# High-Fidelity Pfu
# Product Handling Guide

| | |
|---|---|
| Shipping | On Dry/Blue Ice |
| Catalog numbers | MDX003 |
| Batch No. | See vial |
| Concentration | 2 U/μL |

**Store at –20 °C**

meridian BIOSCIENCE™

**Storage and stability:**
High-Fidelity Pfu 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:**
Meridian operates under ISO 13485 Management System. 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 and further manufacturing use only.

## Description

High-Fidelity Pfu is a high-fidelity DNA polymerase, supplied with separate 10x Pfu Reaction Buffer and $MgCl_2$. The 3' - 5' proofreading exonuclease activity of High-Fidelity Pfu has an error rate of 3.0 x $10^{-6}$ 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 $MgCl_2$ Solution |

## Users Guidelines

The Pfu Reaction Buffer, 10x comprises of 600 mM Tris-HCl, 60 mM $(NH_4)_2SO_4$, 100 mM KCl, 20 mM $MgSO_4$, pH 8.3 at 25 °C.

The $Mg^{2+}$ 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 μM each. As a starting point, we recommend using 0.4 μM final concentration (*i.e.* 4 pmol of each primer per 20 μL reaction volume).

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

## PCR reaction set-up

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 μL | 1x |
| Template | As required | As required |
| 50 mM $MgCl_2$ Solution | Optional | Optional |
| 100 mM dNTP Mix | 0.2 μL | 1 mM |
| 20 μM Forward Primer | 0.4 μL | 400 nM |
| 20 μM Reverse Primer | 0.4 μL | 400 nM |
| High-Fidelity Pfu | 0.4 μL | 0.05 U/μL |
| Water ($ddH_2O$) | ≤ 20 μL | |

## 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 °C | 3 min | 1 |
| Denaturation | 95 °C | 15 s | 25-35 |
| Annealing | User determined | 15 s | |
| Extension | 72 °C | 1.5 - 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.

| Related Products | Cat. No. |
|---|---|
| High-Specificity Pfu HS Mix | MDX006 |
| Glycerol-Free High-Fidelity Pfu (HC) | MDX203 |
| NGS High-Fidelity Pfu Buffer, 10x | MDX038 |
| dNTP Mix, 100mM (Lithium) | MDX051 |
| dNTP Mix, 100mM (Sodium) | MDX084 |

## Technical Support

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

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