

## T4 Polynucleotide Kinase (3' phosphatase minus)



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M0236S 001121214121

# M0236S



**200 units 10,000 U/ml Lot: 0011212**

**RECOMBINANT Store at -20°C Exp: 12/14**

**Description:** T4 Polynucleotide Kinase catalyzes the transfer and exchange of P<sub>i</sub> from the γ position of ATP to the 5' -hydroxyl terminus of double- and single-stranded DNA and RNA, as well as nucleoside 3'-monophosphates (1-5). This modified version exhibits full kinase activity with no 3' phosphatase activity (6,7).

**Source:** An *E. coli* strain that carries a plasmid encoding the modified T4 Polynucleotide Kinase gene.

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### Applications:

- 5' phosphorylation of DNA/RNA for subsequent ligation
- End-labeling of DNA or RNA (2)
- 5' phosphorylation of 3' phosphorylated mono nucleotides to generate a substrate (pNp) that can be added to the 3' end of DNA or RNA by ligase activity (8)
- 5' end labeling of 3' phosphorylated oligos (9)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 0.1 μM ATP and 50% glycerol.

### Reagents Supplied with Enzyme:

10X T4 Polynucleotide Kinase Reaction Buffer (RNase free).

### Reaction Conditions:

1X T4 Polynucleotide Kinase Reaction Buffer. Incubate at 37°C.

### 1X T4 Polynucleotide Kinase Reaction Buffer:

70 mM Tris-HCl  
10 mM MgCl<sub>2</sub>  
5 mM DTT  
pH 7.6 @ 25°C

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5 mM DTT  
pH 7.6 @ 25°C

**Note:** T4 Polynucleotide Kinase requires ATP for activity, but its supplied reaction buffer does not contain ATP because it interferes with radiolabeling reactions. Typically, a kinase reaction is followed by a ligation reaction. To simplify this process, T4 Polynucleotide Kinase is optimized for use in T4 DNA Ligase reaction buffer (which contains the appropriate amount of ATP). We recommend performing the kinase reaction in ligase buffer for 30 minutes; you can then proceed to ligation without a buffer change or heat inactivation.

**Unit Definition:** One unit is defined as the amount of enzyme catalyzing the incorporation of 1 nmol of acid-insoluble [<sup>32</sup>P] in 30 minutes at 37°C (1).

**Unit Assay Conditions:** 1X T4 Polynucleotide Kinase Reaction Buffer, 66 μM [γ-<sup>32</sup>P] ATP (5 x 10<sup>6</sup> cpm/μmol), 0.26 mM 5'-hydroxyl-terminated salmon sperm DNA.

**Quality Assurance:** Free of exonuclease, phosphatase, endonuclease and RNase activities. Each lot is tested under 5'-end-labeling conditions to assure maximal transfer of [<sup>32</sup>P].

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### Quality Control Assays

**16-Hour Incubation:** A 50 μl reaction containing 1 μg of λ DNA and 150 units of enzyme incubated for 16 hours showed no degradation.

**Exonuclease Activity:** Incubation of 100 units of enzyme with 1 μg sonicated [<sup>3</sup>H] DNA (10<sup>6</sup> cpm/μg) for 4 hours at 37°C in 50 μl reaction buffer released < 0.1% radioactivity.

**Endonuclease Activity:** Incubation of 100 units of enzyme with 1 μg φX174 RF I DNA for 4 hours at 37°C in 50 μl reaction buffer resulted in < 10% conversion to RF II.

**RNase Activity:** Incubation of 50 units of 2 μg MS2 phage RNA for 1 hour at 37°C in 50 μl 1X T4 Polynucleotide Kinase Reaction Buffer followed by agarose gel electrophoresis showed no degradation.

(See other side)

CERTIFICATE OF ANALYSIS

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**Other Activities:** <sup>32</sup>P end labeling of 5′-hydroxyl terminated d(T)<sub>8</sub> with 25 units for 30 minutes at 37°C in 50 µl 1X T4 Polynucleotide Kinase Reaction Buffer followed by 20% acrylamide gel electrophoresis revealed that less than 1% of the product had been degraded by exonuclease or phosphatase activities.

**Heat Inactivation:** 65°C for 20 minutes.

**Notes On Use:** [<sup>33</sup>P] ATP may be substituted for [<sup>32</sup>P] ATP.

For radioactive labeling, use 1–50 pmol of 5′ termini in a 50 µl reaction containing 1X T4 Polynucleotide Kinase Reaction Buffer, 50 pmol of gamma-[<sup>32</sup>P] ATP and 20 units of T4 Polynucleotide Kinase. Incubate at 37°C for 30 minutes.

For non-radioactive phosphorylation use up to 300 pmol of 5′ termini in a 50 µl reaction containing 1X T4 Polynucleotide Kinase Reaction Buffer, 1 mM ATP and 10 units of T4 Polynucleotide Kinase. Incubate at 37°C for 30 minutes. 1X T4 DNA Ligase Reaction

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Buffer contains 1 mM ATP and can be substituted in non-radioactive phosphorylations (T4 Polynucleotide Kinase exhibits 100% activity in this buffer).

Fresh buffer is required for optimal activity (in older buffers, loss of DTT due to oxidation lowers activity).

The efficiencies of blunt and recessed 5′-end phosphorylation can be improved by heating to 70°C for 5 minutes, then chilling on ice prior to kinase addition and by adding PEG-8,000 to 5% (w/v) (2).

The following levels of inhibition of T4 Polynucleotide Kinase are observed when the supplied reaction buffer is supplemented with:

50 mM NaCl - no inhibition  
100 mM NaCl - 30% inhibition  
150 mM NaCl - 50% inhibition  
7 mM phosphate - 50% inhibition  
7 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> - 75% inhibition

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Since Polynucleotide Kinase is inhibited by ammonium ions, DNA should not be precipitated in the presence of ammonium ions prior to phosphorylation.

Dephosphorylation prior to end-labeling can be avoided by utilizing the exchange reaction, although this results in lower specific activity labeling (4). Sufficient incorporation levels can be attained using the supplied buffer supplemented with 100 µM ADP in the final reaction. However, higher levels of incorporation with the exchange reaction are achieved when using the buffer described in (2).

Gaps can be phosphorylated with elevated levels of ATP. Nicks are not phosphorylated efficiently.

CTP, GTP, TTP, UTP, dATP or dTTP can be substituted for ATP as a phosphate donor (1).

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## References:

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