

Glycerol-Free DNA Pol I Klenow Fragment exo- (HC)

Shipping:	Blue Ice
Catalog number:	MDX218
Batch Number:	See vial
Concentration:	50 U/μL

Store at -20°C



Storage and stability:

Glycerol-Free DNA Pol I Klenow Fragment exo- (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:

Please refer to the material safety data sheet for further information.

Quality control specifications:

Meridian operates under ISO 13485 Quality Management System. Glycerol-Free DNA Pol I Klenow Fragment exo- (HC) activity is assayed by measuring primer extension with single stranded DNA, in comparison to a reference enzyme. Glycerol-Free DNA Pol I Klenow Fragment exo- (HC) is tested for activity, purity and nuclease contamination prior to test release.

Notes:

For research or further manufactured use only.

Description

Meridian Glycerol-Free DNA Pol I Klenow Fragment exo- (HC) is an N-terminal truncation of *E.coli* DNA Polymerase I which retains polymerase activity, has lost 5'-3' exonuclease activity and has mutations (D355A, E357A) which abolish the 3'-5' exonuclease activity. It is designed for dA and dT tailing for adapter ligation in next generation sequencing library preparation. Glycerol-Free DNA Pol I Klenow fragment exo- can also be used for DNA labelling and dideoxy DNA sequencing. It is supplied with a 5x Reaction Buffer containing excipients required for lyophilization.

Components

Table 1

Product Name
Glycerol-Free DNA Pol I Klenow Fragment exo- (HC), 50 U/μL
Lyo-Ready™ Klenow Reaction Buffer, 5x

Protocol for A-Tailing

- Assemble the reaction in a microcentrifuge tube on ice as indicated in Table 2. The volumes shown are for a 20 μL reaction.
- Incubate the reaction at 37°C for 30 minutes.
- Purify the resulting DNA sample.

Table 2

Reagent	Volume	Final Concentration
Blunt-ended DNA	X μL	1-5 μg
Lyo-Ready Klenow Reaction Buffer	4 μL	1x
dATP	X μL	0.1 mM
DNA Pol I Klenow Fragment exo- (HC)	X μL	0.25 – 0.5 U/μL
Water	Up to 20 μL	

Notes

- 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.
- Blunt-ended DNA should be purified prior to the reaction.
- Dilutions of Klenow Fragment 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:

- Combine reagents in the order shown in Table 3. The volumes shown are sufficient for 10 x 20 μL reactions.
- Vortex thoroughly and pulse-spin in a microcentrifuge.
- Refer to the MDX218 Lyophilization and Post-Lyophilization User Guideline for recommended cycling conditions for lyophilization.
- Seal and store lyophilized material at room temperature until ready for use.
- Rehydrate the lyophilized material in the reaction vials with 20 μL solution containing template, dNTPs, primers or probes, as required.
- Briefly vortex and pulse-spin in a microcentrifuge.
- Proceed with intended use.

Table 3

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

Associated Products

Product	Cat. No.
Glycerol-Free T4 DNA Ligase (HC)	MDX200
Glycerol-Free T7 RNA Polymerase (HC)	MDX201
Glycerol-Free RNase H (HC)	MDX202
Glycerol-Free T4 DNA Polymerase (HC)	MDX207
Glycerol-Free DNA Pol I Klenow Fragment (HC)	MDX208

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 exo- (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

Technical Support

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