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

T4 Polynucleotide Kinase, 3’-phosphatase free

from phage T4 am N81 pse T1 infected *Escherichia coli* BB

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ATP: 5’-dephosphopolynucleotide 5’-phosphotransferase

Cat. No. 10 709 557 001 200 U

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	T4 Polynucleotide Kinase, 3′-phosphatase free	Solution containing 10 U/µl of enzyme in storage buffer: 50 mM Tris-HCl, 1 mM dithiothreitol (DTT), 0.1 mM EDTA, 1 µM ATP, 50% glycerol (v/v), pH approximately 7.5 at +25°C.	1 vial, 200 U
2	T4 Polynucleotide Kinase, 3′-phosphatase free, Phosphorylation Buffer, 10x conc.	500 mM Tris-HCl, 100 mM $MgCl_2$, 1 mM EDTA, 50 mM dithiothreitol (DTT), 1 mM spermidine, pH 8.2 at +25°C.	1 vial, 1 ml

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	T4 Polynucleotide Kinase, 3′-phosphatase free	Store at −15 to −25°C.
2	Phosphorylation Buffer, 10x conc.	

1.3. Additional Equipment and Reagent required

For 5′-end labeling

- 20 pmol [γ ^{32}P]-ATP, 10 µM (aqueous solution)
- Dephosphorylated DNA fragment solution (20 pmol 5 ′-OH-termini)
- Double-distilled water

1.4. 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.

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

2. How to Use this Product

2.1. Protocols

5′-end-labeling of free 5′-OH-termini

1. Add to the dephosphorylated DNA-fragment solution (20 pmol 5′-OH-termini) on ice:

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

Reagent	Volume [µl]
Phosphorylation Buffer, 10x conc.	2
20 pmol [γ ^{32}P]-ATP, ≥10 µM (aqueous solution)	X
Double-distilled water	add to a final volume of 19 µl
10 U T4 Polynucleotide Kinase, 3′-phosphatase free	1
Total Volume	**19**

2. Mix and centrifuge briefly.

3. Incubate for 30 minutes at +37°C.

4. Stop the reaction by cooling in an ice bath.

Labeling yield

Determine the yield of the labeling reaction by trichloroacetic acid precipitation.

2.2. Parameters

Purity

≥2 µg of T4 Polynucleotide Kinase, 3′-phosphatase free migrates as a single band in SDS polyacrylamide gel electrophoresis.

Specific Activity

≥ 40 x 10^3 U/mg

Unit Definition

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

Volume Activity

10 x 10^3 U/ml

3. Additional Information on this Product

3.1. Test Principle

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. It also exchanges 5′terminal phosphate groups.

3.2. Quality Control

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

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Trademarks

All product names and trademarks are the property of their respective owners.

4.4. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

4.5. Regulatory Disclaimer

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

4.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.7. Contact and Support

To ask questions, solve problems, suggest enhancements or 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

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