

# Klenow Fragment (3' → 5' exo-)

User Manual | Recombinant, Research Grade

Cat. No. EV-MOL-004 | Version 1.0 | April 2026

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## 1. Overview

Klenow Fragment (EV-MOL-004) is an exonuclease-deficient, recombinant derivative of *E. coli* DNA Polymerase I produced in *E. coli* BL21(DE3). It consists of the large C-terminal fragment (527 amino acids) with the N-terminal 5' → 3' exonuclease domain deleted and the 3' → 5' proofreading exonuclease abolished by the D424A and E479A mutations. The result is a pure, 5' → 3' DNA polymerase with no exonuclease activity of any kind, approximately 68 kDa in molecular weight.

EV-MOL-004 is the definitive enzyme for fill-in of 5' overhangs, DNA probe labeling by random priming, and Sanger dideoxy sequencing. Because the 3' → 5' exonuclease is abolished, the enzyme cannot degrade chain-terminating ddNTPs or incorporated labeled nucleotides, enabling clean and uniform product generation. Each lot is tested for fill-in activity, exo- verification, and nuclease contamination.

## 2. Catalytic Mechanism

Klenow Fragment (exo-) catalyzes template-directed DNA synthesis strictly in the 5' → 3' direction. The D424A/E479A double mutation in the exonuclease active site permanently abolishes the 3' → 5' proofreading activity. The enzyme extends from a 3'-OH primer terminus, incorporating dNTPs complementary to the template strand. No strand displacement or 5' → 3' exonuclease activity is present. Optimal temperature is 37°C. The enzyme is not thermostable and must not be used in thermocycling protocols.

## 3. Substrate Specificity

Substrate Type	Efficiency	Conditions
5' overhang dsDNA	High	All 4 dNTPs at 33–100 μM; 37°C, 15 min
3' overhang dsDNA	None (exo- cannot chew back)	Use T4 DNA Pol (EV-MOL-002) for 3' overhangs
Random-primed DNA (labeling)	High	Random hexamers; α- <sup>32</sup> P-dNTP; 37°C, 60 min
Nicked dsDNA (nick translation)	High	With DNase I; 37°C, 30–60 min

## 4. Reaction Conditions & Protocol

#### 4.1 Recommended Reaction Setup

Component	Volume
DNA substrate (up to 1 µg)	x µL
10x Klenow Buffer (EV-MOL-004-RB)	2 µL
dNTPs (33 µM each, for labeling; 100 µM for fill-in)	1 µL
Klenow Fragment exo- (EV-MOL-004)	1 µL (5 units)
Nuclease-free water	to 20 µL

- 5' fill-in: all 4 dNTPs at 100 µM; 37°C for 15 min
- Random primer labeling: random hexamers +  $\alpha$ -<sup>32</sup>P-dNTP; 37°C for 60 min
- Sanger sequencing: ddNTPs at 1:4 to 1:8 ratio vs dNTPs; 37°C for 10–15 min
- Inactivation: 75°C for 20 min or spin-column purification

## 5. Unit Definition

One unit (U) is defined as the amount of Klenow Fragment (exo-) required to incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 37°C in a 50 µL reaction containing primed activated calf thymus DNA and 33 µM each dNTP.

## 6. Quality Control

Test	Specification
Purity (SDS-PAGE)	>95% (single band at ~68 kDa)
Molecular Weight	~68 kDa (SDS-PAGE)
Concentration	5 U/µL
5' Fill-in Activity	Complete blunting of 5' overhangs (100 µM dNTPs, 37°C, 15 min)
3'→5' Exo- Verification	<5% substrate degradation (200 U, no dNTPs, 37°C, 1 h)
Strand Displacement (absent)	No displacement detected (nick-translation assay)
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)	7.2–7.6
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

- Fill-in of 5'-overhang restriction termini for blunt-end cloning
- Random primer labeling with radioactive ( $\alpha$ -<sup>32</sup>P-dCTP) or non-radioactive (DIG-dUTP, biotin-dUTP) dNTPs
- Sanger dideoxy chain-termination sequencing
- Nick translation with DNase I for probe labeling
- EMSA probe preparation by 3'-end fill-in with labeled dNTPs

## 9. Troubleshooting

Problem	Possible Cause	Suggested Action
Incomplete fill-in	Insufficient dNTP concentration	Increase all 4 dNTPs to 100 $\mu$ M; extend to 30 min
Low probe specific activity	Old $\alpha$ - <sup>32</sup> P-dNTP or insufficient enzyme	Use fresh isotope; increase enzyme to 10 U
Heterogeneous labeling in random priming	Suboptimal hexamer annealing	Denature DNA at 95°C, snap-cool; use 250 ng/ $\mu$ L hexamers
ddNTP removal in Sanger	This enzyme is exo-, so should not occur; check ddNTP concentration	Verify fresh dNTPs; confirm D424A/E479A lot by spec sheet

## 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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