



Promega

Technical Bulletin

T4 Polynucleotide Kinase

INSTRUCTIONS FOR USE OF PRODUCTS M4101 AND M4103.



www.promega.com

T4 Polynucleotide Kinase

All technical literature is available on the Internet at: www.promega.com/tbs/
 Please visit the web site to verify that you are using the most current version of this
 Technical Bulletin. Please contact Promega Technical Services if you have questions on use
 of this system. E-mail: techserv@promega.com

1. Description.....	1
2. Product Components and Storage Conditions	1
3. Standard Applications	2
A. 5' End-Labeling Protocol, Forward Reaction.....	2
B. Non-Radioactive Kinase Reaction	3
C. Exchange Reaction Using Protruding 5' Phosphoryl Termini as Templates.....	4
4. Product Specifications	5
5. Composition of Buffers and Solutions	5
6. References	6

1. Description

T4 Polynucleotide Kinase (T4 PNK) catalyzes the transfer of the γ -phosphate from ATP to the 5'-terminus of polynucleotides or to mononucleotides bearing a 3'-phosphate group (1). T4 PNK is widely used to end-label short oligonucleotide probes (2), DNA (3) and RNA (4) molecules. Under certain conditions, the reaction with polynucleotides can be made reversible, permitting exchange of the γ -phosphate of ATP with the 5' terminal phosphate of a polynucleotide, thus circumventing the need to dephosphorylate the substrate with alkaline phosphatase (5).

2. Product Components and Storage Conditions

Product	Size	Cat.#
T4 Polynucleotide Kinase	100 units	M4101
	1,000 units	M4103

For Laboratory Use. Cat.# M4101 includes:

- 1 vial T4 Polynucleotide Kinase
- 1 vial Kinase 10X Reaction Buffer

Storage Conditions: Store the T4 Polynucleotide Kinase and the Kinase 10X Reaction Buffer at -20°C .

3. Standard Applications

3.A. 5' End-Labeling Protocol, Forward Reaction

Materials to Be Supplied by the User

(Solution compositions are provided in Section 5.)

- 0.5M EDTA
- ethanol, 100%
- phenol:chloroform:isoamyl alcohol (25:24:1)
- TE buffer
- 7.5M ammonium acetate or 3M sodium acetate (pH 5.5)
- [γ - 32 P]ATP (at 3,000Ci/mmol, 10mCi/ml)

Dephosphorylate the DNA to be labeled prior to performing the labeling reaction. Labeling of protruding 5' ends is more efficient than the labeling of blunt ends or recessed 5' ends. Alternative protocols may be used to increase the labeling efficiency of these templates (6,7).

1. Assemble the following reaction in a sterile microcentrifuge tube:

5' ends of DNA (dephosphorylated)	1-50pmol
Kinase 10X Buffer	5 μ l
[γ - 32 P]ATP (at 3,000Ci/mmol, 10mCi/ml, 50pmol total)	15 μ l
T4 Polynucleotide Kinase	10-20u
deionized water to a final volume of	50 μ l



Do not dissolve DNA in, or precipitate from, buffers containing ammonium salts prior to treatment with T4 PNK (6). Ammonium ions are strong inhibitors of T4 Polynucleotide Kinase.

2. Incubate at 37°C for 10 minutes.
3. Stop the reaction by adding 2 μ l of 0.5M EDTA.
4. Add one volume of phenol:chloroform:isoamyl alcohol (25:24:1). Vortex for 1 minute and spin at full speed in a microcentrifuge for 2 minutes.
5. Transfer the upper, aqueous phase to a fresh tube. Add one volume of chloroform:isoamyl alcohol (24:1). Vortex for 1 minute and spin at full speed in a microcentrifuge for 2 minutes.
6. Transfer the upper, aqueous phase to a fresh tube. Add 0.5 volume of 7.5M ammonium acetate or 0.1 volume of 3M sodium acetate.
7. Add 2 volumes of 100% ethanol, mix and place at -70°C for 30 minutes.
8. Spin at full speed in a microcentrifuge for 5 minutes.
9. Decant the supernatant, and resuspend the DNA in 50 μ l of TE buffer.

The labeled DNA can be separated from unincorporated labeled nucleotides with the Wizard® DNA Clean-Up System (Cat.# A7280), chromatography or an additional ethanol precipitation.

3.B. Non-Radioactive Kinase Reaction (8)

Materials to Be Supplied by the User

(Solution compositions are provided in Section 5.)

- 0.5M EDTA
- ethanol, 100%
- phenol:chloroform:isoamyl alcohol (25:24:1)
- TE buffer
- 7.5M ammonium acetate or 3M sodium acetate (pH 5.5)
- 0.1mM ATP stock

Phosphorylation of insert DNA may be required for ligation with a nonphosphorylated vector. The following reaction may be used for non-radioactive phosphorylation of insert DNA.

1. Assemble the following reaction components in a sterile tube:

insert DNA (average size of 1.5kb)	250ng
Kinase 10X Buffer	4 μ l
0.1mM ATP	2 μ l
T4 Polynucleotide Kinase	10–20u
deionized water to a final volume of	40 μ l

Note: For the non-radioactive labeling of oligonucleotides, use 100pmol of oligo and increase the ATP to a final concentration of 1mM in Step 1.

2. Incubate at 37°C for 30 minutes.
3. Stop the reaction by adding 2 μ l of 0.5M EDTA.
4. Phenol extract and ethanol precipitate as in Section 3.A, Steps 4–8.
5. Decant the supernatant, and resuspend the DNA in 50 μ l of TE buffer.

3.C. Exchange Reaction Using Protruding 5' Phosphoryl Termini as Templates (6)

Materials to Be Supplied by the User

(Solution compositions are provided in Section 5.)

- exchange reaction 10X buffer
- 100% ethanol
- phenol:chloroform:isoamyl alcohol (25:24:1)
- chloroform:isoamyl alcohol (24:1)
- 5mM ADP
- 7.5M ammonium acetate or 3M sodium acetate (pH 5.5)
- [γ - 32 P]ATP (at 3,000Ci/mmol, 10mCi/ml)
- 0.5M EDTA
- TE buffer

The exchange reaction of T4 Polynucleotide Kinase is much less efficient than the forward reaction described above. Also, the buffer conditions for the exchange reaction are different, so the provided Kinase 10X Buffer cannot be used.

1. Assemble the following reaction in a sterile microcentrifuge tube:

5' ends of DNA (5' phosphorylated)	1-50pmol
exchange reaction 10X buffer	5 μ l
5mM ADP	3 μ l
[γ - 32 P]ATP (at 3,000Ci/mmol, 10mCi/ml, 100pmol total)	30 μ l
T4 Polynucleotide Kinase	20u
deionized water to a final volume of	50 μ l

2. Incubate at 37°C for 30 minutes.
3. Stop the reaction by adding 2 μ l of 0.5M EDTA.
4. Phenol extract and ethanol precipitate as in Section 3.A, Steps 4-8.
5. Decant the supernatant, and resuspend the DNA in 50 μ l of TE buffer.

The labeled DNA may be separated from unincorporated labeled nucleotides with our Wizard® DNA Clean-Up System (Cat.# A7280), chromatography or an additional ethanol precipitation.

Notes:

1. 1 mole of 5' ends = 0.5 mole of DNA (6).
2. The final ATP concentration in these reactions should be at least 1 μ M (6).

4. Product Specifications

Source: T4 PNK is purified from *E. coli* cells expressing a recombinant clone.

Molecular Weight: 132kDa. T4 PNK is a tetramer of identical monomers of apparent molecular weight of 33kDa (9).

Unit Definition: One unit is defined as the amount of T4 Polynucleotide Kinase required to catalyze the transfer of 1nmol of phosphate to the 5'-OH end of a polynucleotide from [γ -³²P]ATP in 30 minutes at 37°C.

Inhibitor: Ammonium ions and low levels of phosphate buffers (7).

Optimum pH Range: 7.4 to 8.0 in Tris-HCl buffer, with optimal activity obtained at pH 7.6 (10).

Maximum Activity: Obtained at 37°C (pH 7.6) in the presence of 10mM Mg²⁺ and thiol reagents (5mM DTT or 10mM 2-mercaptoethanol), with a minimum of 1 μ M ATP and 5:1 ratio of ATP over 5'-OH ends (9,10).

Physical Purity: The purity is \geq 90% as judged by SDS-polyacrylamide gels with Coomassie[®] blue staining.

Unit Definition Assay Buffer: 40mM Tris-HCl (pH 7.5), 10mM MgCl₂, 5mM DTT, 0.1mM [γ -³²P]ATP and 0.5 μ g/ μ l 5'-OH polynucleotide end concentration.

Storage Buffer: T4 Polynucleotide Kinase is supplied in 20mM Tris-HCl (pH 7.5), 25mM KCl, 2mM DTT, 0.1mM EDTA, 0.1 μ M ATP, 50% (v/v) glycerol.

5. Composition of Buffers and Solutions

exchange reaction 10X buffer

500mM imidazole-HCl (pH 6.6)
100mM MgCl₂
50mM DTT
1mM spermidine
1mM EDTA

TE buffer

10mM Tris-HCl (pH 8.0)
1mM EDTA

phenol:chloroform:isoamyl alcohol (25:24:1)

Mix equal parts of TE buffer and phenol, and allow the phases to separate. Then mix 1 part of the lower, phenol phase with 1 part of chloroform:isoamyl alcohol (24:1).

6. References

1. Richardson, C.C. (1965) Phosphorylation of nucleic acid by an enzyme from T4 bacteriophage-infected *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **54**, 158-65.
2. Agarwal, K. *et al.* (1981) A general method for detection and characterization of an mRNA using an oligonucleotide probe. *J. Biol. Chem.* **256**, 1023-8.
3. Maxam, A.M. and Gilbert, W. (1980) Sequencing end-labeled DNA with base-specific chemical cleavages. *Methods Enzymol.* **65**, 499-560.
4. Donis-Keller, H., Maxam, A.M. and Gilbert, W. (1977) Mapping adenines, guanines, and pyrimidines in RNA. *Nucleic Acids Res.* **4**, 2527-38.
5. Berkner, K.L. and Folk, W.R. (1977) Polynucleotide kinase exchange reaction: Quantitative assay for restriction endonuclease-generated 5'-phosphoryl termini in DNA. *J. Biol. Chem.* **252**, 3176-84.
6. Maniatis, T. *et al.* (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 122.
7. Ausubel, F.M. *et al.* (1987) *Current Protocols in Molecular Biology*, John Wiley and Sons, New York, 3.10.4-3.10.5.
8. *Protocols and Applications Guide*, Third Edition (1996) Promega Corporation, 186.
9. Perbal, B. (1988) *A Practical Guide to Molecular Cloning*, 2nd edition, John Wiley and Sons, 86.
10. *The Enzymes* (1981) Vol. XIV, Nucleic Acids, Part A, 3rd edition, P.D. Boyer, ed., Academic Press, 308.

© 1991-1993, 1995, 2000, 2002, 2007, 2009 Promega Corporation. All Rights Reserved.

Wizard is a registered trademark of Promega Corporation.

Coomassie is a registered trademark of Imperial Chemical Industries, Ltd.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our Web site for more information.

All prices and specifications are subject to change without prior notice.

Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.