ExactiFi™ Hot-Start DNA Polymerase, 1 U/µl

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0300501	100 Units	100 µl HiFi Hot-Start DNA Polymerase, 1 U/µl 1 ml 5X HiFi Reaction Buffer 250 µl dNTP Mix (10 mM each) 500 µl 50 mM $MgCl_2$

COMPONENT	COMPOSITION
HiFi Hot-Start DNA Polymerase	HiFi Hot-Start DNA Polymerase, 1 U/µl, in storage buffer containing 50 % (v/v) glycerol
HiFi Reaction Buffer	Optimized reaction buffer without magnesium ions
dNTP Mix (10 mM each)	Aqueous solution (pH 7.0) containing 10 mm each: dATP, dCTP, dGTP, dTTP sodium salts
MgCl2	50 mM MgCl2 in water

STORAGE −20 °C (until expiry date – see product label)

FEATURES

- Exceptional fidelity, approximately 50 times higher than Taq polymerase
- Amplifies targets up to 5–10 kb in size
- Produces blunt-end PCR products
- Efficient amplification of both low- and high-complexity targets
- High resistance to inhibitors for robust performance across a variety of sample types

APPLICATIONS

- High-fidelity PCR
- Generation of PCR products for blunt cloning
- Highest fidelity PCR with ultra-low error rates, suitable for NGS

DESCRIPTION

biotechrabbit™ ExactiFi™ Hot-Start DNA Polymerase is a highly purified thermostable recombinant proofreading DNA polymerase. ExactiFi exhibits approximately 50 times higher accuracy than Taq DNA polymerase and amplifies targets up to 5–10 kb in size.

The enzyme catalyzes template-dependent nucleotide polymerization in the 5'→3' direction. Additionally, the 3'→5' exonuclease (proofreading) activity corrects nucleotide incorporation errors, thereby increasing fidelity and accuracy of DNA polymerization. The enzyme has no 5'→3' exonuclease activity and no detectable reverse transcriptase activity, producing blunt-end PCR products.

PROTOCOL

Prevention of PCR contamination

When assembling the amplification reactions, care should be taken to eliminate the possibility of contamination with undesired DNA. Use separate clean areas for preparation of samples and reaction mixtures and for cycling. Wear fresh gloves. Use sterile tubes and pipette tips with aerosol filters for PCR setup. Use only water and reagents that are free of DNA and nucleases.
With every PCR setup, perform a contamination control reaction that does not include template DNA.

Standard PCR setup

The High-Fidelity PCR protocol using biotechrabbit 5X HiFi Reaction Buffer provides excellent results for most applications. In such cases, optimization of template purification (see biotechrabbit nucleic acid purification kits), primer design and annealing temperature is recommended.
The best conditions for each primer-template can be optimized with the following:

- Choosing the optimal quantities of template and primers
- Determining optimal concentrations of the enzyme and magnesium ions
- Optimizing cycling conditions

If unspecific amplification occurs, the amount of DNA Polymerase and the primer concentration can be reduced. Correspondingly, these can be increased when yield is low.

Optimizing magnesium concentration

Many applications use the standard concentration of 1 mM $MgCl_2$. However, reactions with increased amounts of template (e.g., genomic DNA), primer and nucleotides might require higher $MgCl_2$ concentrations (1–3 mM). A separate 50 mM $MgCl_2$ solution is supplied with the enzyme and can be used to adjust the $MgCl_2$ concentration according to the table below:

Final concentration of $MgCl_2$ in a 50 µl reaction, mM	1.0	1.5	2.0	2.5	3.0
Volume of 50 mM $MgCl_2$ solution to add, µl	1.0	1.5	2.0	2.5	3.0

Basic Protocol

Thaw on ice and mix all reagents well, especially the $MgCl_2$ solution and dNTPs. Keep all reagents and reactions on ice. When setting up multiple reactions, prepare a master mix of water, buffer, dNTPs and polymerase. Prepare enough master mix for one more than the actual number reactions. Pipet the master mix into thin-walled 0.2 ml PCR tubes. Add template and primers separately if they are not used in all reactions.

COMPONENT	VOLUME	FINAL CONCENTRATION
5X HiFi Reaction Buffer	10 µl	1X
50 mM $MgCl_2$	1 µl	1 mM
	Higher than 2 mM $MgCl_2$ might increase yield but reduce fidelity	
10 mM dNTP Mix	1 µl	200 µM
Forward primer	Variable	0.2–1 µM
Reverse primer	Variable	0.2–1 µM
Template DNA	Variable	10 pg–1 µg
	Use 0.01–1 ng for plasmid or phage DNA and 0.1–1 µg for genomic DNA	
HiFi Hot-Start DNA Polymerase (1 U/µl)	1 µl	1 U
Nuclease free water	Variable	
Total volume	50 µl	

Mix and centrifuge briefly to collect the liquid in the bottom of the tube.
Place in the PCR cycler.

Cycling Program

STEP	TEMPERATURE	TIME	CYCLES
Initial activation	94 °C	2 min	1
Denaturation	94 °C	10 s	29
Annealing*	55–73 °C	10 s	29
	**Recommended annealing temperature is 5 °C above T_m of primers, or use gradient PCR to optimize the annealing temperature*		
Extension	72 °C	60 s	29
Final extension	72 °C	5 min	1
	To extend all incomplete PCR products		
Storage in the cycler	4 °C	Indefinitely	1

Add loading dye solution (see DNA Loading Dye, 6X, cat. no. BR0800301) to the reactions to analyze PCR products on a gel or store them at −20 °C.

CERTIFICATE OF ANALYSIS

Quality Control

Functional assay

Human genomic DNA was amplified using the ExactiFi™ Hot-Start DNA Polymerase and specific primers to produce a distinct band of 1100 bp.

Self-priming activity

Standard PCR is carried out without primers, using the ExactiFi™ Hot-Start DNA Polymerase and human genomic DNA. No products were amplified.

Exonuclease assay

Linearized lambda/HindIII fragments are incubated with the ExactiFi™ Hot-Start DNA Polymerase in a 50 µl reaction mixture for 4 h at 37 °C. No degradation of DNA was observed.

Endonuclease assay

lambda DNA is incubated with the ExactiFi™ Hot-Start DNA Polymerase in a 50 µl reaction mixture for 4 h at 37 °C. No degradation of DNA was observed.

Nick Activity

Supercoiled plasmid DNA is incubated with the ExactiFi™ Hot-Start DNA Polymerase in a 50 µl reaction mixture for 4 h at 37 °C. No conversion of covalently closed circular DNA to nicked DNA was detected.

E. coli DNA contamination assay

A sample of the denatured ExactiFi™ Hot-Start DNA Polymerase is analyzed with specific primers targeting the 16S rRNA gene in qPCR for the presence of contaminating E. coli DNA. No E. coli DNA was detectable.

Quality confirmed by: Head of Quality Control

SAFETY INSTRUCTIONS

For safety instructions please see Safety Data Sheets (SDS)
Sicherheitshinweise finden Sie in den Sicherheitsdatenblättern (SDB) unter
http://www.biotechrabbit.com/support/documentation.html

USEFUL HINTS

Visit Applications at www.biotechrabbit.com for more products and product selection guides.
Most biotechrabbit products are available in custom formulations and bulk amounts.
In case any customization is required, please contact biotechrabbit via oem@biotechrabbit.com.

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Certified Quality-System mdc ISO 13485 ISO 9001

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