T4 Polynucleotide Kinase (PNK)

Manual

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

Important!

-20°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

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Table of Contents

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).

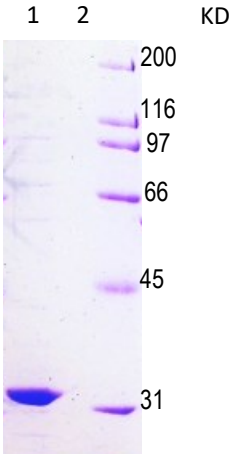

Figure: Lane 1. T4 PNK
Lane 2. Protein Marker

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 [32P]-post labeling assay (5, 6).
- Removal of 3'-phosphate groups (2).

Product Components:

- T4 Polynucleotide Kinase (PNK)
- 10x T4 PNK Reaction Buffer

Storage Temperature:

–20 °C

Storage Buffer:

50 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM β-mercaptoethanol, 1 mM DTT, 25% (v/v) glycerol

10x T4 PNK Reaction Buffer:

500 mM Tris-HCl, 100 mM $MgCl_2$, 50 mM dithiothreitol, pH 7.5 @ 25°C

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.

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

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.

Related Products:

- *Taq* DNA Polymerase (Cat.# 3243)
- *Taq* DNA Polymerase 2x Premix (Cat.# 3249)
- *Taq* DNA Ligase (Cat.# 3219)
- T4 DNA Ligase (Cat.# 3212)
- T4 DNA Polymerase (Cat.# 3222)

Ordering Information:

- Order online within the USA. Place orders on **www.intactgenomics.com** using our secure Shopping Cart.
- Order by email, phone, or fax.
  Email: **sales@intactgenomics.com**
  Phone: (314) 942-3655 **|** Toll-free : 855-835-7172 **|** Fax: (314) 942-3656
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References:

1. Berkner, K.L., Folk, W.R., Polynucleotide kinase exchange reaction, J. Biol. Chem., 252, 3176-3184, 1977.

2. Richardson, C.C., Bacteriophage T4 polynucleotide kinase, The Enzymes (Boyer, P.D., ed.), 14, 299-314, Academic Press, San Diego, 1981.

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.

4. Current Protocols in Molecular Biology, vol. 1 (Ausubel, F.M., et al., ed.), John Wiley & Sons, Inc., Brooklyn, New York, 3.10.2-3.10.5, 1994-2004.

5. Phillips, D.H., Detection of DNA modifications by the 32P-postlabelling assay, Mutation Res., 378, 1-12, 1997.

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