T4 Polynucleotide Kinase, 3´-phosphatase free

From phage T4 am N81 pse T1 infected *Escherichia coli BB*
ATP: 5'-dephosphopolynucleotide 5'-phosphotransferase

Cat. No. 10 709 557 001 200 units
Cat. No. 10 838 292 001 1,000 units

Version 19
Content version: February 2020

Store at −15 to − 25°C

Product overview

Storage buffer
50 mM Tris-HCl, 1 mM dithiothreitol, 0.1 mM EDTA, 1 µM ATP, 50% glycerol (v/v), pH approx. 7.5 (+25°C).

Volume activity
10×10^3 U/ml. T4 Polynucleotide kinase (PNK), 3´-phosphatase free, is assayed acc. to (1).

One unit is the enzyme activity which catalyzes the incorporation of 1 nmol [^{32}P] into acid-precipitable products within 30 min at +37°C.

Specific activity
$\geq 40 \times 10^3$ U/mg acc. to (1, 2).

Source
T4 PNK, 3´-phosphatase free, is prepared from phage T4 *am* N81 *pse* T1-infected *E. coli BB* cells.

Purity
≥ 2 µg of PNK, 3´-phosphatase free, migrate as a single band in SDS polyacrylamide gel electrophoresis according to (3).

Supplied phosphorylation buffer
Phosphorylation buffer for direct phosphorylation, 10 × conc.:

500 mM Tris-HCl, 100 mM $MgCl_2$, 1 mM EDTA, 50 mM dithiothreitol, 1 mM spermidine, pH 8.2 (at +25°C).

Properties
This phage derivative lacks 3´-phosphatase activity. The enzyme catalyzes the transfer of the terminal phosphatase group of ATP to the 5´-hydroxylated terminus of DNA or RNA (1). It also exchanges 5´terminal phosphate groups (4).

Application
T4 PNK, 3´-phosphatase free, is of interest for phosphorylating both 5´- and 3´-termini of RNA.

The added 3´-phosphate prevents cyclization or self addition of the RNA. Another use of T4 PNK, 3-phosphatase free, is the 5´[^{32} P]-terminal labeling of 3´-CMP to give 5´[^{32} P]pCp. This substrate is commonly used for 3´end labeling of RNA with T4 RNA ligase (5).

Note: The 3´-phosphatase activity of the wild-type kinase is not observed under optimal incubation conditions with the mutant T4 PNK.

Stability
The undiluted enzyme is stable at −15 to −25°C until the expiration date printed on the label.

Protocols and required material

Calculation of pmol ends
Use the length and the concentration of your input DNA to calculate pmol ends.

The conversion formula is described in our "Lab FAQS" described in Lab FAQS (http://www.roche.com/labfaqs) under commonly used formulas.

Additional reagents required
- 20 pmol [γ-^{32} P] ATP, ≥10 µM (aqueous solution)
- dephosphorylated DNA-fragment solution (20 pmol 5´-OH-termini).

Protocol
Standard assay for 5´-end-labeling of free 5´-OH-termini.

Step	Action
1	Pipet the following components to a reaction vial on ice: **Reagent** / **Volume** Dephosphorylated DNA-fragment (20 pmol 5´-OH-termini) / variable 20 pmol [γ-^{32} P] ATP, (aqueous solution), ≥10 µM / variable Phosphorylation buffer, 10 × conc. / 2 µl • Add double dist. water to a final volume of 19 µl.
2	• Add 10 U T4 PNK 3´-phosphatase free. • Mix well.
3	Incubate 30 min at +37°C.
4	Stop the reaction by cooling in an ice bath.

Determination of labeling efficiency
The yield of the labeling reaction can be determined by trichloracetic acid precipitation.

Quality control

For lot-specific certificates of analysis, see section, **Contact and Support**.

Changes to previous version

Update of the chapter Quality Control.

References

1 Richardson, C. C. (1968) *Proc. Natl. Acad. Sci. USA* **54**, 158.
2 Bradford, M. (1976) *Anal. Biochem.* **72**, 248.
3 Laemmli, U. K. (1970) *Nature* **227**, 680.
4 Chaconas, G. & v. d. Sande, J. H. (1980), *Methods Enzymol.* **65**, 680.
5 Otsuka, E. *et al.* (1981), *Proc. Natl. Acad. Sci. USA* **78**, 5439.
6 Cameron, V. & Uhlenbeck, O. C. (1977) *Biochemistry* **16**, 5120.
7 Cameron, V. *et al.* (1978) *Nucleic Acids Res.* **5**, 825.
8 Soltis, D. A. & Uhlenbeck, O. C. (1982) *J. Biol. Chem.* **257**, 11332–11339.
9 Soltis, D. A. & Uhlenbeck, O. C. (1982) *J. Biol. Chem.* **257**, 11340–11345.
10 Vance J.R. & Wilson T.E. (2001) Uncoupling of 3'-Phosphatase and 5'-Kinase Functions in Budding Yeast. *J. Biol. Chem.* **276,** 15073-15081.
11 Vance J.R. & Wilson T.E. (2001) Repair of DNA Strand Breaks by the Overlapping Functions ofLesion-Specific and Non-Lesion-Specific DNA 3' Phosphatases. *Molecular and Cellular Biol.* **21**, 7191-7198.

Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

Trademarks

Disclaimer of License

For patent license limitations for individual products please refer to: List of biochemical reagent products

Contact and Support

To ask questions, solve problems, suggest enhancements and report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany