Green Hot Start PCR Master Mix Direct-Load, 2×

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0200501	100 rxn of 50 µl	2 × 1.25 ml Green Hot Start PCR Master Mix Direct-Load
BR0200502	500 rxn of 50 µl	10 × 1.25 ml Green Hot Start PCR Master Mix Direct-Load
BR0200504	2000 rxn of 50 µl	40 × 1.25 ml Green Hot Start PCR Master Mix Direct-Load

COMPONENT	COMPOSITION
Green Hot Start PCR Master Mix Direct-Load	Optimized 2× Green Hot Start PCR Master Mix containing electrophoresis tracking dyes (yellow and blue) and density reagent for direct gel loading.
STORAGE	–20°C (until expiry date – see product label)

FEATURES

- Optimized Green Hot Start PCR Master Mix for fast setup and direct loading on the gel
- Exceptionally pure Hot Start *Taq* DNA Polymerase and highest quality dNTPs
- High product yields and robustness in a wide application range

APPLICATIONS

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

DESCRIPTION

biotechrabbit™ Green Hot Start PCR Master Mix Direct Load is a perfect choice for a fast reaction setup that reduces the time required for calculation and pipetting and eliminates the need for buffer optimization. It is designed for low-background, high-throughput PCR amplification of 0.2–5 kb DNA targets. Additionally the special formulation allows reactions to be loaded directly onto gels after amplification without adding additional loading dye.

Green Hot Start PCR Master Mix Direct-Load contains two dyes (blue and yellow) that separate during electrophoresis, allowing migration progress to be monitored. Reactions with Green Hot Start PCR Master Mix Direct-Load 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 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
- Optimizing cycling conditions

BASIC PROTOCOL

- The Master Mix is designed to be used without any optimization as it has all necessary reaction components in optimal amounts for successful PCR.
- Thaw on ice and mix all reagents well.
- Keep all reagents and reactions on ice.
- 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
Green Hot Start PCR Master Mix Direct-Load, 2×	25 µl	1×
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	
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

- Reactions assembled with the Green Reaction Buffer have sufficient density for direct loading onto agarose gels. Do not add any loading dyes for gel loading.

CERTIFICATE OF ANALYSIS

Quality Control

Functional assay

Human genomic DNA was amplified using the Green Hot Start PCR Master Mix Direct-Load and specific primers to produce a distinct band.

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.

CONTACT BIOTECHRABBIT

biotechrabbit GmbH
Volmerstr. 9a
12489 Berlin, Germany

info@biotechrabbit.com
support@biotechrabbit.com
www.biotechrabbit.com

Phone: +49 30 555 7821-10
Fax: +49 30 555 7821-99

Certified Quality-System mdc ISO 13485 ISO 9001

valid from 27.04.2018