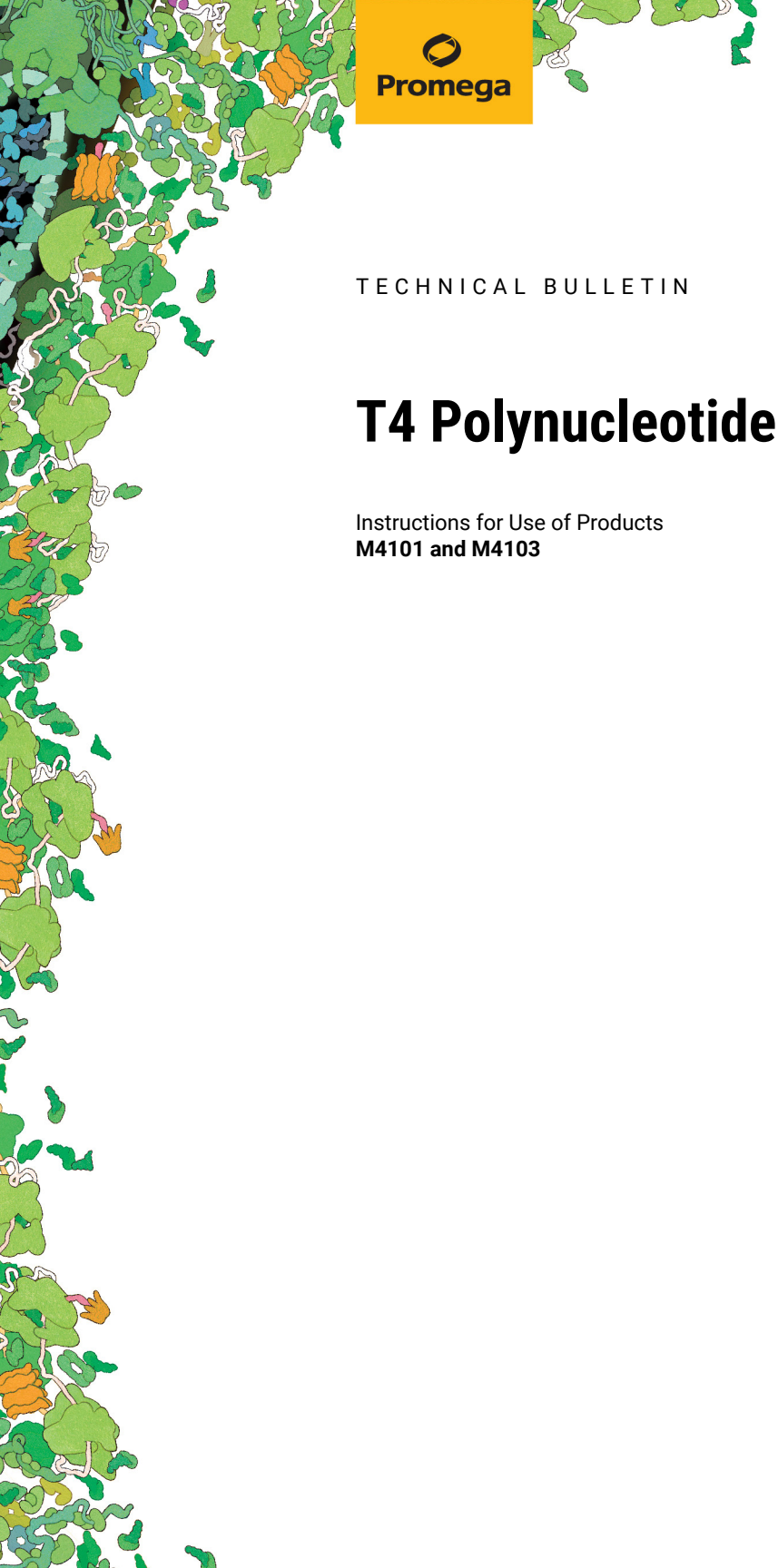

TECHNICAL BULLETIN

T4 Polynucleotide Kinase

Instructions for Use of Products
M4101 and M4103

Revised 10/25
TB519

Promega

T4 Polynucleotide Kinase

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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). In addition to this 5´-kinase activity, T4 PNK also has 3´-phosphatase activity, which converts 3´-phosphate ends to 3´-hydroxyl termini. Under certain conditions, the reaction is reversible, permitting exchange of the γ phosphate of ATP with the 5´-terminal phosphate of a polynucleotide.

