Glycerol-Free High-Fidelity Pfu (HC) Product Handling Guide

Shipping:	On Dry/Blue Ice
Catalog numbers:	MDX203
Batch No.:	See vial
Concentration:	20 U/μL

Store at –20 °C

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Storage and stability:
Glycerol-Free High-Fidelity Pfu (HC) is shipped on dry/blue ice. On arrival store at -20 °C for optimum stability. Repeated freeze/thaw cycles should be avoided. Thawing during transportation does not affect the product performance. 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 High-Fidelity Pfu (HC) activity is assayed by measuring the rate of *in-vitro* transcription from a DNA template sequence in comparison to a reference enzyme.
The High-Fidelity Pfu and its components are extensively tested for activity, processivity, efficiency, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Notes:
For research or further manufactured use only

Description

Glycerol-Free High-Fidelity Pfu (HC) is a high-fidelity, thermostable DNA-dependent DNA polymerase from *Pyrococcus furiosus,* The 3' - 5' proofreading exonuclease activity of Pfu polymerase makes it suitable for applications that require high-fidelity DNA synthesis or blunt-ended PCR fragments. Glycerol-Free High-Fidelity Pfu (HC) is supplied in a glycerol-free storage buffer and is accompanied by a 5x Reaction Buffer that contains magnesium, dNTPs and excipients required for lyophilization. Glycerol-Free High-Fidelity Pfu (HC) enables flexible and scalable reaction volumes, and is suitable for applications such NGS library amplification, gene expression, cloning and site-directed mutagenesis.

Kit components

Table 1

Component
Glycerol-Free High-Fidelity Pfu (HC), 20 U/μL
Pfu Polymerase Dilution Buffer
Lyo-Ready™ Pfu Reaction Buffer, 5x

Users Guidelines

PCR reaction setup

1. Dilute Glycerol-Free High-Fidelity Pfu (HC) to 2 U/μL using Pfu Polymerase Dilution Buffer.
2. Prepare a master mix of Glycerol-Free High-Fidelity Pfu (HC) and assay-specific primers (see recommended composition in Table 2).

Table 2

Reagent	Volume	Final Reaction Concentration
Pfu Reaction Buffer, 5x	5 μL	1x
DNA Template*	X μL	As required
20 μM Forward Primer**	0.5 μL	400 nM
20 μM Reverse Primer**	0.5 μL	400 nM
Glycerol-Free High-Fidelity Pfu (HC) 2 U/μL	1 μL	0.08 U/μL
Water	Up to 25 μL	

**For DNA templates with low structural complexity, such as plasmid DNA, we recommend using 25 pg - 5 ng DNA per 25 μL reaction volume. For eukaryotic genomic DNA, we recommend a starting amount of 80 ng DNA per 25 μL reaction, this can be varied between 2.5 ng - 250 ng.*

***Forward and reverse primers are generally used at the final concentration of 200 to 600 nM each. As a starting point, we recommend using 400 nM final concentration (i.e. 5 pmol of each primer per 25 μL reaction volume).*

Lyophilization Protocol

Assemble the reaction in a microcentrifuge tube in ice the order outlined below:

1. Combine reagents in the order shown in Table 3.
2. Vortex thoroughly and pulse-spin in a microcentrifuge.
3. Keep the mixture on ice until ready to dispense.
4. Aliquot 15 μL of the mixture into appropriate vessel and spin down.
5. Refer to the "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 solution containing DNA templates into the tube containing lyophilized material and add nuclease-free water up to 25 μL.
8. Run the PCR reaction using the conditions described in Table 4.

Table 3

Reagent	Volume	Final Reaction Concentration
Pfu Reaction Buffer	5 μL	1x
Glycerol-Free High-Fidelity Pfu (HC) 2 U/μL	1 μL	0.08 U/μL
20 μM Forward Primer (optional)	0.5 μL	400 nM
20 μM Reverse Primer (optional)	0.5 μL	400 nM
Water	Up to 15 μL	

PCR amplification

The PCR conditions in Table 3 are suitable for amplicons of up to 1 kb.

Table 4

Step	Temperature	Time	Cycles
Initial denaturation	95 °C	3 min	**1**
Denaturation	95 °C	15 s	25-35
Annealing	50-72 °C	15 s	
Extension	72 °C	15 - 30 sec/kb	
Final extension (optional)	72 °C	4 - 10 min	1

****For multiplex PCR we suggest using 55 °C as a starting annealing temperature. If further optimization is required we recommend using a temperature gradient to determine the optimal annealing temperature needed for the multiplex PCR. Since multiplex PCR generally requires a longer extension step, we suggest starting with a minimum of 90 s and increasing it if required.*

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 High-Fidelity Pfu (HC) added to Lyo-Ready™ Pfu 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

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.