Tinzyme Co., Limited

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T4 Polynucleotide Kinase

Product Number: PC71

Shipping and Storage

-20°C

Components

Component	PC71 500U	PC71 2500U
T4 Polynucleotide Kinase (10U/μl)	50 μl	250 μl
10×T4 PNK Reaction Buffer	150 μl	800 μl

Description

T4 Polynucleotide Kinase, abbreviated as T4 PNK, is expressed in Escherichia coli. The expressed gene is derived from T4 bacteriophage and can catalyze the phosphorylation of ATP γ- The 5'- hydroxy terminus and 3' - monophosphate nucleosides of the nucleotide chain (double stranded or single stranded DNA or RNA) undergo transfer and exchange, while also possessing 3'phosphatase activity, which can hydrolyze the 3'-phosphate group from the 3' phosphate terminus of the oligonucleotide, deoxygenated 3'-monophosphate nucleoside, and deoxygenated 3'- diphosphate nucleoside.The T4 polynucleotide kinase can be used for 5' end labeling or phosphorylation of oligonucleotides, DNA, or RNA; Catalyze the phosphorylation of single nucleotide 5'and remove the 3' terminal phosphate group. Heating this product at 75℃ for 10 minutes can inactivate it, and adding EDTA can also inactivate it. Metal ion chelating agents, phosphates, ammonium ions, KCl greater than 50 mM, and NaCl can significantly inhibit its activity.

Unit definition

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

Quality control

After multiple column purification, the purity was detected by SDS-PAGE to be over 99%; After testing, there was no contamination of nucleic acid endonuclease, exonuclease, phosphatase, and RNA enzyme activities.

Protocol

DNA 5 'terminal phosphorylation

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

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

2. After setting the reaction system according to the above table, gently mix and centrifuge to precipitate the liquid.
3. Incubate at 37℃ for 30 minutes.
4. Add 1μL EDTA at 0.5 M/pH8.0 was used to terminate the reaction.

DNA 5 'end labeling

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

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

2. After setting the reaction system according to the above table, gently mix and centrifuge to precipitate the liquid.
3. Incubate at 37℃ for 30 minutes.
4. Add 1μl EDTA at 0.5 M/pH8.0 was used to terminate the reaction.

Please refer to relevant literature for other purposes.

Note

1. Due to the strong inhibitory effect of ammonium salts on the activity of T4 Polynucleotide Kinase, the DNA obtained from ammonium salt precipitation 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. Enzymes should be stored in an ice box or ice bath when used, and should be immediately stored at -20℃ after use.