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Version: 03/2024

T4 Polynucleotide Kinase

Cat. No. : CW2671S (500 U)
CW2671M (2500 U)

Storage Condition: -20 ℃

Components

Component	CW2671S 500 U	CW2671M 2500 U
T4 Polynucleotide Kinase (10 U/μL)	50 μL	250 μL
10×T4 PNK Reaction Buffer	150 μL	800 μL

Introduction

T4 nucleotide Poly Kinase, T4 PNK for short, is expressed by E. coli. The source of the expressed gene is T4 bacteriophage. It can catalyze the transfer and exchange of phosphate between the γ-position of ATP and the 5'-hydroxyl end of oligonucleotide chains (double-stranded or single-stranded DNA or RNA) and 3'-monophosphate nucleosides. At the same time, it also has 3' phosphatase activity, which can hydrolyze the 3'-phosphate group from the 3' phosphate end of oligonucleotides, deoxy3'-monophosphate nucleosides and deoxy3'-diphosphate nucleosides. The T4 polynucleotide kinase can be used for 5' end labeling or phosphorylation of oligonucleotides, DNA or RNA, and catalyzing 3' phosphorylation of single nucleotide 5' phosphorylation and removal of 3' end phosphate groups, etc. This product can be inactivated by heating at 75 ℃ for 10 minutes and can also be inactivated by adding EDTA. Metal ion chelators, phosphates, ammonium ions, KCl and NaCl in concentrations greater than 50 mM can significantly inhibit its activity.

Active Definition

The amount of enzyme required to transfer 1 nmol of γ-phosphate group on ATP to the 5'-OH end of DNA within 30 minutes at 37 ℃ is defined as 1 activity unit.

Quality Control

After several column purifications, the purity of SDS-PAGE was more than 99%. No contamination by endonuclease, exonuclease, phosphatase, and RNase activities was detected.

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Protocol

Phosphorylation of DNA 5' ends

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

Reagent	50 μL System
DNA to be phosphorylated	1-20 pmol (5' ends)
10×T4 PNK Reaction Buffer	2 μL
0.1mM ATP	1 μL
T4 Polynucleotide Kinase (10 U/μL)	1 μL
ddH_2O	to 20 μL

2. After setting up the reaction system according to the table above, mix gently and then centrifuge to precipitate the liquid.
3. Incubate at 37 ℃ for 30 minutes.
4. Add 1 μL of 0.5 M/pH 8.0 EDTA to stop the reaction.

Labeling of DNA 5' ends

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

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

2. After setting up the reaction system according to the table above, mix gently and then centrifuge to precipitate the liquid.
3. Incubate at 37 ℃ for 30 minutes.
4. Add 1 μL of 0.5 M / pH 8.0 EDTA to stop the reaction.

For other uses, please refer to the relevant documents.

Notes

1. Since ammonium salts can strongly inhibit the activity of T4 Polynucleotide Kinase, the DNA precipitated by ammonium salts cannot be used for the labeling reaction of T4 Polynucleotide Kinase.
2. PEG can promote the rate and efficiency of 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 when used and should be stored at -20 ℃ immediately after use.