Taq DNA Polymerase, lyophilized, 5 U/µl

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0101202	500 U	Lyo *Taq* DNA Polymerase 100 µl *Taq* Reconstitution Buffer 2 × 1.8 ml 10× Reaction Buffer with $MgCl_2$
BR0101203	2500 U	5 × Lyo *Taq* DNA Polymerase 5 × 100 µl *Taq* Reconstitution Buffer 7 × 1.8 ml 10× Reaction Buffer with $MgCl_2$

COMPONENT	COMPOSITION
Lyo *Taq* DNA Polymerase	Lyophilized *Taq* DNA Polymerase, 5 U/µl
Taq Reconstitution Buffer	Optimized storage buffer for reconstituting Lyo *Taq* DNA Polymerase containing 50% (v/v) glycerol
10× Reaction Buffer with $MgCl_2$	Optimized PCR reaction buffer including 15 mM $MgCl_2$

LYO TAQ DNA POLYMERASE RECONSTITUTION

1) Transfer the whole content of one vial *Taq* Reconstitution Buffer to one vial Lyo *Taq* DNA Polymerase
2) Mix well – the lyophilisate will dissolve within seconds
3) Store the reconstituted *Taq* DNA Polymerase, 5 U/µl, at -20°C

STORAGE

Store at room temperature or below (until expiry date – see product label)
Reconstituted *Taq* DNA Polymerase: store at –20°C

FEATURES

- Room-temperature stable enzymes and mixes
- Optimal concentration of $MgCl_2$ included in Reaction Buffer
- High product yields and robustness in a wide application range
- Exceptionally pure *Taq* DNA Polymerase for both routine and demanding PCR applications

APPLICATIONS

- Ambient shipment and room-temperature storage
- Routine and applied PCR up to 5 kb
- RT-PCR
- TA cloning

DESCRIPTION

biotechrabbit™ Lyo *Taq* DNA Polymerase is a freeze-dried versions of its well-established liquid equivalent. The stabilized format allows shipment and storage without cooling.
Taq DNA Polymerase is a first-choice thermostable DNA polymerase for all routine PCR applications, ensuring high product yields from various templates for targets up to 5 kb in size. The convenient version provides optimal MgCl2 concentration in the Reaction Buffer, saving time during reaction setup.
biotechrabbit *Taq* DNA Polymerase is a thermostable, highly processive 5' →3' DNA polymerase that has low 5'→3' exonuclease activity and lacks 3' →5' exonuclease (proofreading) activity. The latter activity allows incorporation of modified nucleotides into DNA.
The enzyme also exhibits deoxynucleotidyltransferase activity, resulting in the addition of an extra A overhang at the 3' ends of PCR products, allowing easy cloning of PCR products into vectors with T overhangs.

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 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

- Thaw on ice and mix all reagents well, especially the dNTPs (i.e. 10 mM dNTP Mix, cat. no. BR0600201).
- 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. Alternatively, use biotechrabbit Lyo PCR ReadyMix, 2× (cat. no. BR0101101)
- 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× Reaction Buffer with $MgCl_2$	5 µl	1×
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	
Taq DNA Polymerase (5 U/µl) (reconstituted lyophilisate)	0.2–0.5 µl	1–2.5 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*	(55-68°C)	15–30 s	25–35
	**Recommended annealing temperature is 5°C below Tm of primers, or 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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