

# High-Fidelity Pfu Product Handling Guide

Shipping:	On Dry/Blue Ice
Catalog numbers:	MDX003
Batch No.:	See vial
Concentration:	2 U/ $\mu$ L

Store at  $-20\text{ }^{\circ}\text{C}$



## Storage and stability:

High-Fidelity Pfu is shipped on dry/blue ice. On arrival store at  $-20\text{ }^{\circ}\text{C}$  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:

Bioline operates under ISO 13485 Management System. The High-Fidelity Pfu 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

High-Fidelity Pfu is a high-fidelity PCR product containing a hot-start antibody, separate 10x Pfu Reaction Buffer and  $\text{MgCl}_2$ . The 3' - 5' proofreading exonuclease activity of High-Fidelity Pfu has an error rate of  $3.0 \times 10^{-6}$  and generates blunt-ended amplicons up to 5 kb in length making it ideal for high yields in NGS library amplification.

## Kit components

Table 1

Component
High-Fidelity Pfu
Pfu Reaction Buffer, 10x
50 mM $\text{MgCl}_2$ Solution

## Users Guidelines

The Pfu Reaction Buffer, 10x comprises of 600 mM Tris-HCl, 60 mM  $(\text{NH}_4)_2\text{SO}_4$ , 100 mM KCl, 20 mM  $\text{MgSO}_4$ , pH 8.3 at  $25\text{ }^{\circ}\text{C}$ .

The  $\text{Mg}^{2+}$  concentration in the 1x Pfu Reaction Buffer is 2 mM, this is the optimum concentration for High-Fidelity Pfu for most PCR reactions and should only be adjusted if necessary.

Forward and reverse primers are generally used at the final concentration of 0.2-0.6 mM each. As a starting point, we recommend using 0.4 mM final concentration (i.e. 4 pmol of each primer per 20  $\mu$ L reaction volume).

For DNA templates with low structural complexity, such as plasmid DNA, we recommend using 50 pg - 10 ng DNA per 50  $\mu$ L reaction volume. For eukaryotic genomic DNA, we recommend a starting amount of 200 ng DNA per 50  $\mu$ L reaction, this can be varied between 5 ng - 500 ng.

## PCR reaction setup

Prepare a master mix of High-Fidelity Pfu and assay-specific primers (see recommended composition in Table 2).

Table 2

Reagent	Volume	Final Concentration
Pfu Reaction Buffer, 10x	2 $\mu$ L	1x
Template	As required	As required
20 $\mu$ M Forward Primer	0.4 $\mu$ L	400 nM
20 $\mu$ M Reverse Primer	0.4 $\mu$ L	400 nM
Fast High-Fidelity Pfu	0.4 $\mu$ L	0.05 U/ $\mu$ L
Water (ddH <sub>2</sub> O)	$\leq$ 20 $\mu$ L	

## PCR amplification

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

Table 3

Step	Temperature	Time	Cycles
Initial denaturation	95 $^{\circ}\text{C}$	3 min	1
Denaturation	95 $^{\circ}\text{C}$	15 s	25-35
Annealing	User determined	15 s	
Extension	72 $^{\circ}\text{C}$	1.5 - 30 sec/kb	
Final extension (optional)	72 $^{\circ}\text{C}$	4 - 10 min	1

For multiplex PCR we suggest using 55  $^{\circ}\text{C}$  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](mailto:mbi.tech@meridianlifescience.com)

Bioline Reagents Ltd  
UNITED KINGDOM

Tel: +44 (0)20 8830 5300  
Fax: +44 (0)20 8452 2822

Meridian Life Science Inc.  
USA

Tel: +1 901 382 8716  
Fax: +1 901 382 0027

Bioline GmbH  
GERMANY

Tel: +49 (0)337 168 1229  
Fax: +49 (0)3371 68 1244

Bioline (Aust) Pty. Ltd  
AUSTRALIA

Tel: +61 (0)2 9209 4180  
Fax: +61 (0)2 9209 4763

Bioline France  
FRANCE

Tel: +33 (0)1 42 56 04 40  
Fax: +33 (0)9 70 06 62 10