biotechrabbit
leap and lead

Green Hot Start *Taq* DNA Polymerase Direct-Load, 5 U/µl

LOT: See product label EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0200701	100 U	20 µl Hot Start *Taq* DNA Polymerase 1.8 ml 5× Green Direct-Load Reaction Buffer 1.5 ml 50 mM $MgCl_2$
BR0200702	500 U	100 µl Hot Start *Taq* DNA Polymerase 2 × 1.8 ml 5× Green Direct-Load Reaction Buffer 1.5 ml 50 mM $MgCl_2$
BR0200703	2500 U	500 µl Hot Start *Taq* DNA Polymerase 14 × 1.8 ml 5× Green Direct-Load Reaction Buffer 5 × 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× Green Direct-Load Reaction Buffer	Optimized PCR buffer without magnesium ions for direct gel loading contains electrophoresis tracking dyes (yellow and blue) and density reagent.
50 mM $MgCl_2$	50 mM $MgCl_2$ in water

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

FEATURES

- Green Reaction Buffer formulation for direct loading on the gel right after PCR
- High PCR specificity and sensitivity
- Hot Start *Taq* DNA Polymerase for demanding sensitive PCR applications and high yields

APPLICATIONS

- High-sensitivity PCR and immediate gel analysis
- High-specificity hot-start PCR up to 5 kb
- Amplification of low-copy-number targets
- RT-PCR and TA cloning

DESCRIPTION

biotechrabbit™ Green Hot Start *Taq* DNA Polymerase Direct-Load 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 that allows direct electrophoresis and ensures high product yields from various templates.
The 5× Reaction Buffer of Green Hot Start DNA Polymerase is recommended for any amplification reaction that will be visualized by agarose gel electrophoresis. The Buffer contains two dyes (blue and yellow) that separate during electrophoresis, allowing the migration progress to be monitored. Reactions assembled with the 5× Reaction Buffer have sufficient density for direct loading onto agarose gels.

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 *Taq* 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 1.5 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	1.5	1.75	2.0	2.5	3.0
Volume of 50 mM $MgCl_2$ solution to add, µl	1.5	1.75	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. Alternatively, use biotechrabbit PCR Green Direct-Load Hot Start Master Mix, 2× (cat. no. BR0200501)

- 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× Green Direct-Load Reaction Buffer	10 µl	1×
50 mM $MgCl_2$	Variable (standard 1.5 µl)	1.5–3 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	
Hot Start *Taq* DNA Polymerase (5 U/µl)	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°C	15–30 s	25–35
		Approximately 5°C below T_m of primers	
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

- Reactions assembled with the Green Direct-Load Reaction Buffer have sufficient density for direct loading onto agarose gels. Do not add any loading dyes for gel loading.
- Analyze PCR reactions on the gel or store them at –20°C up to they are analyzed.

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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