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T4 Polynucleotide Kinase, Cloned

Cat. Nos. P0505H, P0501K, and P0503K

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1. Introduction

T4 Polynucleotide Kinase (T4 PNK) catalyzes the transfer of the γ-phosphate of ATP to the 5′ terminus of single- and double-stranded DNA or RNA molecules that have a 5′ hydroxyl. The enzyme also removes the 3′ phosphate from 3′-phosphoryl polynucleotides, deoxyribonucleoside 3′-monophosphates, and deoxyribonucleoside 3′,5′-diphosphates to form a 3′ hydroxyl. T4 PNK has many uses, including labeling the 5′ ends of DNA or RNA with ^{32}P for use in sequencing, as hybridization probes, or in transcript mapping with S1 nuclease or Mung Bean Nuclease. The enzyme can also be used to phosphorylate oligodeoxynucleotide linkers or other DNA molecules prior to ligation and to end-label oligodeoxynucleotide primers for use in sequencing reactions. T4 PNK may also be used in an exchange reaction to radiolabel the 5′ ends of DNA or RNA molecules that have unlabeled 5′ phosphates.

T4 PNK is available in 500-, 1,500-, and 3,000-Unit sizes at a concentration of 10 U/µl. A 10X Reaction Buffer is also provided. A 10 mM ATP Solution is available separately.

2. Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: T4 PNK is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.1 mM EDTA, 1 mM dithiothreitol (DTT), and 0.1% Triton® X-100.

Unit Definition: One unit catalyzes the transfer of 1 nmol of phosphate from γ-[^{32}P]-labeled ATP to the 5′-hydroxyl ends of micrococcal nuclease–treated calf thymus DNA in 30 minutes at 37°C.

10X Reaction Buffer: 330 mM Tris-acetate (pH 7.5), 660 mM potassium acetate, 100 mM magnesium acetate, and 5 mM DTT.

ATP is not included in the 10X Reaction Buffer and must be added separately.

Quality Control: T4 PNK is function-tested in a reaction containing 50 mM Tris-HCl (pH 7.5), 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, 0.1 mM γ-[^{32}P]-ATP, 0.2 mg/ml of micrococcal nuclease-treated calf thymus DNA, and varying amounts of enzyme.

Contaminating Activity Assays: T4 PNK is free of detectable DNA exo- and endonuclease, and RNase activities.

3. Related Products

The following products are also available:

- ATP Solution (10 mM)
- T4 DNA Ligase
- T4 RNA Ligase
- APex™ Heat-Labile Alkaline Phosphatase
- Mung Bean Nuclease
- Tobacco Acid Pyrophosphatase
- RNA 5′ Polyphosphatase
- Fast-Link™ DNA Ligation Kits
- Ampligase® Thermostable DNA Ligase

4. Protocol I: End-labeling with T4 PNK

The following protocol was developed for end-labeling primers for use in many applications requiring end-labeled DNA.

1. Combine the following reaction components on ice in the order given:

		Final Concentration
x µl	deionized water	---
12.5 pmol	primer	0.5 pmol/µl
2.5 µl	10X T4 PNK Reaction Buffer	1X
0.5 µl	γ-[^{32}P]-ATP (80 µCi; 12 pmol; > 7,000 Ci/mmol)	~0.5 pmol/µl
3 U	T4 Polynucleotide Kinase	0.12 U/µl
25 µl	Total reaction volume	

2. Incubate at 37°C for 30 minutes.
3. Inactivate the T4 PNK by incubating at 70°C for 5 minutes.

End-labeled primers can be used in cycle sequencing reactions without separating unincorporated nucleotides from the labeled primer. Primers should be stored at –20°C and may be used for up to 2 weeks after labeling.

5. Protocol II: Phosphorylation of oligodeoxynucleotides with T4 PNK

The following protocol describes the nonradioactive phosphorylation of oligodeoxynucleotides used in ligation amplification assays with Ampligase Thermostable DNA Ligase. The protocol can also be used for other applications requiring the addition of a phosphate to the 5′ end of a DNA strand.

1. Combine the following reaction components on ice in the order given:

		Final Concentration
x µl	deionized water	---
10 µl	oligonucleotide at 1.0 µg/µl*	0.2 µg/µl
5 µl	10X T4 PNK Reaction Buffer	1X
5 µl	10 mM ATP Solution	1 mM
3 U	T4 Polynucleotide Kinase	0.06 U/µl
50 µl	Total reaction volume	

2. Incubate at 37°C for 30 minutes.
3. Inactivate the T4 PNK by incubating at 70°C for 5 minutes.

*To determine the micrograms of double-stranded DNA needed in other applications, multiply the length of the DNA fragment by 0.00034. For example, add 1.7 µg of a 5,000-bp restriction fragment (5,000 x 0.00034 = 1.7).

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