T4 Polynucleotide Kinase

T4 Polynucleotide Kinase

(T4 bacteriophage of Escherichia coli)

Cat. No.	Size
E1261-01	1 000 u
E1261-02	5 000 u

Unit Definition:
One unit is defined as the amount of enzyme required to transfer 1 nmol of gamma-phosphate from ATP to the 5'-OH termini of salmon sperm DNA fragments in 30 min at 37°C (1).

Storage Conditions:
Store at -20°C

Storage Buffer:
50 mM Tris-HCl (pH 7.6 at 22°C), 25 mM KCl, 1 mM dithiothreitol, 0.1 µM ATP, 0.1 mM EDTA and 50 % (v/v) glycerol.

Assay Conditions:
70 mM Tris-HCl (pH 7.6 at 22°C), 10 mM $MgCl_2$, 5 mM dithiothreitol, 27 nmol of DNA-phosphorus (5'-OH terminated salmon sperm DNA) and 70 nM [alpha-^{32}P]ATP. Reaction volume is 100 µl.

Note: T4 Polynucleotidekinase is active in a broad range of reaction buffers, given the presence of 1 mM ATP final concentration. T4 PNK reactions can be conducted without the need to purify DNA from previously conducted reactions. Specifically, T4 PNK is compatible with 1x buffers for T4 DNA ligase + 1 mM ATP. The included 10x T4 PNK reaction buffer does not contain ATP. 1 mM ATP must be added to T4 PNK buffer.

Polynucleotide kinase catalyzes the phosphorylation of 5' hydroxy-termini of double- and single stranded DNA or RNA.

Description:

- Catalyzes the transfer of the gamma-phosphate of ATP to a 5'-OH terminus in DNA or RNA.
- Contains 3'-phosphatase activity (1).
- Used for 5'-end labeling of nucleic acids prior to DNA or RNA sequencing (2, 3).
- Phosphorylates synthetic linkers and fragments of DNA or RNA prior to ligation.
- Labels 5'-termini prior to partial restriction enzyme digestion.

T4 Polynucleotidekinase Reaction Protocol

For cloning DNA fragments by ligating with dephosphorylated vector DNA, the fragments should have phosphates on their 5' termini.
Since phosphorylation of PCR products by T4 polynucleotide kinase is inefficient, primers should be phosphorylated prior to PCR reaction.

Phosphorylation of PCR primers

- Mix the following solutions for each primer required for PCR.

Primer (100 pmol/ul)	5 µl
10x T4 PNK buffer	2.5 µl
10 mM ATP	2.5 µl
H_2O	14 µl
T4 Polynucleotide Kinase (10U/ul)	1 µl

- If even larger amounts of kinased primer are required, use 15 µl primer (100 pmol/ul), 5 µl buffer, 3 µl PNK, 0.5 µl 100 mM ATP, 26.5 µl dd water .
- Incubate for 30 min -1 h at 37°C. Store frozen at -20°C (Final concentration of primers: 20 pmol/ul). Note: 1x EURx T4 DNA Ligase buffer (Cat. No. E1060) contains 1 mM ATP and can be substituted in non-radioactive phosphorylation reactions (T4 PNK exhibits 100 % activity in this buffer).
- Optional: Inactivation of kinase: 5 min at 70°C, primers can be directly used after ethanol precipitation without prior heat inactivation; other protocols suggest direct usage of primers even without the precipitation step.

Quality Control:

All preparations are tested for contaminating endonuclease, exonuclease and nonspecific RNase activities, along with functional testing in end-labeling reactions.

References:

1. *Richardson, C.C. (1971) Progress in Nucleic Acids Research and Molecular Biology 2, 815-828.*
2. *Maxam, A. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 560-56.*
3. *Donis-Keller, H. (1980) Nucleic Acids Res. 8, 3133-3142.*