

T4 polynucleotide Kinase (10 U/μL)

Product description

T4 Polynucleotide Kinase is a polynucleotide 5'-hydroxyl kinase that catalyzes the transfer of the γ-phosphate group of ATP to the 5'-hydroxyl termini of oligonucleotide chains (double-stranded or single-stranded DNA or RNA) and to 3'-monophosphate nucleotides, and this reaction is reversible. It possesses 3'-phosphatase activity, hydrolyzing the 3'-phosphate group from the 3'-phosphate termini of oligonucleotides, deoxy-3'-monophosphate nucleotides, and deoxy-3'-diphosphate nucleotides. In the presence of ADP, T4 PNK exhibits 5'-phosphatase activity, catalyzing the exchange of the terminal 5'-phosphate groups between 5'-P-oligo/polynucleotides and ATP. It is suitable for DNA library construction and end-labeling for probe preparation.

Specifications

Cat NO.	12902ES76 / 12902ES86 / 12902ES92
Size	500 U / 2,500 U / 10,000 U
Concentration	10 U/μL
Source	Recombinantly expressed in <i>E. coli</i>
Enzyme Activity Definition	The amount of enzyme required to catalyze the incorporation of 1 nmol of [γ-32P] ATP into acid-precipitable material in 30 minutes at 37°C.

Components

Components No.	Name	12902ES76	12902ES86	12902ES92
12902-A	T4 polynucleotide Kinase (10 U/μL)	50 μL	250 μL	1 mL
12902-B	10×T4 PNK Buffer	500 μL	1 mL	2×1 mL

[Note]: The 10×T4 PNK Buffer does not contain ATP. Customers need to add ATP separately, with a recommended final concentration of 1 mM, or use T4 DNA ligase buffer.

Storage

This product should be stored at -25~-15°C for 1 year.

Instructions

5' End Labeling of DNA/RNA:

1. Set up the reaction system as shown in the following table:

Component	Volume
Dephosphorylated DNA or Oligonucleotide	1~20 pmol (5'-termini) or 10-50 pmol
10×T4 PNK Buffer	2 μL
[γ-32P or γ-33P]-ATP (3,000 Ci/mmol)	20 pmol
T4 polynucleotide Kinase (10 U/μL)	1 μL
H ₂ O	to 20 μL

2. After setting up the reaction, mix gently and incubate at 37°C for 30 minutes.
3. Add 1 μ L of 0.5 M EDTA (pH 8.0), mix well, and incubate at 75°C for 10 minutes to terminate the reaction.
4. Subsequently, purify the labeled DNA using phenol-chloroform extraction and ethanol precipitation, or an appropriate DNA purification kit.

5' End Phosphorylation of DNA:

1. Set up the reaction system as shown in the following table:

Component	Volume
Linear dsDNA or Oligonucleotide	1~20 pmol (5'-termini) or 10-50 pmol
10×T4 PNK Buffer	2 μ L
ATP (10 mM)	2 μ L
T4 polynucleotide Kinase (10 U/ μ L)	1 μ L
H ₂ O	to 20 μ L

2. After setting up the reaction, mix gently and incubate at 37°C for 20 minutes.
3. Incubate at 75°C for 10 minutes to terminate the reaction.
4. Subsequently, purify the phosphorylated DNA using phenol-chloroform extraction and ethanol precipitation, or an appropriate DNA purification kit.

Notes

1. This product is intended for research use only.
2. For your safety and health, please wear a lab coat and disposable gloves when handling this product.