Glycerol-Free DNA Pol I Klenow Fragment (HC) Product Handling Guide

Shipping:	On Dry/Blue Ice
Catalog number:	MDX208
Batch No.:	See vial
Concentration:	50 U/µL

Store at –20 °C

meridian BIOSCIENCE™

Storage and stability:
Glycerol-Free DNA Pol I Klenow Fragment (HC) is shipped on Blue Ice and should be stored at -20°C upon receipt. Repeated freeze/thaw cycles should be avoided. .

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 DNA Pol I Klenow Fragment (HC) activity is assayed by measuring primer extension single stranded DNA, in comparison to a reference enzyme. Glycerol-Free DNA Pol I Klenow Fragment (HC) is tested for activity, purity and nuclease contamination prior to test release.

Notes:
For research or further manufactured use only.

Description

Glycerol-Free DNA Pol I Klenow Fragment (HC) is an N-terminal truncation of *E.coli* DNA Polymerase I which retains polymerase and 3'-5' exonuclease activity but has lost 5'-3' exonuclease activity. It is designed for DNA blunting by 3' overhang removal and fill-in of 5' overhangs for adapter ligation in next generation sequencing library preparation. Glycerol-Free DNA Pol I Klenow Fragment can also be used for DNA labelling and dideoxy DNA sequencing. It is supplied with a 5x Lyo-Ready™ Reaction Buffer containing excipients required for lyophilization.

Kit components

Table 1

Component
Glycerol-Free DNA Pol I Klenow Fragment (HC), 50 U/µL
Lyo-Ready™ Klenow Reaction Buffer, 5x^{T}

^{T} Reaction Buffer is only supplied with the sample size Glycerol-Free DNA Pol I Klenow Fragment (HC)

Users Guidelines

Protocol for DNA blunting

1. Assemble the reaction in a microcentrifuge tube on ice as indicated in Table 2. The volumes shown are for a 20 µL reaction.
2. Incubate the reaction at 25°C for 15 minutes.
3. Stop reaction by heating for 20 minutes at 75°C.

Table 2

Reagent	Volume	Final concentration
DNA	X µL	1 to 5 µg
Lyo-Ready™ Klenow Reaction Buffer, 5x	4 µL	1x
dNTP mix*	x µL	0.2 mM
DNA Pol I Klenow Fragment (HC)	X µL	1 - 5 units
Water	Up to 20 µL	

Notes

- The recommended starting concentration of Klenow Fragment (HC) is 1 U per 1 µg DNA.
- Each dNTP should be added to a final concentration of 0.05 mM.
- Klenow is suitable for use in a variety of buffers, in such cases titration of enzyme may be required between 1-5 U per 1 µg DNA.
- Dilutions of Klenow Fragment or DNA should be prepared in 1x Lyo-Ready™ Klenow Reaction Buffer.
- Always vortex Lyo-Ready™ Klenow Reaction Buffer, 5x before use.
- Avoid multiple freeze/thawing of enzyme and buffer.

Lyophilization Protocol

Assemble the reaction in a microcentrifuge tube in the order outlined below:

1. Combine reagents in the order shown in Table 3. The volumes shown are sufficient for 10 x 20 µL reactions.
2. Vortex thoroughly and pulse-spin in a microcentrifuge.
3. Refer to the MDX208 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.
5. Rehydrate the lyophilized material in the reaction vials with 20 µL solution containing template, dNTPs, primers or probes, as required.
6. Briefly vortex and pulse-spin in a microcentrifuge.
7. Proceed with intended use.

Table 3

Reagent	Volume	Final concentration
Lyo-Read Klenow Reaction Buffer, 5x	40 µL	1x
DNA Pol I Klenow Fragment (HC)	x µL	0.25 –0.5 U/µL
Water	Up to 200 µL	

Associated products

Product	Cat. No.
Glycerol-Free T4 Ligase (HC)	MDX200
Glycerol-Free T7 RNA Polymerase (HC)	MDX201
Glycerol-Free RNase H (HC)	MDX202
Glycerol-Free T4 DNA Polymerase (HC)	MDX2087

Meridian Life Science Inc.
USA

Tel: +1 901 382 8716
Fax: +1 901 382 0027

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 DNA Pol I Klenow Fragment (HC) added to Lyo-Ready™ Klenow 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