL-007	Size	100 U / 500 U	Storage	-20°C

1. Overview

EV5™ High-Fidelity DNA Polymerase (EV-MOL-007) is Enzovera's proprietary fusion enzyme produced in E. coli Rosetta(DE3). It consists of the Sso7d processivity domain from Sulfolobus solfataricus fused N-terminally to Pfu DNA Polymerase from Pyrococcus furiosus via a flexible Gly-Ser linker. The 873 amino acid, ~99 kDa fusion protein combines the proofreading fidelity of wild-type Pfu with the dramatically enhanced processivity conferred by the Sso7d double-stranded DNA-binding domain, delivering Q5-class performance.

EV5™ achieves an error rate below 1 per 10^7 bp, an extension rate of 1 kb/min (15 sec/kb in fast protocol), and reliable amplification of targets up to 20 kb. It generates blunt-ended products compatible with all sequence-independent assembly and blunt cloning workflows. Each lot is tested for PCR fidelity, processivity, long-range amplification, and nuclease contamination before release.

2. Catalytic Mechanism

The Sso7d domain binds double-stranded DNA non-specifically and non-covalently with sub-nanomolar affinity, effectively tethering the polymerase holoenzyme to the template throughout synthesis. This reduces the rate of polymerase dissociation by more than 10-fold relative to wild-type Pfu, enabling synthesis of products exceeding 20 kb and dramatically faster extension rates. The Pfu polymerase domain retains its 3'→5' proofreading exonuclease, ensuring error rates below 1 × 10□⁷ per bp. No 5'→3' exonuclease or strand displacement is present.

3. Substrate Specificity

Substrate Type	Efficiency	Conditions
Genomic DNA (standard PCR)	Very High	72°C extension, 1 kb/min; 30 cycles
Long-range PCR (up to 20 kb)	High	Extend to 2 min/kb; 68°C extension temperature
GC-rich templates (>65% GC)	High	Add GC Enhancer Buffer 1× final; 98°C denaturation
cDNA PCR (from RT reaction)	High	Standard conditions; works with ParaScript RT EV-MOL-008

4. Reaction Conditions & Protocol

4.1 Recommended Reaction Setup

Component	Volume
Template DNA (1 pg – 100 ng)	x µL
5× EV5 HF Buffer (EV-MOL-007-RB)	4 µL
dNTPs (10 mM each)	0.4 µL
Forward primer (10 µM)	1 µL
Reverse primer (10 µM)	1 µL
EV5™ DNA Polymerase (EV-MOL-007)	0.5 µL (1 unit)
Nuclease-free water	to 20 µL

- Initial denaturation: 98°C for 30 s
- Cycling (30–35 cycles): 98°C 10s | Tm−5°C 30s | 72°C 1 min/kb (standard) or 15 s/kb (fast protocol)
- Final extension: 72°C for 2 min
- For GC-rich templates: add GC Enhancer Buffer to 1× final concentration
- Products are blunt-ended; A-tail with Taq if TA cloning is required

5. Unit Definition

One unit (U) is defined as the amount of EV5™ 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 ~99 kDa)
Molecular Weight	~99 kDa (SDS-PAGE)
Concentration	2 U/µL
PCR Activity (1 kb)	Single band from 1 ng human genomic DNA, 30 cycles
Long-range PCR (10 kb)	Successful amplification of 10 kb target from genomic DNA
Error Rate	<1 × 10■■ per bp (lacZ fidelity assay)
Blunt-end Fidelity	No non-templated 3’-A addition detected
Thermostability	>95% activity after 98°C, 35 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, expression construct amplification, and library generation
- Long-range PCR of targets from 5 to 20 kb
- NGS library preparation requiring faithful amplification of genomic or cDNA templates
- Site-directed mutagenesis by overlap extension or whole-plasmid inverse PCR
- Amplification of GC-rich, AT-rich, or secondary-structure-forming templates
- Any application where Taq-level error rates are unacceptable

9. Troubleshooting

Problem	Possible Cause	Suggested Action
No product from GC-rich template	Secondary structure	Add GC Enhancer to 1×; use 98°C denaturation; reduce primer Tm
Smearing in long-range PCR	Extension temp too high or time too short	Lower extension to 68°C; extend to 2 min/kb
Multiple bands	Non-specific priming	Raise annealing temp 2°C; use touch-down PCR protocol
Low fidelity (confirmed by sequencing)	Incorrect buffer or Mg²■ concentration	Use supplied 5× EV5 HF Buffer; do not modify Mg²■ without optimization

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.