Originally described for its use in end-labeling nucleic acids, T4 PNK is commonly used in next-generation sequencing (NGS) and RNA-seq library preparation, as well as DNA cloning and repair workflows. In these applications, it phosphorylates DNA or RNA fragments to enable adapter ligation or to generate ligatable ends from synthetic or PCR products. T4 PNK can also label DNA and RNA with modified nucleotides.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
T4 Polynucleotide Kinase	**100 units**	**M4101**

For Laboratory Use. Includes:

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

PRODUCT	SIZE	CAT.#
T4 Polynucleotide Kinase	**1,000 units**	**M4103**

For Laboratory Use. Includes:

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

Storage Conditions: Store the T4 Polynucleotide Kinase and the Kinase 10X Reaction Buffer at –30°C to –10°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 (2,3).

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

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

Do not dissolve DNA in, or precipitate from, buffers containing ammonium salts prior to treatment with T4 PNK (2). Ammonium ions are strong inhibitors of T4 Polynucleotide Kinase.

2. Incubate at 37°C for 10 minutes.
3. Stop the reaction by adding 2µl of 0.5M EDTA.
4. Add one volume of phenol:chloroform:isoamyl alcohol (25:24:1). Vortex for 1 minute, and centrifuge 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 centrifuge 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. Centrifuge at full speed in a microcentrifuge for 5 minutes.
9. Decant the supernatant, and resuspend the DNA in 50µl 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 (4)

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	4µl
0.1mM ATP	2µl
T4 Polynucleotide Kinase	10–20u
deionized water to a final volume of	40µl

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 2µl 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 50µl of TE buffer.

3.C. Exchange Reaction Using Protruding 5´ Phosphoryl Termini as Templates (2)

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´ dephosphorylated)	1–50pmol
exchange reaction 10X buffer	5µl
5mM ADP	3µl
[γ-^{32}P]ATP (at 3,000Ci/mmol, 10mCi/ml, 100pmol total)	30µl
T4 Polynucleotide Kinase	20u
deionized water to a final volume of	50µl

2. Incubate at 37°C for 30 minutes.
3. Stop the reaction by adding 2µl 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 50µl 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:

a. 1 mole of 5´ ends = 0.5 mole of DNA (2).
b. The final ATP concentration in these reactions should be at least 1µM (2).

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 (5).

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-^{32}P]ATP in 30 minutes at 37°C.

Inhibitor: Ammonium ions and low levels of phosphate buffers (3).

Optimum pH Range: 7.4 to 8.0 in Tris-HCl buffer, with optimal activity obtained at pH 7.6 (6).

Maximum Activity: Obtained at 37°C (pH 7.6) in the presence of 10mM Mg^{2+} and thiol reagents (5mM DTT or 10mM 2-mercaptoethanol), with a minimum of 1µM ATP and 5:1 ratio of ATP over 5´-OH ends (5,6).

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

Unit Definition Assay Buffer: 40mM Tris-HCl (pH 7.5), 10mM $MgCl_2$, 5mM DTT, 0.1mM [γ-^{32}P]ATP and 0.5µg/µl 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, 0.1µM ATP, 50% (v/v) glycerol.

5. Composition of Buffers and Solutions

exchange reaction 10X buffer

500mM	imidazole-HCl (pH 6.6)
100mM	$MgCl_2$
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. Maniatis, T. *et al*. (1982) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 122.
3. Ausubel, F.M. *et al*. (1987) *Current Protocols in Molecular Biology*, John Wiley and Sons, New York, 3.10.4–3.10.5.
4. *Protocols and Applications Guide*, Third Edition (1996) Promega Corporation, 186.
5. Perbal, B. (1988) *A Practical Guide to Molecular Cloning*, 2nd edition, John Wiley and Sons, 86.
6. *The Enzymes* (1981) Vol. XIV, Nucleic Acids, Part A, 3rd edition, P.D. Boyer, ed., Academic Press, 308.

7. Summary of Changes

The following changes were made to the 10/25 revision of this document:

1. Updated the Description in Section 1 and deleted references in Section 6.
2. Individually listed both catalog numbers and associated product components in Section 2.
3. Moved the document into a new template.
4. Made minor text edits.