Glycerol-Free RNase H (HC)

Shipping:	Blue Ice
Catalog number:	MDX202
Batch Number:	See vial
Concentration:	50 U/µL

Store at –20°C

meridian BIOSCIENCE™

Storage and stability:
Glycerol-Free RNase H (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 RNase H (HC) activity is assayed by measuring the rate at which RNA is hydrolyzed in a RNA-DNA hybrid when compared to a reference enzyme. Glycerol-Free RNase H (HC) is tested for purity and nuclease contamination prior to release.

Notes:
This reagent has been manufactured under 13485 Quality Management System and is suitable for further manufacturing use as an IVD component.

Description

Glycerol-Free RNase H (HC) is a non-sequence-specific endonuclease enzyme that specifically catalyzes the hydrolysis of the phosphodiester bonds of RNA in RNA-DNA substrates. RNase H is typically used to remove RNA template following reverse transcription or cDNA synthesis. Glycerol-Free RNase H (HC) is supplied in a glycerol-free storage buffer and is accompanied by a 5x Reaction Buffer that contains the excipients required for lyophilization. Glycerol-Free RNase H (HC) facilitates flexible and scalable reaction volumes and is suitable for the hydrolysis of RNA in RNA-DNA hybrids at a commercial scale and for the production of room temperature stable product.

Components

Table 1

Product Name	
Glycerol-Free RNase H (HC), 50 U/µL	1 x 20 µL
Lyo-Ready™ RNase H Reaction Buffer, 5x	1 x 2 mL

Protocol for *RNase H digestion*

1. RNase H will only cleave the RNA in a RNA-DNA hybrid.
2. Combine reagents in the order shown in Table 2. The volumes shown are for a 20 µL reaction.
3. Briefly vortex and spin down in a microcentrifuge.
4. Incubate the reaction at 37°C for 30 minutes.
5. The reaction can be stopped with 1 µL of 0.5M EDTA or by heating at 65°C for 10 minutes.

Table 2

Reagent Name	Volume	Final Reaction Concentration
Lyo-Ready™ RNase H Reaction Buffer, 5x	5 µL	1 x
RNA-DNA template DNA	X µL	0.1-2 µg
Glycerol-Free RNase H (HC)	X µL	0.1-0.5 U
Water	up to 25 µL	

Notes

- The recommended concentration of RNase H (HC) is 0.1-0.5 U per 25 µL reaction.
- Dilutions of RNase H should be prepared in 1x Lyo-Ready™ RNase H Reaction Buffer.
- Always vortex Lyo-Ready™ RNase H Reaction Buffer, 5x before use.
- Avoid multiple freeze/thawing of enzyme and buffer.
- RNase H is suitable for use in a variety of buffers, in such cases titration of enzyme may be required between 0.1-0.5 U per reaction.

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 25 µL reactions.
2. Vortex thoroughly and pulse-spin in a microcentrifuge.
3. Keep the mixture on ice until ready to dispense.
4. Aliquot 20 µL of the mixture into an appropriate vessel and spin down.
5. Refer to the MDX202 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. Reconstitute the lyophilized material in 20 µL nuclease-free water and add 0.1-2 µg of RNA-DNA template DNA in 5 µL.
8. Briefly vortex and spin down in a microcentrifuge.
9. Incubate the reaction at 37°C for 30 minutes.

Table 3

Reagent Name	Volume	Working Concentration
Lyo-Ready™ RNase H Reaction Buffer, 5x	50 µL	1.25x
Glycerol-Free RNase H (HC)	X µL	0.1-0.5 U/rxn
Water	up to 200 µL	

Associated Products

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

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

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 RNase H (HC) added to Lyo-Ready™ RNase H 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