Thermo
SCIENTIFIC

PRODUCT INFORMATION

T4 Polynucleotide Kinase (T4 PNK)

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Lot: _ Expiry Date: _

Components	#EK0031	#EK0032
T4 Polynucleotide Kinase (T4 PNK), 10 U/µL	500 U	2500 U
10X Reaction Buffer A *(for forward reaction)*	0.4 mL	2 × 1 mL
10X Reaction Buffer B *(for exchange reaction)*	0.2 mL	1 mL
24% PEG 6000 Solution	0.2 mL	1 mL

Store at -20 °C

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Description

T4 Polynucleotide Kinase (T4 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 (forward reaction). The reaction is reversible. In the presence of ADP T4 Polynucleotide Kinase exhibits 5'-phosphatase activity and catalyzes the exchange of phosphate group between 5'-P-oligo-/polynucleotides and ATP (exchange reaction) (1). The enzyme is also a 3'-phosphatase (2).

Applications

- Labeling 5'-termini of nucleic acids (3, 4) (*see* protocols on back page) to be used as:
  - probes for hybridization,
  - probes for transcript mapping,
  - markers for gel-electrophoresis,
  - primers for DNA sequencing,
  - primers for PCR.
- 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).

Source

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

Molecular Weight

The enzyme is a homotetramer. It consists of four identical subunits of 28.9 kDa.

Definition of Activity Unit

One unit of the enzyme transfers 1 nmol of γ-phosphate from ATP to 5'-OH DNA in 30 min at 37 °C.

Enzyme activity is assayed in the following mixture: 100 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, 5 mM DTT, 0.5 mM 5'-OH DNA, 0.05 mM ATP and 0.1 MBq/mL [γ-^{33}P]-ATP.

Storage Buffer

The enzyme is supplied in: 20 mM Tris-HCl (pH 7.5), 25 mM KCl, 0.1 mM EDTA, 2 mM DTT and 50% (v/v) glycerol.

10X Reaction Buffer A (for forward reaction)

500 mM Tris-HCl (pH 7.6 at 25 °C), 100 mM $MgCl_2$, 50 mM DTT, 1 mM spermidine.

10X Reaction Buffer B (for exchange reaction)

500 mM imidazole-HCl (pH 6.4 at 25 °C), 180 mM $MgCl_2$, 50 mM DTT, 1 mM spermidine and 1 mM ADP.

Inhibition and Inactivation

- Inhibitors: metal chelators, phosphate and ammonium ions, KCl and NaCl at a concentration higher than 50 mM.
- Inactivated by heating at 75 °C for 10 min or by addition of EDTA.

Note

- 5'-termini of nucleic acids can be labeled by either the forward or the exchange reaction (1).
- Polyethylene glycol (PEG) and spermidine improve the rate and efficiency of the phosphorylation reaction (7). PEG is used in the exchange reaction.
- As T4 Polynucleotide Kinase is inhibited by ammonium ions, use sodium acetate to precipitate DNA prior to phosphorylation (1, 2).
- Activity in Thermo Scientific Buffers, % (in comparison to activity in buffer A):

Buffers	Activity, %
for restriction enzymes: Thermo Scientific FastDigest, FastDigest™ Green, O, G, 1X Thermo Scientific Tango, 2X Tango™, BamHI, EcoRI	100
B, R, KpnI	75-100
Ecl136II, PacI, SacI	50-75
for *Taq* buffer with KCl	100
for RT buffers	100

(continued on back page)

Protocol for DNA/RNA 5'-end labeling by T4 PNK in the forward rection

1. Prepare the following reaction mixture:

Dephosphorylated DNA *or* Oligonucleotide	1-20 pmol of 5'-termini 10-50 pmol
10X reaction buffer A	2 µL
[γ-^{32}P or γ-^{33}P]-ATP	20 pmol
T4 Polynucleotide Kinase	1 µL (10 U)
Water, nuclease-free (#R0581)	to 20 µL
Total volume	20 µL

2. Incubate at 37 °C for 30 min.
3. Add 1 µL 0.5 M EDTA (pH 8.0) and incubate at 75 °C for 10 min.
4. Separate labeled DNA from unincorporated label by gel filtration on Sephadex G-50.

Protocol for DNA 5'-end labeling by T4 PNK in the exchange reaction

1. Prepare the following reaction mixture:

Linear DNA	1-20 pmol of 5'-termini
10X reaction buffer B	2 µL
[γ-^{32}P or γ-^{33}P]-ATP	40 pmol
24% (w/v) PEG 6000 solution	4 µL
T4 Polynucleotide Kinase	1 µL (10 U)
Water, nuclease-free (#R0581)	to 20 µL
Total volume	20 µL

2. Incubate at 37 °C for 30 min.
3. Add 1 µL 0.5M EDTA (pH 8.0) and incubate at 75 °C for 10 min.
4. Separate labeled DNA from unincorporated label by gel filtration on Sephadex G-50.

Note

- If ethanol solution of [γ-^{32}P or γ-^{33}P]-ATP is used, dry the required amount of ATP under vacuum and dissolve in water, nuclease-free.
- The ATP concentration should be at least 1 µM in the forward reaction and at least 2 µM in the exchange reaction (3, 4).

Protocol for Phosphorylation of DNA

1. Prepare the following reaction mixture:

Linear ds DNA *or* Oligonucleotide	1-20 pmol of 5'-termini 10-50 pmol
10X reaction buffer A for T4 Polynucleotide Kinase	2 µL
ATP, 10 mM*	2 µL
T4 Polynucleotide Kinase	1 µL (10 U)
Water, nuclease-free (#R0581)	to 20 µL
Total volume	**20 µL**

* Prepare 10 mM ATP solution by combining 10 µL of 100 mM ATP solution (#R0441) and 90 µL of Water, nuclease-free.

2. Mix thoroughly, spin briefly and incubate at 37 °C for 20 min.
3. Heat at 75 °C for 10 min.

Note

Visit www.thermofisher.com for molar calculations.

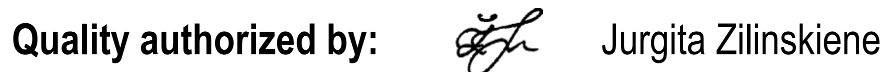
CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable degradation was observed after incubation of supercoiled plasmid DNA with T4 Polynucleotide Kinase.

Ribonuclease Assay

No detectable degradation was observed after incubation of [3H]-RNA with T4 Polynucleotide Kinase.



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