

Glycerol-Free T4 Polynucleotide Kinase Product Handling Guide

Shipping:	On Dry or Blue Ice
Catalog number:	MDX206
Batch No.:	See vial
Concentration:	20 U/ μ L

Store at -20°C



Storage and stability:

Glycerol-Free T4 Polynucleotide Kinase is shipped on blue ice. On arrival store at -20°C for optimum stability. Repeated freeze/thaw cycles should be avoided. Thawing during transportation does not affect the product performance. Solutions should be mixed/equilibrated after each thawing to avoid phasing.

Expiry:

When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Safety precautions:

Read and understand the SDS (Safety Data Sheets) before handling the reagents. Hardcopies of the SDS will be provided with the first shipment, thereafter they will be available upon request.

Quality Control:

Meridian operates under ISO 13485 Quality Management System. Glycerol-Free T4 Polynucleotide Kinase is assayed by measuring phosphorylation in oligonucleotides using molecular beacon DNA probe in comparison to a reference enzyme in a phosphorylation-ligation coupled enzyme reaction. Glycerol-Free T4 Polynucleotide Kinase is tested for activity, purity and nuclease contamination prior to test release.

Notes:

For research and further manufacturing use only.

Description

Glycerol-Free T4 Polynucleotide Kinase (PNK) is specifically formulated for use in the DNA end-repair step of next-generation sequencing library preparation. When used for this application, PNK phosphorylates the 5' ends of blunt-ended DNA, facilitating the ligation of DNA fragments to adapters necessary for the sequencing reaction. It is also commonly employed in various DNA molecular biology applications, including end-labeling, cloning, and ligation. Glycerol-Free T4 PNK is provided with a 5x Lyo-Ready™ T4 PNK Reaction Buffer, which includes the necessary excipients for lyophilization.

Kit Components

Table 1

Component
Glycerol-Free T4 Polynucleotide Kinase, 20 U/ μ L
Lyo-Ready™ T4 PNK Reaction Buffer, 5x ^T

^T Reaction Buffer is only supplied with the sample size of Glycerol-Free T4 Polynucleotide Kinase.

User Guidelines

Phosphorylation of 5' termini

1. Prepare the reaction mixture as indicated in table 2.

Table 2

Reagent	Volume	Working
Glycerol-Free T4 Polynucleotide Kinase (HC)	0.5 μ L	0.5 U/ μ L
Lyo-Ready™ T4 PNK Reaction Buffer, 5x ^T	4 μ L	1x
DNA template	x μ L	Up to 300 pmol of 5' termini
Water	Up to 20 μ L	

^T Buffer already contains phosphate donor for PNK. If using a custom Reaction Buffer, ensure it contains at least 1 mM of phosphate donor per reaction; add separately if necessary.

2. Mix thoroughly, spin briefly and incubate at 37°C for 30 min.
3. Stop the reaction by adding EDTA to a final concentration of 0.5 M, or by heating at 75°C for 20 min.

Notes

- T4 PNK can use ATP, dATP, or dNTP as phosphate donors.
- Always vortex the Lyo-Ready™ T4 PNK Reaction Buffer, 5x before use.

Lyophilization Protocol

1. Assemble the reaction in a microcentrifuge tube on ice as indicated in table 3. The volumes shown are for a 20 μ L reaction.
2. Vortex thoroughly and pulse-spin in a microcentrifuge.
3. Refer to the «Lyophilization and Post-Lyophilization User Guideline» for recommended cycling conditions for lyophilization.
4. Seal and store lyophilized material at room temperature until ready for use.

Table 3

Reagent	Volume
Glycerol-Free T4 Polynucleotide Kinase, 10 U/ μ L	0.5 μ L
Lyo-Ready™ T4 PNK Reaction Buffer, 5x	4 μ L
Water	Up to 20 μ L

5. Rehydrate the lyophilized master mix in the reaction vials with 20 μ L solution containing the DNA template.
6. Mix thoroughly, spin briefly and start the phosphorylation reaction by incubating at 37°C for 30 min.
7. Stop the reaction by adding EDTA to a final concentration of 0.5 M, or by heating at 75°C for 20 min.

Associated Products

Products Name	Cat. No.
Glycerol-Free T4 Ligase (HC)	MDX200
Glycerol-Free High-Fidelity Pfu (HC)	MDX203
Glycerol-Free T4 DNA Polymerase (HC)	MDX207
Glycerol-Free DNA Pol I Klenow Fragment (HC)	MDX208
Glycerol-Free Taq HS, 50 U/ μ L	MDX011
dNTP Mix, 10 mM	MDX086

Lyophilization & Post-Lyophilization User Guideline



The guidelines in this document can help users avoid problems in lyophilization. For storage and stability, expiry and general handling of these product pre-lyophilization, please refer to the individual Product Handling Guides.

Safety precautions:

Read and understand the SDS (Safety Data Sheets) before handling the reagents. Copies of these SDSs are available on our website or upon request.

There are several advantages for lyophilization, including room temperature shipping and storage, extended shelf-life and increased flexibility in sample volume. In order to be compatible with lyophilization however, enzyme preparations must be glycerol-free and include specialized lyophilization-excipients that preserve the mixture as it is exposed to various lyophilization conditions including freezing, temperature ramps, vacuum and dehydration. An ideal lyophilization formulation should stabilize an enzyme in a freeze-dried format and allow very fast rehydration and reactivation of the enzyme preparations, without impacting its performance post rehydration.

Lyophilization

- The lyophilization cycle protocol in table 1 is suitable for lyophilization of the Glycerol-Free T4 Polynucleotide Kinase (HC) added to Lyo-Ready™ T4 PNK Reaction Buffer, 5x in standard reaction tubes and plates. These parameters are provided as a guidance only and should be optimized to different user formats and systems.
- An annealing step can be added during the freezing step to assist crystallization of amorphous material.
- Combined primary and secondary drying time can be extended up to 24 hours.
- For product containing excipients, there should be no need to add any further excipients to assist lyophilization.

Table 1. Lyophilization guidelines

Step	Temperature	Time	Description
Freezing	+4°C	10 min	Hold
	-45°C	1.0°C/min	Ramp
Primary Drying	-45°C	180 min	Hold
	-40°C	0.5°C/min	Ramp
	-40°C	720 min	Hold
Secondary Drying	+25°C	0.5°C/min	Ramp
	+25°C	240 min	Hold

Post-Lyophilization

For maximum shelf-life, we suggest packaging lyophilized material under inert gas conditions (e.g. nitrogen or argon) and insert a desiccant sachet to improve stability. Pouches should be heat-sealed and labelled.

Technical Support

For any technical enquiries, please contact our Technical Support team via email at: mbi.tech@meridianlifescience.com