NGS High-Fidelity Pfu Buffer, 10x Product Handling Guide

Shipping:	On Dry or Blue Ice
Catalog number:	MDX038
Batch No.:	See vial
Concentration:	10x

Store at –20 °C

meridian BIOSCIENCE™

Storage and stability:
NGS High-Fidelity Pfu Buffer, 10x is shipped on dry or 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 SDS will be provided with the first shipment, thereafter they will be available upon request.

Quality control:
Bioline operates under ISO 13485 Quality Management System. NGS High-Fidelity Pfu Buffer, 10x and its components are extensively tested for activity, processivity, efficiency, heat activation sensitivity, 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 further manufacturing use as an IVD component.

Description

NGS High-Fidelity Pfu Buffer, 10x is designed for the amplification of DNA libraries, if required after ligation of the adapters. The NGS High-Fidelity Pfu Buffer contains dNTPs and Mg and requires only the addition of a high fidelity polymerase (such as High-Fidelity Pfu (MDX003)), primers and DNA template for robust amplification.

Kit Components

Table 1.

Reagent
NGS High-Fidelity Pfu Buffer, 10x

User Guidelines

Preparation

Prepare the amplification Primer Mix by diluting the primers in nuclease-free water to a final concentration of 2.5 µM each. Store at -20 °C and thaw on ice before use.

NOTE: The amplification primers should be compatible with the adapter system used.

Master mix preparation

Assemble the following reaction on ice using the volumes shown in Table 2. Ensure optimal mixing by pipetting up and down.

Table 2

Reagent	Volume
Purified adapter-ligated library	30 µL
High-fidelity Taq DNA Polymerase	2 µL
NGS High-Fidelity Pfu Buffer, 10x	5 µL
Primer Mix	5 µL
Water	As required
Total volume	**Up to 50 µL**

Assay setup

PCR conditions in Table 3 are guidelines, further optimization may be needed.

Table 3

Temperature	Time	Cycles
98 °C	3 min	1
98 °C	30 s	See Tables 4 and 5
65 °C	30 s	
72 °C	1 min	
72 °C	10 min	1
4 °C	Hold	

Table 4 shows the recommended number of PCR cycles to obtain approximately 100 ng of amplified library from 0.5-200 ng of purified adapter -ligated DNA.

Table 4

Amount of adapter-ligated DNA after post ligation clean-up	Estimated number of PCR cycles
<200 ng	1-2
20-40 ng	5-6
6-19 ng	6-7
2-5 ng	10-11
<1 ng	13-14

Table 5 shows the suggested number of PCR cycles required to obtain approximately 100 ng of amplified library from different input DNA.

Table 5

Input DNA into end-repair reaction	Estimated number of PCR cycles
1 µg	1
100 ng	5-6
50 ng	6-7
10 ng	9-10
1 ng	13-14

Related Products	Cat. No.
NGS ER Enzyme Mix	MDX040
NGS End-Repair Buffer, 5x	MDX036
NGS Ligase Buffer, 5x	MDX036
High-Fidelity Pfu	MDX003
NGS Library Quantification	MDX039

Technical Support

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

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