T4 Polynucleotide Kinase

Catalog: RK20524
Size: 250 U / 500 U / 2,500 U
Concentration: 10,000 U/ml
Components:

T4 Polynucleotide Kinase (10,000 U/ml)	RM20514
10X T4 PNK Reaction Buffer	RM20122

Product Description

T4 Polynucleotide Kinase (T4 PNK) 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. T4 Polynucleotide Kinase also catalyzes the removal of 3′-phosphoryl groups from 3′-phosphoryl polynucleotides, deoxynucleoside 3′-monophosphates and deoxynucleoside 3′-diphosphates.

T4 PNK is applicable to end-labeling DNA or RNA for probes and DNA sequencing, addition of 5′-phosphates to oligonucleotides to allow subsequent ligation and removal of 3′-phosphoryl groups.

Product Source: An *E.coli* strain that carries the cloned T4 Polynucleotide Kinase gene.

Unit Definition: One Richardson 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 T4 PNK 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.

Storage Conditions: 10 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, 0.1 µM ATP pH7.4 @ 25℃
Storage Temperature: -20℃

Reaction Conditions:
1X T4 PNK Reaction Buffer, Incubate at 37℃
1X T4 PNK Reaction Buffer:
70 mM Tris-HCl, 10 mM $MgCl_2$, 5 mM DTT, pH7.6 @ 25℃
Heat Inactivation: 65℃ for 20 min.

Instruction

➢ **Radioactive Labeling with T4 PNK or T4 PNK (3′ phosphatase minus)**

1. Set-up the following reaction:

Nuclease-free Water	up to 50 µl
T4 PNK Reaction Buffer (10X)	5 µl
DNA	Up to 50 pmol of 5′termini
ATP (10 mM)	50 pmol of [γ-^{32}P] ATP
T4 PNK	2 µl (20 units)

2. Incubate at 37℃ for 30 minutes.
3. Heat inactivate by incubating at 65℃ for 20 minutes.

Note: [^{33}P] ATP may be substituted for [^{32}P] ATP.

➢ **Non-radioactive Phosphorylation with T4 PNK or T4 PNK (3′phosphatase minus)**

1. Set-up the following reaction in a microcentrifuge tube on ice:

Nuclease-free Water	up to 50 µl
T4 PNK Reaction Buffer (10X)	5 µl
DNA	up to 300 pmol of 5′termini
ATP (10 mM)	5 µl
T4 PNK	1 µl (10 units)

2. Incubate at 37℃ for 30 minutes.
3. Heat inactivate by incubating at 65℃ for 20 minutes.

Notes: *ATP is not supplied; 1X T4 DNA Ligase Buffer contains 1 mM ATP and can be substituted in non-radioactive phosphorylations (T4 Polynucleotide Kinase exhibits 100% activity in this buffer).*

➢ **End-labeling Protocol**

5′ overhangs can be end-labeled radioactively with T4 Polynucleotide Kinase (PNK), or filled in using the Klenow fragment with labeled dNTPs:

-1 µl ^{32}P ATP (3,000 Ci/mmol, 5 mCi/ml)
-2 µl T4 PNK Reaction Buffer (10X)
-1 or 2 µl DNA ladder (1 µg)
-1 µl T4 PNK

Protocol:

1. Add distilled water to a reaction volume of 20 µl.
2. Incubate for 30 minutes at 37 ℃.
3. 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. The signal strength is about twice that signal when ADP is added to 100 µM.

Notes:

1. 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.
2. Dephosphorylation prior to end-labeling can be avoided by utilizing the exchange reaction, although this results in lower specific activity labeling.
3. Sufficient incorporation levels can be attained using the supplied buffer supplemented with 100 µM ADP in the final reaction.

QC Process:

- Purity is above 95% detected by SDS-PAGE.
- No exonuclease, nuclease, RNase contamination.
- No residual host genomic DNA detected by PCR.