

Phi29 DNA Polymerase

User Manual | Recombinant, Research Grade

Cat. No. EV-MOL-005 | Version 1.0 | April 2026

Cat. No.	EV-MOL-005	Size	500 U / 2,500 U	Storage	-20°C
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1. Overview

Phi29 DNA Polymerase (EV-MOL-005) is a recombinant, research-grade enzyme produced in *E. coli* Rosetta(DE3) from the cloned bacteriophage phi29 DNA polymerase gene. It is a 413 amino acid, 66.7 kDa monomeric enzyme with extraordinary processivity (>70 kb per binding event), strand displacement activity, and an intrinsic 3' → 5' proofreading exonuclease. All three activities function isothermally at 30°C, without thermocycling.

EV-MOL-005 is the gold standard enzyme for Rolling Circle Amplification (RCA) and Whole Genome Amplification (WGA). Its processivity surpasses that of all other characterized wild-type DNA polymerases, enabling synthesis of DNA strands exceeding 70 kilobases from a single primer-binding event. Each lot is tested for RCA activity, processivity, exonuclease proofreading, and nuclease contamination before release.

2. Catalytic Mechanism

Phi29 DNA Polymerase synthesizes DNA in the 5' → 3' direction with a unique protein-primed initiation mechanism *in vivo*. For *in vitro* applications, random hexamer primers (exonuclease-resistant, phosphorothioate-modified) prime synthesis at multiple sites on circular or linear templates. As the polymerase synthesizes, it simultaneously displaces the downstream strand without requiring a helicase or clamp loader. Displaced strands serve as additional templates for further priming, creating an exponential amplification cascade (Multiple Displacement Amplification). The active 3' → 5' proofreading exonuclease maintains a low error rate ($\sim 1 \times 10^{-6}$ per bp) throughout.

3. Substrate Specificity

Substrate Type	Efficiency	Conditions
Circular DNA (RCA)	Very High	30°C, 16–18 h; exo-resistant hexamers; isothermal
Genomic DNA (WGA/MDA)	Very High	30°C, 2–18 h; >1 µg output from <1 ng input
Linear dsDNA (limited)	Moderate	30°C; lower efficiency than circular template
ssDNA viral genomes	High	30°C, 16 h; suitable for circular ssDNA virus detection

4. Reaction Conditions & Protocol

4.1 Recommended Reaction Setup

Component	Volume
Template DNA (1 pg – 500 ng)	x μ L
10x Phi29 Buffer (EV-MOL-005-RB)	2 μ L
Exo-resistant random hexamers (500 μ M)	1 μ L
dNTPs (10 mM each)	1 μ L
Phi29 DNA Polymerase (EV-MOL-005)	1 μ L (10 units)
Nuclease-free water	to 20 μ L

- Denature template at 95°C for 3 min; snap-cool on ice before adding enzyme
- Isothermal amplification: 30°C for 2–18 h (longer = more product)
- Inactivation: 65°C for 10 min
- Use only exonuclease-resistant random hexamers (phosphorothioate-modified at 3' terminus)

5. Unit Definition

One unit (U) is defined as the amount of Phi29 DNA Polymerase required to incorporate 0.5 nmol of dTMP into acid-insoluble material in 10 minutes at 30°C in a 50 μ L reaction containing 10 μ g/mL poly(dA)-oligo(dT) template-primer and 33 μ M [methyl-³H]-dTTP.

6. Quality Control

Test	Specification
Purity (SDS-PAGE)	>95% (single band at ~66.7 kDa)
Molecular Weight	~66.7 kDa (SDS-PAGE)
Concentration	10 U/ μ L
RCA Activity	>100-fold amplification of 1 ng plasmid in 4 h at 30°C
Processivity	>70 kb products on circular template (gel analysis)
3'→5' Proofreading Exonuclease	Confirmed activity on 3'-mismatched substrate
Exonuclease Contamination	No detectable degradation (λ DNA, 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 μ g RNA (200 U, 2 h, 37°C)
pH (formulation buffer)	7.2–7.6

Sterility

No microbial growth (7-day incubation)

7. Storage & Stability

- **Storage temperature:** -20°C (avoid repeated freeze-thaw; aliquot upon receipt)
- **Supplied in:** 10 mM Tris-HCl (pH 7.4), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol
- **Stability:** 24 months from date of manufacture when stored correctly
- **Shipping:** On dry ice

8. Applications

- Rolling Circle Amplification (RCA) of plasmids, minicircles, and padlock probes
- Whole Genome Amplification (WGA) by Multiple Displacement Amplification (MDA)
- Amplification of low-input or single-cell genomic DNA prior to sequencing
- Detection of circular ssDNA viral genomes (e.g., parvoviruses, geminiviruses)
- Isothermal amplification prior to array hybridization or SNP genotyping

9. Troubleshooting

Problem	Possible Cause	Suggested Action
Low amplification yield	Inadequate denaturation or hexamer quality	Ensure 95°C denaturation; use only phosphorothioate-modified hexamers
High background in no-template control	Hexamer self-priming or contamination	Use stringent negative controls; work in dedicated low-contamination area
Short product size distribution	Reaction too short or high enzyme:template ratio	Extend incubation to 16 h; reduce enzyme to 5 U
Low fidelity / sequencing errors	Insufficient proofreading	Ensure DTT is fresh in buffer; avoid temperatures above 37°C

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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