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T4 Polynucleotide Kinase

Catalog Number: E106-1, E106-2

Table 1. Kit Components and Storage

Kit Component	E106-1 (500 units)	E106-2 (2500 units)	Storage	Stability
T4 PNK (10 units/µL)	50 µL	250 µL	-20 °C, avoid repeated free-thaw	The product is stable for 12 months when stored as directed.
10× Reaction Buffer	250 µL	1.25 mL		

Product Description

T4 Polynucleotide Kinase catalyzes the transfer and exchange of Pi from the γ position of ATP to the 5´ -hydroxyl terminus of polynucleotides (double-and single-stranded DNA and RNA) and nucleoside 3´-monophosphates. T4 Polynucleotide Kinase also catalyzes the removal of 3´-phosphoryl groups from 3´-phosphoryl polynucleotides, deoxynucleoside 3´-monophosphates and deoxynucleoside 3´-diphosphates.

The enzyme is available in 500 and 2500 unit sizes at a concentration of 10 U/µL. The enzyme is supplied with a 10× Reaction Buffer.

Applications

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

Product Specifications

- **Storage Buffer:** 10 mM Tris-HCl (pH 7.4 at 25 °C), 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.1 µM ATP, and 50% (v/v) glycerol.
- **Unit Definition:** One unit is defined as the amount of enzyme catalyzing the incorporation of 1 nmol of acid-insoluble [^{32}P] in a total reaction volume of 50 µl in 30 minutes at 37°C in 1X Reaction Buffer with 66 µM [γ-^{32}P] ATP (5 x 10^6 cpm/µmol) and 0.26 mM 5´-hydroxyl-terminated salmon sperm DNA.

Phosphorylation Protocol with T4 PNK

1. Assemble the following reaction in a microcentrifuge tube on ice:

10× Reaction Buffer	5 µL
10 mM ATP	5 µL
DNA	up to 300 pmol of 5' termini
Nuclease-free water	to 49 µL
T4 PNK	1 µL
Total volume	50 µL

2. Mix gently and spin down for a few seconds.
3. Incubate at 37 °C for 30 min.
4. Stop the reaction by heating at 65 °C for 20 min.

End-labeling Protocol with T4 PNK

1. Assemble the following reaction in a microcentrifuge tube on ice:

10× Reaction Buffer	2 µL
^{32}P ATP (3,000 Ci/mmol, 5 mCi/ml)	1 µL
DNA	1 µg
Nuclease-free water	to 19 µL
T4 PNK	1 µL
Total volume	20 µL

2. Mix gently and spin down for a few seconds.
3. Incubate at 37 °C for 30 min.
4. Run the samples for 50 to 60 minutes at 100V in TBE buffer in a 4-20% acrylamide gel (10 cm x 10 cm). A 20 minutes exposure gives very readable signals.