Glycerol-Free T7 RNA Polymerase (HC)

Shipping:	Blue Ice
Catalog number:	MDX201
Batch Number:	See vial
Concentration:	1,000 U/µL

Store at –20°C

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Storage and stability:
Glycerol-Free T7 RNA Polymerase (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 T7 RNA Polymerase (HC) activity is assayed by measuring the rate of in-vitro transcription from a DNA template containing the T7 promoter sequence when compared to a reference enzyme. Glycerol-Free T7 RNA Polymerase (HC) is tested for purity, exo- and endo-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 T7 RNA Polymerase (HC) is a DNA-dependent RNA polymerase with strict specificity to DNA template containing the double-stranded T7 promoter sequence. T7 RNA polymerase can efficiently incorporate standard or modified ribonucleoside triphosphates (NTPs) making it an ideal enzyme for *in-vitro* synthesis of labeled or unlabeled RNA for research, diagnostic or clinical applications. Glycerol-Free T7 RNA Polymerase (HC) is supplied in a glycerol-free storage buffer and is accompanied by a 5x Transcription Buffer that contains excipients required for lyophilization, $MgCl_2$ and RNase Inhibitor. Glycerol-Free T7 RNA Polymerase (HC) enables flexible and scalable reaction volumes, and is suitable for preparation of mRNA on a commercial scale, point-of-care diagnostic applications or production of room temperature stable product.

Components

Table 1

Product Name
Glycerol-Free T7 RNA Polymerase (HC), 1,000 U/µL
Lyo-Ready™ Transcription Buffer, 5x

Protocol for *in vitro* transcription

1. To synthesize a uniform length of RNA, use linear dsDNA such as cleaved plasmid or PCR product as template for *in vitro* transcription reactions.
2. Assemble the reaction at room temperature in the order shown in Table 2. The volumes shown are for a 50 µL reaction.
3. Vortex thoroughly and pulse-spin in a microcentrifuge
4. Incubate the reaction at 37°C for 1-2 hours.

Table 2

Reagent Name	Volume	Final Reaction Concentration
Lyo-Ready™ Transcription Buffer, 5x	10 µL	1 x
NTPs, 25 mM each*	1 µL	0.5 mM
Linear template DNA*	X µL	0.2-1 µg
Glycerol-Free T7 RNA Polymerase (HC)	0.25 µL	5 U/µL
Water	up to 50 µL	

*not supplied

Notes

- Ensure that equipment and materials used are free of RNase contamination.
- For higher yield, NTPs can be increased to a final reaction concentration of 1 mM
- For higher yield, the reaction can be incubated overnight at 37°C
- Always vortex Lyo-Ready™ Transcription Buffer, 5x before use.
- Avoid multiple freeze/thawing of enzyme and buffer.
- Avoid the use of DNA solutions with a concentration of EDTA higher than 0.1 mM.

Lyophilisation 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 50 µL reactions.
2. Vortex thoroughly and pulse-spin in a microcentrifuge.
3. Keep the mixture on ice until ready to dispense.
4. Aliquot 12.5 µL of the mixture into appropriate vessel and spin down.
5. Refer to the « MDX201 Lyophilization and Post-Lyophilization User Guideline » for recommended cycling conditions for lyophilization.
6. Seal and store lyophilised material at room temperature until ready for use.
7. Add 0.2-1 µg of linear template DNA to each tube containing lyophilized material and add nuclease-free water up to 50 µL.
8. Briefly vortex and spin down in a microcentrifuge
9. Incubate the reaction at 37°C for 1-2 hours

Table 3

Reagent Name	Volume	Working Concentration
Lyo-Ready™ Transcription Buffer, 5x	100 µL	4x
NTPs, 25 mM each*	10 µL	2 mM
Glycerol-Free T7 RNA Polymerase (HC)	2.5 µL	20 U/µL
Water	Up to 125 µL	

*not supplied

Associated Products

Product	Cat. No.
Glycerol-Free T4 Ligase (HC)	MDX200
Glycerol-Free RNase H (HC)	MDX202

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 T7 RNA Polymerase (HC) added to Lyo-Ready™ Transcription 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.

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