

T4 Polynucleotide Kinase Kit (10 U/μL)

Table of Contents

Product Description	1
Product Applications	1
Unit Definition and Buffer Composition	1
Storage and Handling	1
Heat Inactivation	1
Kit Contents	1
Phosphorylation Protocols	2
Revision History	2

Product Description

T4 Polynucleotide Kinase (T4 PNK) catalyzes the transfer of the terminal gamma-phosphate from ATP to the 5'-OH group of double- and single-stranded DNA, RNA and nucleoside 3'-monophosphate molecules. T4 PNK also exhibits 3'-phosphatase activity and 5'-ADP phosphatase activity.

Product Applications*

- Radioactive or non-radioactive labeling of 5'-termini of nucleic acids (i.e., probes, primers, or markers)
- 5'-phosphorylation of nucleic acid substrates for downstream use in ligation
- Removal of 3'-phosphate groups

*Watchmaker Genomics has not tested or validated T4 Polynucleotide Kinase in all applications listed.

Unit Definition and Buffer Composition

- One unit of T4 Polynucleotide Kinase is defined as the amount of enzyme catalyzing the incorporation of 1 nmol of phosphate onto a DNA substrate from an ATP donor in 30 minutes at 37°C
- Enzyme Storage Buffer: 10 mM Tris-HCl pH 7.4, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50% Glycerol, 0.1 μM ATP
- 10X T4 PNK Reaction Buffer: 700 mM Tris-HCl, pH 7.6 at 25°C, 100 mM MgCl₂, 50 mM DTT

Storage and Handling

T4 Polynucleotide Kinase Kits are shipped on ice packs. Upon receipt, store all kit components at -25°C to -15°C. Keep all components and reaction mixes on ice or a cooled reagent block during routine use. Take care to homogenize solutions thoroughly before use and during reaction setup. Do not vortex the kinase. When stored and handled as indicated, the product will retain full performance until the expiry date printed on the kit box.

Heat Inactivation

65°C for 20 minutes

Kit Contents

Kit	Kit Code	Description	Component Volumes	
			250 μL (2.5 kU)	1000 μL (10 kU)
T4 Polynucleotide Kinase Kit (10 U/μL)	7K0046-250UL	T4 Polynucleotide Kinase (10 U/μL)	250 μL	-
		10X T4 PNK Reaction Buffer	1250 μL	
T4 Polynucleotide Kinase (10 U/μL)	7K0018-250UL	T4 Polynucleotide Kinase (10 U/μL)	250 μL	1000 μL
	7K0018-1ML			

For larger volumes, higher concentrations, and custom formats, contact the **Sales Team** at sales@watchmakergenomics.com.

Phosphorylation Protocols

1. End labeling DNA/RNA for DNA sequencing and probes

1.1 On ice, combine components as specified:

Component	Final Concentration	Volume (per 50 μL reaction)
DNA/RNA	Variable	1 to 50 pmol 5' termini
10X T4 PNK Reaction Buffer	1X	5 μL
[γ - ³² P]ATP ¹ (3000 Ci/mmol, 10 mCi/μL)	1 pmol/μL (50 pmol total)	15 μL
T4 Polynucleotide Kinase (10 U/μL)	0.4 U/μL	2 μL (20 Units)
Nuclease-free water	–	Up to 50 μL

¹[γ -³³P]ATP may be substituted for [γ -³²P]ATP.

1.2 Incubate the reaction as follows:

Step	Temperature (°C)	Time (min)
Phosphorylation	37	30
Heat inactivation	65	20

2. Non-radioactive phosphorylation of nucleic acid 5' termini

2.1 On ice, combine components as specified:

Component	Final Concentration	Volume (per 50 μL volume)
DNA/RNA	Variable	Up to 300 pmol 5' termini
10X T4 PNK Reaction Buffer	1X	5 μL
ATP (10 mM)	1 mM	5 μL
T4 Polynucleotide Kinase (10 U/μL)	0.2 U/μL	1 μL (10 Units)
Nuclease-free water	–	Up to 50 μL

2.2 Incubate the reaction as follows:

Step	Temperature (°C)	Time (min)
Phosphorylation	37	30
Heat inactivation	65	20

Revision History

Version	Description	Date
1.0	• First protocol release	09/2023
1.1	• Correction to reaction buffer part name • Correction to ATP final concentration in Protocol 1 • Inclusion of heat inactivation conditions and Enzyme Storage Buffer components	01/2024



WATCHMAKER
GENOMICS

5744 Central Avenue, Suite 100
Boulder, CO 80301

www.watchmakergenomics.com

For Research Use Only. Not for use in diagnostic procedures.

This content is covered by patents, trademarks, and/or copyrights owned or controlled by Watchmaker Genomics, Inc.

For more information, please visit watchmakergenomics.com/licenses. The use of these products may require you to obtain additional third party intellectual property rights for certain applications.

© 2024 Watchmaker Genomics, Inc.

For Technical Support, please contact
support@watchmakergenomics.com.

PTD-7 WMTG114