Pfu DNA Polymerase, 2.5 U/µl

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0300101	100 U	40 µl *Pfu* DNA Polymerase 400 µl 10× *Pfu* Reaction Buffer 1.5 ml 5× PCR Enhancer*
BR0300102	500 U	200 µl *Pfu* DNA Polymerase 2 × 1.2 ml 10× *Pfu* Reaction Buffer 1.5 ml 5× PCR Enhancer*

COMPONENT	COMPOSITION
Pfu DNA Polymerase	*Pfu* DNA Polymerase, 2.5 U/µl, in storage buffer containing 50% (v/v) glycerol
10× *Pfu* Reaction Buffer	Optimized PCR reaction buffer including magnesium ions
5× PCR Enhancer*	Proprietary PCR enhancer mix

* For reaction optimization. Sufficient PCR Enhancer for all reactions to be ordered separately (BR1900201).

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

FEATURES

- Accurate PCR for demanding applications
- Approximately ten times higher accuracy than *Taq* DNA Polymerase
- Proof-reading for increased fidelity

APPLICATIONS

- High-fidelity PCR
- Generation of PCR products for blunt cloning
- Site directed mutagenesis

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DESCRIPTION

biotechrabbit™ *Pfu* DNA Polymerase is a highly purified thermostable recombinant proofreading DNA polymerase. *Pfu* DNA Polymerase exhibits approximately 10 times higher accuracy than *Taq* DNA polymerase and amplifies targets up to 3–4 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 and produces blunt-end PCR products.
For the most demanding applications, the supplied 5× PCR Enhancer can be optionally used for improving results when using templates with GC-rich sequences and complex structures.

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 standard PCR protocol using biotechrabbit reaction buffer provides excellent results for most applications. Optimization might be necessary for certain conditions, such as the amplification of long targets, high GC or AT content, strong template secondary structures or insufficient template purity. 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.

BASIC PROTOCOL

- Optionally, use 5× PCR Enhancer to increase the yield and to lower the background in more complicated PCR reactions (low amounts of template, impure or GC-rich template).
- 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.

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- Alternatively, use biotechrabbit *Pfu* PCR Master Mix, 2× (cat. no. BR0300201)
- 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
10× *Pfu* Reaction Buffer	5 µl	1×
5× PCR Enhancer (optional)*	10 µl	1×
* For reaction optimization. PCR Enhancer for all reactions to be ordered separately (BR1900201).		
10 mM dNTP Mix	1 µl	200 µM
Forward primer	Variable	0.2–1 µM
Reverse primer	Variable	0.2–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	
Pfu DNA Polymerase (2.5 U/µl)	0.5–1 µl	1.25–2.5 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	95°C	2 min	1
Denaturation	95°C	30 s	25–35
Annealing	55°C	30–45 s	25–35
	Approximately 5°C below T_m of primers		
Extension	72°C	2 min/kb	25–35
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, 6×, cat. no. BR0800301) to the reactions to analyze PCR products on a gel or store them at −20°C.
- For cloning, always purify the PCR product from a gel (see BR0700501 GenUP™ PCR/Gel Extraction Kit).

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CERTIFICATE OF ANALYSIS

Unit Definition

One unit catalyzes the incorporation of 10 nmol of deoxyribonucleotides into a polynucleotide fraction in 30 minutes at 72°C.

Quality Control

Functional assay

Human genomic DNA was amplified using the DNA Polymerase and specific primers to produce a distinct band of 750 bp.

Self-priming activity

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

Exonuclease/Endonuclease assay

lambda DNA is incubated with the 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 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 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 SDS 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.

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Certified Quality-System mdc ISO 13485 ISO 9001

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