Glycerol-Free T4 DNA Polymerase (HC)
Product Handling Guide

Shipping:	On Dry/Blue Ice
Catalog number:	MDX207
Batch No.:	See vial
Concentration:	20 **U/µL**

Store at –20 °C

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Storage and stability:
Glycerol-Free T4 DNA Polymerase (HC) is shipped on dry/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 DNA Polymerase (HC) activity is assayed by measuring primer extension activity, in comparison to a reference enzyme. Glycerol-Free T4 DNA Polymerase (HC) and its components are extensively tested for activity, purity, absence of nuclease contamination and absence of nucleic acid contamination.

Notes:
For research or further manufactured use only.

Description

Glycerol-Free T4 DNA Polymerase (HC) is a DNA-dependent DNA polymerase that catalyzes 5´→ 3´ synthesis of DNA from primed single-stranded DNA template. Glycerol-Free T4 DNA Polymerase (HC) possesses a strong 3'→ 5' exonuclease (proofreading) activity, but lacks 5'→3' exonuclease activity. It is supplied with a 5x Reaction Buffer that contains excipients required for lyophilization. Glycerol-Free T4 DNA Polymerase (HC) enables flexible and scalable reaction volumes, and is suitable for applications such as blunting of DNA ends (fill-in 5'-overhangs or/and removal of 3'-overhangs) or probe labelling.

Kit components

Table 1

Component
Glycerol-Free T4 DNA Polymerase (HC), 20 U/µL
Lyo-Ready™ T4 DNA Pol Reaction Buffer, 5x[T]

[T] Reaction Buffer is only supplied with the sample size Glycerol-Free T4 DNA Polymerase (HC)

Users Guidelines

Blunting of DNA ends (3'-overhang removal/5'-overhang fill-in)

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

Table 2

Reagent	Volume	Working Conc.
Glycerol-Free T4 DNA Polymerase (HC), 20 U/µL*	0.05 to 0.25 µL	1 to 5 U
Lyo-Ready™ T4 DNA Pol Reaction Buffer, 5x	10 µL	1x
10 mM dNTP mix**	0.5 µL	100 µM
Linear DNA template	X µL	1 to 5 µg
Water	Up to 50 µL	

** The recommended T4 DNA polymerase reaction concentration is 1 unit per microgram of template DNA*
*** Not supplied*

2. Mix thoroughly, spin briefly and incubate at 12°C for 15 min, or at room temperature for 5 min.

3. Stop the reaction by adding EDTA to a final concentration of 10 mM, or by heating at 75°C for 10 min.

Notes

- Do not use low levels of dNTPs (<100 µM) in the reaction as, once the dNTPs are exhausted, the exonuclease activity of the enzyme will degrade the DNA.
- Blunt ended DNA cannot serve as template for the reaction.

Lyophilization Protocol

1. Assemble the reaction in a microcentrifuge tube on ice as indicated in table 3. The volumes shown are for a 20 µL reaction.
2. Vortex thoroughly and pulse-spin in a microcentrifuge.
3. Refer to the «MDX207 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 DNA Polymerase (HC), 20 U/µL*	0.05 to 0.25 µL
Lyo-Ready™ T4 DNA Pol Reaction Buffer, 5x	4 µL
Water	Up to 15 µL

** The recommended T4 DNA polymerase reaction concentration is 1 unit per microgram of template DNA*

5. Rehydrate the lyophilized master mix in the reaction vials with 20 µL solution containing the DNA template and dNTPs.
6. Mix thoroughly, spin briefly and start the DNA blunting reaction by incubating at 12°C for 15 min, or at room temperature for 5 min.
7. Stop the reaction by adding EDTA to a final concentration of 10 mM, or by heating at 75°C for 10 min.

Associated products

Product	Cat. No.
Glycerol-Free T4 Ligase (HC)	MDX200
Glycerol-Free High-Fidelity Pfu (HC)	MDX203
Glycerol-Free T4 PNK (HC)	MDX206
Glycerol-Free DNA Pol I Klenow Fragment (HC)	MDX208
dNTP Mix, 10 mM	MDX086

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Technical Support

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

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 DNA Polymerase (HC) added to Lyo-Ready™ T4 DNA Pol 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

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