T4 Polynucleotide Kinase

Item No.: T665879
Storage condition: -20℃

Product Content

Component	T665879-500U	T665879-2500U
T4 Polynucleotide Kinase (10U/μl)	50 μl	250 μl
10×T4 PNK Reaction Buffer	150 μl	800 μl

Product Introduction

T4 Polynucleotide Kinase abbreviated as T4PNK, Chinese name T4 Polynucleotide Kinase, is expressed by Escherichia coli, the source of the expressed gene is T4 bacteriophage, which can catalyze the transfer and exchange of phosphoric acid in the γ-site of ATP and 5'-hydroxy terminus of the oligonucleotide chain (double or single stranded DNA or RNA) and 3'-monophosphate nucleosides, and also possesses 3'-phosphatase activity, which can transfer 3'-phosphate groups from the 3'-phosphate termini of oligonucleotides, deoxy 3'-monophosphate nucleosides and deoxy 3'-diphosphate nucleosides. This T4 polynucleotide kinase can be used for 5' end labeling or phosphorylation of oligonucleotides, DNA or RNA; catalyzing 5' phosphorylation of 3' phosphorylated mononucleotides and removal of 3' end phosphate groups. It can be inactivated by heating at 75℃ for 10 minutes, and can also be inactivated by adding EDTA. Metal ion chelators, phosphate, ammonium ions, KCl and NaCl greater than 50mM can significantly inhibit its activity.

Activity definition

The amount of enzyme required to transfer 1 nmol of γ-phosphate group on the transfer ATP to the 5'-OH end of DNA in 30 min at 37° C is defined as 1 active unit.

quality control

After several column purifications, its purity was greater than 99% by SDS-PAGE; it was detected to be free of contamination by nucleic acid endo- and exonucleases, phosphatases and RNAase activity.

Usage

DNA 5' end phosphorylation

1. Refer to the following table to set up the reaction system

reagents	50μl reaction system
DNA to be phosphorylated	1-20 pmol (5' end)
10×T4 PNK Reaction Buffer	2μl
0.1 mM ATP	1μl
T4 Polynucleotide Kinase (10U/μl)	1μl
ddH_2O	up to 20μl

2. After setting up the reaction system according to the above table, gently mix and then centrifuge to precipitate the liquid.
3. Place at 37° C for 30 minutes of incubation.
4. The reaction was terminated by adding 1 μl of 0.5 M/pH8.0 EDTA.

DNA 5' end labeling

1.Refer to the following table to set up the reaction system

reagents	50μl reaction system
DNA to be phosphorylated	1-20 pmol (5' end)
10×T4 PNK Reaction Buffer	2μl
[γ-^{32}P or γ-^{33}P]-ATP (3,000Ci/mmol)	20pmol
T4 Polynucleotide Kinase (10U/μl)	1μl
ddH_2O	up to 20μl

2.After setting up the reaction system according to the table above, gently mix and then centrifuge to precipitate the liquid.
3.Place at 37° C for 30 minutes of incubation.
4.The reaction was terminated by adding 1 μl of 0.5 M/pH8.0 EDTA. For other uses, please refer to the relevant literature for your own work.

matters needing attention

1. Since ammonium salts can strongly inhibit the activity of T4 Polynucleotide Kinase, the DNA obtained from ammonium salt precipitation cannot be used in the labeling reaction of T4 Polynucleotide Kinase.
2. PEG promotes the rate and efficiency of the phosphorylation reaction, and PEG should be added to the exchange reaction system.
3. The enzyme should be stored in an ice box or on an ice bath, and should be stored at -20℃ immediately after use.