T4 Polynucleotide Kinase (T4 PNK)

User Manual | Recombinant, Research Grade

Cat. No. EV-MOL-003 | Version 1.0 | April 2026

Cat. No.	EV-MOL-003	**Size**	500 U / 2,500 U	**Storage**	-20°C

1. Overview

T4 Polynucleotide Kinase (EV-MOL-003) is a recombinant, research-grade bifunctional enzyme produced in E. coli Rosetta(DE3) from the cloned bacteriophage T4 pseT gene. It is a homotetramer (~132 kDa) assembled from four identical 33 kDa subunits. The enzyme possesses two independent catalytic activities: (1) an ATP-dependent 5'-kinase that transfers the γ-phosphate of ATP to 5'-OH termini of DNA and RNA; and (2) a 3'-phosphatase that removes 3'-phosphate groups from DNA and RNA in an ADP-dependent reaction.

EV-MOL-003 is the standard reagent for 5'-end labeling with [γ-^{32}P]-ATP, phosphorylation of synthetic oligonucleotides prior to ligation, and NGS library end-repair. Each lot is tested for kinase activity, phosphatase activity, and nuclease contamination before release.

2. Catalytic Mechanism

5'-Kinase reaction: T4 PNK catalyzes ATP + 5'-OH-DNA → ADP + 5'-phosphate-DNA. This is the forward kinase reaction used for end-labeling and oligonucleotide phosphorylation. The reaction requires ATP and Mg²□ and proceeds optimally at 37°C.

3'-Phosphatase reaction: T4 PNK also removes 3'-phosphate groups from DNA and RNA termini, converting non-ligatable 3'-phosphate ends (generated by certain restriction enzymes or chemical fragmentation) to ligatable 3'-OH termini. This reaction is ADP-dependent at physiological pH.

3. Substrate Specificity

Substrate Type	Efficiency	Conditions
5'-OH DNA or RNA	High	ATP 1 mM; Mg²■ 10 mM; 37°C, 30–60 min
Synthetic oligos (phosphorylation)	High	37°C, 30 min; verify by gel shift or TLC
3'-Phosphate DNA/RNA	High	200 µM ADP; same buffer; 37°C, 30 min
Nick-containing dsDNA	Moderate	Combined T4 PNK + T4 Ligase workflow

4. Reaction Conditions & Protocol

4.1 Recommended Reaction Setup

Component	Volume
DNA or RNA substrate (up to 1 µg)	x µL
10× T4 PNK Buffer (EV-MOL-003-RB)	2 µL
[γ-^{32}P]-ATP or cold ATP (10 mM)	1 µL
T4 PNK (EV-MOL-003)	1 µL (10 units)
Nuclease-free water	to 20 µL

- 5'-kinase (forward): 37°C for 30–60 min; 1 mM ATP final
- 3'-phosphatase: substitute ATP with 200 µM ADP; 37°C, 30 min
- Inactivation: 65°C for 20 min or heat-phenol extraction
- For NGS end-repair: combine with T4 DNA Polymerase (EV-MOL-002) in same reaction

5. Unit Definition

One unit (U) is defined as the amount of T4 PNK required to catalyze the transfer of ≥90% of the γ-phosphate from 1 nmol of [γ-^{32}P]-ATP to a 5'-OH oligonucleotide substrate in 30 minutes at 37°C in a 20 µL reaction containing 1× T4 PNK Buffer.

6. Quality Control

Test	Specification
Purity (SDS-PAGE)	>95% (single band at ~33 kDa monomer)
Molecular Weight	~33 kDa monomer (SDS-PAGE)
Concentration	10 U/µL
5'-Kinase Activity	≥90% γ-^{32}P transfer to 5'-OH oligo (37°C, 30 min)
3'-Phosphatase Activity	≥80% 3'-phosphate removal (ADP, 37°C, 30 min)
ATP Dependence	<5% phosphorylation without ATP
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

- 5’-end radiolabeling of DNA and RNA with [γ-^{32}P]-ATP for gel-shift, footprinting, and hybridization
- Phosphorylation of synthetic oligonucleotides for ligation or primer use
- NGS library end-repair: 5’-phosphorylation and 3’-phosphate removal in a single reaction
- Removal of 3’-phosphate groups to generate ligatable 3’-OH termini
- Exchange labeling of pre-phosphorylated 5’ ends with [γ-^{32}P]-ATP and ADP

9. Troubleshooting

Problem	Possible Cause	Suggested Action
Low labeling efficiency	ATP or enzyme too dilute	Increase ATP to 1 mM; use fresh [γ-^{32}P]-ATP; verify enzyme activity
Incomplete phosphorylation of long oligos	Secondary structure in substrate	Denature at 95°C, snap-cool on ice before reaction
Background in 3’-phosphatase assay	Residual kinase activity	Use ADP instead of ATP; increase ADP to 500 µM
No activity after storage	Freeze-thaw degradation	Aliquot upon receipt; avoid repeated freeze-thaw

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.