Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

T4 Polynucleotide Kinase

#EK0031 500u

Lot: **Quality guaranteed:**

Concentration: 10u/µl

Supplied with: 0.4ml of 10X Reaction Buffer A *(for forward reaction)*
0.2ml of 10X Reaction Buffer B *(for exchange reaction)*
0.2ml of 24% PEG 6000 Solution

Store at –20°C

In total 4 vials.

ISO 9001 ISO 14001
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Description

T4 Polynucleotide Kinase (T4 PNK) is a polynucleotide 5'-hydroxyl kinase that catalyzes the transfer of the γ-phosphate from ATP to 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 PNK exhibits 5'-phosphatase activity and catalyzes the exchange of terminal of 5'-phosphate groups (exchange reaction) (1). The enzyme is also a 3'-phosphatase (2).

Source

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

Unit Definition

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

Activity Assay

100mM Tris-HCl (pH 8.0), 10mM $MgCl_2$, 5mM DTT, 0.5mM 5'-OH DNA, 0.05mM ATP and 0.1MBq/ml [γ-^{33}P]-ATP.

Storage Buffer

20mM Tris-HCl (pH 7.5), 25mM KCl, 0.1mM EDTA, 2mM DTT and 50% glycerol.

10X Reaction Buffer A *(for forward reaction)*

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

10X Reaction Buffer B *(for exchange reaction)*

0.5M imidazole-HCl (pH 6.4 at 25°C), 0.18M $MgCl_2$, 50mM DTT, 1mM spermidine, 1mM EDTA and 1mM ADP.

Applications

- Labeling 5'-termini of nucleic acids (3, 4) (*see* protocols on back page) for production of:
  – probes for hybridization,
  – probes for transcript mapping,
  – markers for gel-electrophoresis,
  – primers for DNA sequencing,
  – primers for PCR.
- 5'-phosphorylation of oligonucleotide linkers and DNA or RNA prior to ligation.
- Detection of DNA modification by the [^{32}P]-postlabeling assay (5, 6).
- Removal of 3'-phosphate groups (2).

Inactivation

By heating at 70°C for 10min or by the 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 reactions (7). PEG should be added to the exchange reaction mixture (*see* protocol on back page).
- T4 Polynucleotide Kinase is inhibited by phosphate and ammonium ions or by KCl and NaCl at a concentration >50mM. The DNA should not be precipitated in the presence of ammonium ions prior to the phosphorylation reaction (3, 4).
- Activity in Fermentas REase Buffers, % (in comparison to activity in buffer A)

B	G	O	R	Tango™		BamHI	Ecl136II, SacI	EcoRI	KpnI
				1X	2X				
75-100	100	100	75-100	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 1µg of pBR322 DNA in 50µl of activity assay buffer for 4 hours at 37°C.

Exodeoxyribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 50 units of enzyme with 1µg of sonicated *E.coli* [^{3}H]-DNA in 50µl of activity assay buffer for 4 hours at 37°C.

Ribonuclease Assay

0% of the total radioactivity was released into trichloroacetic acid-soluble fraction after incubation of 50 units of enzyme with 1µg of [^{3}H]-RNA in 50µl of activity assay buffer for 4 hours at 37°C.

Functional Assay

T4 Polynucleotide Kinase was tested for labeling 5′-termini of DNA.

Quality authorized by: Jurgita Zilinskiene

Protocol for Labeling 5'-termini of DNA by Forward Reaction

❶ Prepare the following reaction mixture:

dephosphorylated DNA	1-20pmol of 5'-termini
10X reaction buffer A	2µl
[γ-^{32}P or γ-^{33}P]-ATP	20pmol
water, nuclease-free	to 19µl
T4 Polynucleotide Kinase (10u)	1µl

Incubate at 37°C for 30 minutes.

❷ Add 1µl 0.5M EDTA (pH 8.0) and extract with an equal volume of chloroform.

❸ Separate labeled DNA from unincorporated label by gel filtration on Sephadex G-50.

Protocol for Labeling 5'-protruding Termini of DNA by Exchange Reaction

❶ Prepare the following reaction mixture:

digested DNA	1-20pmol of 5'-termini
10X reaction buffer B	2µl
[γ-^{32}P or γ-^{33}P]-ATP	40pmol
24% (w/v) PEG 6000 solution	4µl
water, nuclease-free	to 19µl
T4 Polynucleotide Kinase (10u)	1µl

Incubate at 37°C for 30 minutes.

❷ Add 1µl 0.5M EDTA (pH 8.0) and extract with an equal volume of chloroform.

❸ 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 1µM in the forward reaction and at least 2µM in the exchange reaction (3, 4).

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.
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.
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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Related Products

Product	Cat. No.
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PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.
Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

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