Glycerol-Free Thermostable DNA Helicase (HC)
Product Handling Guide

Shipping:	Blue Ice
Catalog number:	MDX242
Batch Number:	See vial
Concentration:	50 ng/µL

Store at –20°C

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Storage and stability:
Glycerol-Free Thermostable DNA Helicase (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:
Glycerol-Free Thermostable DNA Helicase (HC) activity is assayed by measuring the rate at which double stranded DNA is unwound when compared to a reference enzyme. Glycerol-Free Thermostable DNA Helicase (HC) is tested for protein concentration, purity and nuclease contamination prior to release.

Notes:
This reagent has been manufactured under 13485 Quality Management System and is suitable for research or further manufacturing use only.

Description

Glycerol-Free Thermostable DNA Helicase (HC) is a multi-domain DNA helicase with the ability to unwind double stranded DNA, without a requirement for an overhang structure. Glycerol-Free Thermostable DNA Helicase (HC) is otherwise known as *Tte* UvrD helicase from the thermophilic organism *Thermoanaerobacter tengcongensis* and functions with a wide range of DNA substrates and exhibits high thermal stability. It is supplied in a glycerol-free storage buffer and is accompanied by a 5x lyophilization compatible Reaction Buffer. Glycerol-Free Thermostable DNA Helicase (HC) facilitates flexible and scalable reaction volumes and is suitable for use in Point-of-Care diagnostic devices.

Components

Table 1

Product Name
Glycerol-Free Thermostable DNA Helicase (HC), 50 ng/µL
Lyo-Ready Thermostable DNA Helicase Reaction Buffer, 5x

Protocol for unwinding double stranded DNA

1. Combine reagents in the order shown in Table 2. The volumes shown are for a 20 µL reaction.
2. Briefly vortex and spin down in a microcentrifuge.
3. Incubate the reaction at 65°C for 10 minutes.
4. The reaction can be stopped by addition of EDTA to 1 mM or by heating at 80°C for 10 minutes.

Table 2

Reagent Name	Volume	Final Reaction Concentration
Lyo-Ready Thermostable DNA Helicase Reaction Buffer, 5x	5 µL	1x
DNA template	X µL	Up to 1 µg
Glycerol-Free Thermostable DNA Helicase (HC)	X µL	1 ng/µL
Water	up to 20 µL	

Notes

- The recommended concentration of Thermostable DNA Helicase (HC) is 0.5-2 ng/µL in a 20 µL reaction.
- Dilutions of Helicase should be prepared in 1x Lyo-Ready Thermostable DNA Helicase Reaction Buffer.
- Always vortex Lyo-Ready Thermostable DNA Helicase Reaction Buffer, 5x before use.
- Avoid multiple freeze/thawing of enzyme and buffer.
- Unwound DNA will reanneal, so trapping or digestion is required to observe the unwound DNA product.

Lyophilization Protocol

Assemble the reaction in a microcentrifuge tube at room temperature 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. Keep the mixture on ice until ready to dispense.
4. Aliquot 10 µL of the mixture into an appropriate vessel and spin down.
5. Refer to the MDX242 Lyophilization and Post-Lyophilization User Guideline for recommended cycling conditions for lyophilization.
6. Seal and store lyophilized material at room temperature until ready for use.
7. Add up to 1 µg of double stranded DNA to each tube containing lyophilized material and add nucelease free water up to 20 µL.
8. Briefly vortex and spin down in a microcentrifuge.
9. Incubate the reaction at 65°C for 10 minutes.

Table 3

Reagent Name	Volume	Working Concentration
Lyo-Ready Thermostable DNA Helicase Reaction Buffer, 5x	40 µL	2x
Glycerol-Free Thermostable DNA Helicase (HC)	4 µL	2 ng/µL
Water	up to 100 µL	

Associated Products

Product	Cat. No.
High Conc. Glycerol-Free Bst	MDX018
dNTP Mix, 100mM, Sodium Salt	MDX084
Glycerol-Free DNA Helicase (HC)	MDX241

Technical Support

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

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Lyophilization & Post-Lyophilization User Guideline

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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 Thermostable DNA Helicase (HC) added to Lyo-Ready Thermostable DNA Helicase 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