Product Manual
Cat. No: #3101

HiFi Library Amplification Mix

Description

HiFi Library Amplification Mix is a high-performance proofreading polymerase mix combined with an aptamer-based hot-start. This 2x ready-to-use mix is tailored for library amplification in NGS workflows and GC-rich PCR applications, delivering reliable amplification of challenging targets with minimal bias.

The HiFi Library Amplification Mix contains the advanced HiFi DNA Polymerase Hot-Start, designed for robust and versatile high-fidelity PCR. The mix contains an optimized blend of buffer, dNTPs, $MgCl_2$, and enhancers, specifically formulated to reduce GC-dependent bias and improve amplification efficiency. This unique composition ensures superior library amplification, resulting in high-quality NGS datasets with increased discrete read counts compared to other high-fidelity mixes.

The HiFi Library Amplification Mix excels in amplifying difficult templates with extreme GC content both high and low. It is also suitable for use in multiplex PCR assays.

Kit Components

Component	S pack*
HiFi Library Amplification Mix (2x)	1.25 mL

*Other pack sizes, bulk orders and customization are available upon request.

The 2x mix includes HiFi DNA Polymerase Hot Start, 6 mM $MgCl_2$, 2 mM dNTPs, along with enhancers and stabilizers. Additional PCR enhancers or $MgCl_2$ should not be added, as the mix has been carefully optimized to ensure high PCR success rates.

Storage and Shipment

Transport with an ice pack. The reagents should be stored at -20°C upon arrival. The reagents are stable until the expiration date if stored at -20°C or for 1 month if stored at 4°C.

Reaction Master Mix Set-Up

The recommended master mix set-up for a 25 µL and 50 µL reaction volume is shown in the table below. Keep all components on ice during reaction set up.

Reagent	Volume 25 µL	Volume 50 µL	Final concentration
HiFi Library Amplification Mix (2x)	12.5 µL	25 µL	1x
[Δ]Forward primer (10 µM)	1 µL	2 µL	400 nM
[Δ]Reverse primer (10 µM)	1 µL	2 µL	400 nM
[ΔΔ]DNA/cDNA Template	X	X	Variable
Nuclease-free Water	Up to 25 µL final volume	Up to 50 µL final volume	

[Δ]For NGS library amplification, primers targeting the ligated adapters (such as P5 and P7 for Illumina platforms) should be used at a concentration ranging from 0.4 µM to 1 µM. For standard end-point PCR, primers should have a predicted melting temperature of 60 °C to 70 °C, calculated using the default settings in Primer 3 (http://bioinfo.ut.ee/primer3/). The recommended final primer concentration in the reaction should be between 0.2 µM and 0.6 µM.

[ΔΔ]For 25 µL reaction volumes use < 100 ng of genomic DNA and < 5 ng of less complex DNA per reaction. For 50 µL reaction volumes use < 200 ng of genomic DNA and < 10 ng of less complex DNA per reaction.

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Instrument and Program Set-Up

3-Step cycling

Cycles	Steps	Temp	Time
1	Pre-denaturation	95°C	1 min
⁼15–15 (for NGS) 25-35 (standard)	‡Denaturation	95°C	15 sec
	‡‡Annealing	55–68°C	15 sec
	‡‡‡Extension	72°C	30 sec

2-Step cycling

Cycles	Steps	Temp	Time
1	Pre-denaturation	95°C	1 min
⁼15–15 (for NGS) 25-35 (standard)	‡Denaturation	95°C	15 sec
	‡‡Annealing	68-72°C	30 sec

⁼Excessive amplification of an NGS library can introduce unwanted artifacts and amplification bias. To minimize these effects, use the lowest number of cycles necessary to achieve sufficient yields for downstream processes. Typical yields between 250 ng and 1000 ng are adequate for most NGS applications. Depending on the DNA input, 5–15 cycles are usually sufficient. For non-NGS applications, 25–35 cycles are recommended.

‡Perform denaturation at 95 °C. For templates with high GC content that result in low yields, increasing the denaturation temperature to 98–100 °C may improve product yield.

‡‡To determine the optimal annealing temperature, we recommend running a temperature gradient experiment. Alternatively, start with an annealing temperature of 60 °C and increase in 2 °C increments if non-specific products are observed.

‡‡‡The optimal extension temperature is 72 °C. Extension time should be adjusted based on amplicon length and template complexity, with 30 seconds per kilobase (kb) being suitable for most applications.

Technical information and support

For technical enquiries or assay development support, please contact us via e-mail at: mdx@medixbiochemica.com.

Additional information and technical resources are available on our website at: info.medixbiochemica.com/resources.