Medix Biochemica

Product Manual
Cat. No: #3000

HiFi DNA Polymerase Hot-Start

Description

HiFi DNA Polymerase Hot-Start is a hot-start, high fidelity hyperthermophilic recombinant DNA polymerase from the hyperthermophilic archaeon Pyrococcus furiosus. HiFi DNA Polymerase Hot-Start exhibits 5' to 3' polymerase activity and 3' to 5' exonuclease activity. A state-of-the-art aptamer-like molecule reversibly blocks its 3' to 5' exonuclease and 5' to 3' polymerase activities at room temperature. This abolishes the formation of primer dimers and non-specific amplification during and after PCR. The enzyme has been further mutated to display higher processivity, which enables shorter PCR extension cycles.

In addition, the optimized buffer chemistry facilitates high sensitivity, yield, and specificity and robust and rapid polymerase processivity. The enzyme is ideal for long, complex, difficult DNA templates and is resistant to PCR inhibitors. HiFi DNA Polymerase Hot-Start has a lower error rate than standard Taq DNA polymerase (100x). The enzyme is suitable for PCR applications where higher accuracy is needed, such as site-directed mutagenesis, sequencing, and cloning. Amplified products generated by HiFi DNA Polymerase Hot-Start are blunt ended. The enzyme is also compatible with fast and standard cycling using a variety of DNA templates.

Kit Components

Component	S pack*	M pack*
HiFi DNA Polymerase Hot-Start (2 U/µL)	0.05 mL	0.25 mL
∞ 5x HiFi Buffer	1.7 mL	3 x 1.7 mL
∞∞ 10x HiFiOpt Enhancer	1.7 mL	2 x 1.7 mL

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

∞The 5x HiFi Buffer has been formulated for robust PCR performance. The buffer contains MgCl2, dNTPs, stabilizers, and enhancers.

∞∞When no amplification is observed due to complex/GC-rich templates, add 10x HiFiOpt Enhancer to the reaction.

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
5x HiFi Buffer	5 µL	10 µL	1x
10x HiFiOpt Buffer	2.5 µL	5 µL	1x
ΔForward primer (10 µM)	1 µL	2 µL	400 nM
ΔReverse primer (10 µM)	1 µL	2 µL	400 nM
HiFi DNA Polymerase Hot-Start (2 U/µL)	0.25 µL	0.5 µL	0.02 U/µL
ΔΔDNA/cDNA Template	X	X	Variable
Nuclease-free Water	Up to 25 µL final volume	Up to 50 µL final volume	

ΔPrimers and probes should be specific to the target DNA/RNA of interest. The recommended Tm for primers is between 56°C and 60°C.

ΔΔFor 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.

Oy Medix Biochemica Ab
Klovinpellontie 3, FI-02180 Espoo
Finland

mdx@medixbiochemica.com
www.medixbiochemica.com
VAT reg. no. FI14631532

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Instrument and Program Set-Up

Cycles	Steps	Temperature	Time
1	Pre-denaturation	95°C	1 min
25–35	Denaturation	95°C	15 sec
	Annealing	60–75°C	15 sec
	**Extension	72°C	30 sec

**The extension time for most applications should be 30 seconds per kb of target region. The optimum extension time should be determined based on complexity of the template.

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.