Glycerol-Free Phi29 DNA Polymerase (HC)

Product Handling Guide

Shipping:	On Dry Ice
Catalog numbers:	MDX240
Batch No.:	See vial
Concentration:	100 U/µL

Store at –80 °C

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Storage and stability:
Glycerol-Free Phi29 DNA Polymerase (HC) is shipped on dry/blue ice. On arrival store at –80 °C for optimum long-term stability. Repeated freeze/thaw cycles should be avoided. Thawing Solutions should be mixed/equilibrated after each thawing to avoid phasing.

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 SDSs will be provided with the first shipment, thereafter they will be available upon request.

Quality control:
Glycerol-Free Phi29 DNA Polymerase (HC) activity is assayed by measuring primer extension activity, in comparison to a reference enzyme. Glycerol-Free Phi29 DNA Polymerase (HC) and its components are extensively tested for activity, purity, absence of nuclease contamination and absence of nucleic acid contamination.

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

Description

Glycerol-Free Phi29 DNA Polymerase (HC) is a highly processive DNA-dependent DNA polymerase with strong strand displacement activity, which enables efficient isothermal DNA amplification from low DNA inputs. Glycerol-Free Phi29 DNA Polymerase (HC) 3′ → 5′ exonuclease activity delivers high fidelity amplification, making it an appropriate solution for sequencing DNA template preparation. It is supplied with a 5x Reaction Buffer that contains excipients required for lyophilization. Glycerol-Free Phi29 DNA Polymerase (HC) enables flexible and scalable reaction volumes, and is suitable for applications such as rolling circle amplification (RCA) and multiple displacement amplification (MDA).

Kit components

Table 1

Component
Glycerol-Free Phi29 DNA Polymerase (HC), 100 U/µL
Lyo-Ready Phi29 DNA Pol Reaction Buffer, 5x[Ŧ]

[Ŧ] Reaction Buffer is only supplied with the sample size Glycerol-Free Phi29 DNA Polymerase (HC)

Users Guidelines

Notes

- **Thaw the enzyme and its reaction buffer on ice.**
- **Avoid repeated freeze-thaw cycles.**
- **Glycerol-Free Phi29 DNA Polymerase (HC) can be stored at -20 °C for short-term storage.**

Rolling Circle Amplification Protocol

1. Prepare the reaction mixture by adding each component in the order as indicated in table 2.

Table 2

Reagent	Volume	Final Conc.
Lyo-Ready Phi29 DNA Pol Reaction Buffer, 5x	10 µL	1x
Exo-resistant Primers (100 µM)**	0.5 µL	1 µM
100 mM dNTP mix*	0.5 µL	1 mM
Circular DNA template***	X µL	variable
Glycerol-Free Phi29 DNA Polymerase (HC), 100 U/µL	0.1 µL	10 U/rxns
100 mM $MgCl_2$ mix*	2.5 µL	5 mM
Water	Up to 50 µL	

** **dNTP mix and $MgCl_2$ are not supplied with MDX240**. Recommended concentrations are shown in the table above. However, customers are advised to optimize the concentration of dNTP mix and $MgCl_2$ for their individual assay needs.*
*** Due to the 3'->5' exonuclease activity of phi29 DNA Polymerase, protection of the 3'-ends of primers with at least two phosphorothioate (PTO) bonds is strongly recommended*
**** For double-stranded DNA template, denature the DNA beforehand by heating at 95°C for 3 minutes and by immediately placing the tube back on ice, prior to addition to the reaction.*

2. Vortex and spin down reaction for 5 seconds before incubating at 30°C for up to 4 hours.
3. Stop the reaction by inactivating the enzyme for 10 min at 65°C.
4. Store RCA products overnight at 4°C, or at -20°C for long-term storage.

Multiple Displacement Amplification Protocol

1. Prepare the reaction mixture by adding each component in the order as indicated in table 3.

Table 3

Reagent	Volume	Final Conc.
Lyo-Ready Phi29 DNA Pol Reaction Buffer, 5x	10 µL	1x
Exo-Resistant Random Primers (500 µM)	5 µL	50 µM
100 mM dNTP mix*	0.5 µL	1 mM
Linear DNA template***	X µL	variable
Glycerol-Free Phi29 DNA Polymerase (HC), 100 U/µL	0.1 µL	10 U/rxns
100 mM $MgCl_2$ mix*	2.5 µL	5 mM
Water	Up to 50 µL	

2. Vortex and spin down reaction for 5 seconds before incubating at 30 °C for up to 4 hours.
3. Stop the reaction by inactivating the enzyme for 10 min at 65 °C.
4. Store MDA products overnight at 4 °C, or at -20 °C for long-term storage.

Lyophilization Protocol

1. Assemble the reaction in a microcentrifuge tube on ice as indicated in table 4. The volumes shown are for a 50 µL reaction.

Table 4

Reagent	Volume	Final Conc.
Lyo-Ready Phi29 DNA Pol Reaction Buffer, 5x	10 µL	1x
Exo-resistant Primers (100 µM)**	0.5 µL	1 µM
100 mM dNTP mix*	0.5 µL	1 mM
Glycerol-Free Phi29 DNA Polymerase (HC), 100 U/µL	0.1 µL	10 U/rxns
Water	Up to 20 µL	

2. Vortex thoroughly and pulse-spin in a microcentrifuge. Pre-freeze the vials at -80 °C before transferring into freeze-dryer.
3. Run a suitable lyophilization cycle (See: MDX240 Lyophilization and Post-lyophilization User Guideline). Seal and store lyophilized material at room temperature until ready for use.
4. Rehydrate the lyophilized master mix on ice with 50 µL solution containing the DNA template and $MgCl_2$.

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 5 is suitable for lyophilization of the Glycerol-Free Phi29 DNA Polymerase (HC) added to Lyo-Ready Phi29 DNA Pol 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.
- The product already contains excipients, therefore, there should be no need to add any further excipients to assist lyophilization.

Table 5. Lyophilization guidelines

Step	Temperature	Time	Description	Vacuum Set Points
Shelf Pre-Freezing	-40°C	n/a	Pre-Freezing	
Freezing	-45 °C	0.5 °C/min	Ramp	
	-45 °C	10 min	Hold	
Evacuation	-55 °C (Condenser)			75 mTorr (100 µbar)
Primary Drying*	-45 °C	180 min	Hold	37.5 mTorr (50 µbar)
	-40 °C	0.5 °C/min	Ramp	
	-40 °C	900 min	Hold	
Secondary Drying	+22 °C	0.5 °C/min	Ramp	37.5 mTorr (50 µbar)
+22 °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

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