T4 Polynucleotide Kinase

NEW ENGLAND BioLabs Inc.
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M0201S 091121214121

M0201S

RR Yes

500 units **10,000 U/ml** **Lot: 0911212**

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

Description: Catalyzes the transfer and exchange of P_i from the γ position of ATP to the 5´-hydroxyl terminus of polynucleotides (double- and single-stranded DNA and RNA) and nucleoside 3´-monophosphates. Polynucleotide Kinase also catalyzes the removal of 3´-phosphoryl groups from 3´-phosphoryl polynucleotides, deoxynucleoside 3´-monophosphates and deoxynucleoside 3´-diphosphates (1).

Source: An *E. coli* strain that carries the cloned T4 Polynucleotide Kinase gene. It is purified by a modification of the method of Richardson (1).

Applications:

- End-labeling DNA or RNA for probes and DNA sequencing (2)
- Addition of 5´-phosphates to oligonucleotides to allow subsequent ligation
- Removal of 3´-phosphoryl groups (3)

Supplied in: 50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 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 $MgCl_2$
5 mM dithiothreitol
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 Richardson unit is defined as the amount of enzyme catalyzing the incorporation of 1 nmol of acid-insoluble [^{32}P] in 30 minutes at 37°C (1).

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

Quality Assurance: Free of exonuclease, phosphatase, endonuclease and RNase activities. Each lot is tested under 5´-end-labeling conditions to assure maximal transfer of [^{32}P].

Quality Control Assays

16-Hour Incubation: A 50 µl reaction containing 1 µg of λ DNA and 500 units of enzyme incubated for 16 hours showed no degradation.

Exonuclease Activity: Incubation of 300 units of enzyme with 1 µg sonicated [^{3}H] DNA (10^5 cpm/µg) for 4 hours at 37°C in 50 µl reaction buffer released < 0.1% radioactivity.

Endonuclease Activity: Incubation of 200 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 100 units with 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.

Physical Purity: Purified to > 95% homogeneity as determined by SDS-PAGE analysis using Coomassie Blue detection.

(See other side)

CERTIFICATE OF ANALYSIS

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Other Activities: ^{32}P end labeling of 5´-hydroxyl terminated d$(T)_8$ with 50 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: [^{33}P] ATP may be substituted for [^{32}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-[^{32}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 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 $(NH_4)_2SO_4$ - 75% inhibition

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).

References:

1. Richardson, C.C. (1981). In P.D. Boyer (Ed.), *The Enzymes* Vol. 14, (pp. 299–314). San Diego: Academic Press.
2. Sambrook, J. et al. (1989) *Molecular Cloning*: *A Laboratory Manual*, (2nd ed.), (pp. 10.59–10.67, 11.31–11.33). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.
3. Cameron, V. and Uhlenbeck, 0.C. (1977) *Biochemistry* 16, 5120–5126.
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