T4 Polynucleotide Kinase

REF: EG25201S

Storage Condition

-20℃

Components

Component	Amount
T4 Polynucleotide Kinase (10 U/μl)	50 μl
10× T4 PNK Buffer	1 ml

Description

T4 Polynucleotide Kinase (T4 PNK) is a polyribonucleotide 5'-hydroxyl kinase that catalyzes the transfer of the γ-phosphate group from ATP to the 5'-hydroxyl terminus of oligonucleotide chains, whether they are double-stranded or single-stranded DNA or RNA. Notably, this reaction is reversible. In addition, T4 PNK exhibits 3'-phosphatase activity, which hydrolyzes the 3'-phosphate group from the 3'-phosphate terminus of oligonucleotides, deoxy-3'-monophosphate nucleotides, and deoxy-3'-diphosphate nucleotides.

Definition of Activity Unit

One unit of activity is defined as the amount of enzyme required to incorporate 1 nmol of [γ-^{32}P] ATP into acid-insoluble precipitate within 30 minutes at 37℃ in 1× T4 PNK Buffer.

Applications

1. 5' phosphorylation of DNA/RNA for subsequent ligation.
2. End labeling DNA or RNA for probes and DNA sequencing.
3. Removal of 3' phosphoryl groups.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 20 μl reaction containing 200 ng of supercoiled plasmid and 10 U of T4 Polynucleotide Kinase incubated for 4 hours at 37 ℃ results in <20% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 20 μl reaction containing 15 ng of dsDNA fragments and 10 U of T4 Polynucleotide Kinase incubated for 16 hours at 37 ℃ results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

RNase Activity

A 10 μl reaction containing 500 ng of RNA and 10 U of T4 Polynucleotide Kinase incubated for 1 hour at 37 ℃ results in >90% of the substrate RNA remains intact as determined by agarose.

Residual Host DNA

Using the third method of the determination of exogenous DNA residue in General Chapter 3407 of ChP(2020) Volume IV, the residual amount of Escherichia coli host cell DNA in this product is less than 1 copy per 10 units.

Protocol

1. 5' -phosphorylation of DNA:

Reagent	Amount
Substrate	1~300 pmol (5' end)
10× T4 PNK Buffer[a]	5 μl
T4 Polynucleotide Kinase	1 μl
ATP (10 mM)	5 μl
ddH_2O	Up to 50 μl

a. The 10× T4 PNK Buffer does not contain radioactive ATP and needs to be prepared separately.

① Mix thoroughly, spin briefly and incubate at 37℃ for 30 min.

② After the reaction is complete, heat at 75 ℃ for 10 minutes to inactivate T4 Polynucleotide Kinase.

2. DNA 5' end labeling:

Reagent	Amount
Substrate	1~50 pmol (5' end)
10×T4 PNK Buffer[b]	5 μl
T4 Polynucleotide Kinase	2 μl
[γ-^{32}P]ATP (10 mM)	0.25 μl
ddH_2O	Up to 50 μl

b. The 10× T4 PNK Buffer does not contain radioactive ATP and needs to be prepared separately.

① Mix thoroughly, spin briefly and incubate at 37℃ for 30 min.

② After the reaction is complete, heat at 75 ℃ for 10 minutes to inactivate T4 Polynucleotide Kinase.

Notice

1. Metal ion chelators, phosphate, ammonium ion, KCl and NaCl over 50mM can significantly inhibit the activity of T4 PNK.
2. Polyethylene glycol (PEG) and spermidine improve the rate and efficiency of the phosphorylation reaction.
3. 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.
4. Keep the enzyme on ice while handling, and store at -20℃ immediately after use.

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