Green PCR Master Mix Direct-Load, 2×

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0100401	100 rxn of 50 µl	2 × 1.25 ml Green PCR Master Mix Direct-Load
BR0100402	500 rxn of 50 µl	10 × 1.25 ml Green PCR Master Mix Direct-Load
BR0100404	2000 rxn of 50 µl	40 × 1.25 ml Green PCR Master Mix Direct-Load

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

FEATURES

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

APPLICATIONS

- High-throughput PCR and immediate gel analysis
- Routine PCR up to 5 kb
- TA cloning

DESCRIPTION

biotechrabbit™ Green 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. Additionally the special formulation allows reactions onto be loaded onto an agarose gel directly after amplification without a separate step for adding loading dye.

Green PCR Master Mix Direct-Load contains two dyes (blue and yellow) that separate during electrophoresis, allowing the migration progress to be monitored. Reactions with the Green PCR Master Mix have sufficient density for direct loading onto agarose gels. Green Reaction Buffer also allows mixtures containing the enzyme to be identified.

The Green PCR Master Mix Direct-Load contains highly purified recombinant biotechrabbit *Taq* DNA Polymerase, extremely high-quality dNTPs and optimized PCR buffer; thus, only template DNA, PCR primers and PCR-grade water need to be added.

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 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 PCR Master Mix Direct-Load 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 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.

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