Hot Start *Taq* DNA Polymerase, 5 U/µl

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0200102	500 U	100 µl Hot Start *Taq* DNA Polymerase 2 × 1.8 ml 5× PCR Reaction Buffer 1.5 ml 50 mM $MgCl_2$

COMPONENT	COMPOSITION
Hot Start *Taq* DNA Polymerase	Hot Start *Taq* DNA Polymerase, 5 U/µl, in storage buffer containing 50% (v/v) glycerol
5× PCR Reaction Buffer	Optimized PCR buffer without magnesium ions
50 mM $MgCl_2$	50 mM $MgCl_2$ in water
STORAGE	–20°C (until expiry date – see product label)

FEATURES

- High PCR specificity and sensitivity
- Exceptionally pure Hot Start *Taq* DNA Polymerase for sensitive PCR applications and high yields
- Antibody-based Hot Start for fast polymerase activation

APPLICATIONS

- Hot-start PCR up to 3 kb
- Amplification of low-copy-number targets
- RT-PCR and TA cloning

DESCRIPTION

biotechrabbit™ Hot Start *Taq* DNA Polymerase is a first-choice hot-start PCR enzyme for all demanding PCR applications. The enzyme ensures high product yields with low background and without primer–dimer formation and nonspecific priming.
The Hot Start *Taq* DNA Polymerase is inactive during reaction setup due to the bound antibody which is quickly released at elevated temperatures, ensuring the enzyme is active only during PCR. There is no need for prolonged heating or denaturation steps.
The optional use of 5× PCR Enhancer improves PCR results in many cases, including impure template or low template abundance.

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 by choosing the optimal quantities of template and primers and optimizing cycling conditions.

Optimizing magnesium concentration

Many applications use the standard concentration of 1.5–2 mM $MgCl_2$. However, reactions with increased amounts of template (e.g., genomic DNA), primer and nucleotides might require higher $MgCl_2$ concentrations (2–3 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	2.00	2.25	2.5	2.75	3.0
Volume of 50 mM $MgCl_2$ solution to add, µl	2.00	2.25	2.5	2.75	3.0

BASIC PROTOCOL

- Optionally, use the supplied 5× PCR Enhancer to increase the yield and reduce 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.

- 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
5× Reaction Buffer	10 µl	1×
50 mM $MgCl_2$	Variable (standard 2 µl)	2 mM
Higher than 2 mM $MgCl_2$ might increase yield but reduce fidelity		
5× PCR Enhancer* (optional)	10 µl	1×
10 mM dNTP Mix (BR0600202)	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 0.05–1 µg for genomic DNA		
Hot Start *Taq* DNA Polymerase (5 U/µl)	0.5 µl	2.5 U
Nuclease free water	Variable	
Total volume	50 µl	

- For total reaction volumes other than 50 µl, scale reagents proportionally.
- 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*	(55–68°C)	15–30 s	25–35
	**Recommended annealing temperature is 2°C above Tm of primers. Use gradient PCR to optimize the annealing temperature.*		
Extension	72°C	30–60 s/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.

CERTIFICATE OF ANALYSIS

Unit Definition

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol of dNTP into acid-insoluble form in 30 minutes at 72°C in the presence of the reaction buffer.

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 assay

Linearized lambda/HindII fragments are incubated with the 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 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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