Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

T4 Polynucleotide Kinase

#EK0032 2500 u

Lot: **Expiry Date:**

Concentration: 10 u/µl

Supplied with: 2 x 1 ml of 10X Reaction Buffer A
(for forward reaction)
1 ml of 10X Reaction Buffer B
(for exchange reaction)
1 ml of 24% PEG 6000 Solution

Store at -20°C

In total 5 vials.

ISO 9001 ISO 14001
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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 Fermentas Buffers, % (in comparison to activity in buffer A)

Fast-Digest®/ Fast-Digest® Green	B, R	O, G	*Taq* with KCl	RT	T4 DNA Ligase	Tango™		BamHI	EcI136II, PacI, SacI	EcoRI	KpnI
						1X	2X				
100	75-100	100	100	100	100	100	100	100	50-75	100	75-100

(continued on back page)

QUALITY CONTROL ASSAY DATA

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 50 units of enzyme with 1 µg of pUC19 DNA in 50 µl of activity assay buffer for 4 hours at 37°C.

Ribonuclease Assay

≤0.5% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 50 units of enzyme with 1 µg of [^{3}H]-RNA in 50 µl of activity assay buffer for 4 hours at 37°C.

Quality authorized by:  Jurgita Zilinskiene

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 extract with an equal volume of chloroform.
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 extract with an equal volume of chloroform.
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

See Appendix on p.522 or visit www.fermentas.com/reviewer for molar calculations.

References

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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 ^{32}P-postlabelling assay, Mutation Res., 378, 1-12, 1997.
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7. Harrison, B., Zimmerman, S.B., T4 polynucleotide kinase: macromolecular crowding increases the efficiency of reaction at DNA termini, Anal. Biochem., 158, 307-315, 1986.