Intact Genomics®

T4 Polynucleotide Kinase (PNK)

Catalog #	3231	3232
Package Size	500 units	2,500 units
Volume	50 µl	250 µl
Concentration	10 units/µl	

Description

T4 Polynucleotide Kinase (PNK) catalyzes the transfer of the γ-phosphate from ATP to the 5'-OH group of single- and double-stranded DNAs and RNAs, oligonucleotides or nucleoside 3'-monophosphates. In the presence of ADP, T4 PNK exhibits 5'-phosphatase activity and catalyzes the exchange of phosphate group between 5'-P-oligonucleotides/polynucleotides and ATP (1). The enzyme is also a 3'-phosphatase (2).

Physical Purity

The purity of this enzyme is >98% homogeneity as determined by SDS-PAGE using Coomassie® Blue staining (see figure below).

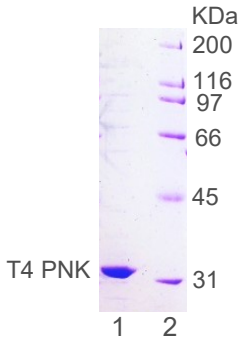

Figure: Lane 1. T4 PNK
Lane 2. Protein marker

Product Source

E. coli cells with a cloned pseT gene of bacteriophage T4.

Applications

- Labeling 5'-termini of nucleic acids (3, 4)
- 5'-phosphorylation of oligonucleotides, PCR products, other DNA or RNA prior to ligation.
- Phosphorylation of PCR primers.
- Detection of DNA modification by the [^{32}P]-postlabeling assay (5, 6).
- Removal of 3'-phosphate groups (2).

Includes

1) T4 Polynucleotide Kinase (PNK)
2) 10X T4 PNK Reaction Buffer

Storage Temperature

–20 °C

Storage Buffer

50 mM Tris-HCl,
50 mM KCl,
1 mM DTT,
0.1 mM EDTA,
50% Glycerol, pH 7.5 @ 25 ºC

10X T4 PNK Reaction Buffer

500 mM Tris-HCl
100 mM MgCl2
50 mM dithiothreitol
pH 7.5 @ 25 °C

Note

T4 PNK requires ATP for activity, but it is not supplied with ATP because it interferes with radiolabeling reactions.

Unit Definition

One unit of T4 Polynucleotide Kinase converts 1 nmol of 32P from [γ-32P]-ATP into an acid-insoluble form in 30 minutes at 37 °C under standard assay conditions.

Inhibition and Inactivation

- Inhibitors: metal chelators, phosphate and ammonium ions, KCl and NaCl at a concentration higher than 50 mM.
- Inactivated by heating at 70°C for 15 min or by addition of EDTA.

Quality Control Assays

T4 PNK is tested in 5´ phosphorylation of nucleic acids and is free from exo- and endonuclease and RNase activities.

For Radioactive Labeling

Use 1–50 pmol of 5´ termini in a 50 µl reaction containing 1X T4 PNK reaction buffer, 50 pmol of gamma-[32P] ATP and 20 units of T4 PNK. Incubate at 37 °C for 60 minutes.

For Non-Radioactive Phosphorylation

Use up to 300 pmol of 5´ termini in a 50 µl reaction containing 1X T4 PNK reaction buffer, 1 mM ATP and 10 units of T4 PNK. Incubate at 37 °C for 60 minutes.

References

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3. Sambrook, J., Russell, D.W., Molecular Cloning: A Laboratory Manual, the third edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001.
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5. Phillips, D.H., Detection of DNA modifications by the 32P-postlabelling assay, Mutation Res., 378, 1-12, 1997.
6. Keith, G., Dirheimer, G., Postlabeling: a sensitive method for studying DNA adducts and their role in carcinogenesis, Curr. Opin. Biotechnol. 6, 3-11, 1995.

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