PCR Master Mix, lyophilized, 2×

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0101101	50 rxn of 50 µl	Lyo PCR Master Mix 1.25 ml PCR Reconstitution Buffer
BR0101102	250 rxn of 50 µl	5 × Lyo PCR Master Mix 5 × 1.25 ml PCR Reconstitution Buffer

COMPONENT	COMPOSITION
Lyo PCR Master Mix	Lyophilized 2× PCR Master Mix
PCR Reconstitution Buffer	Optimized PCR buffer for reconstituting Lyo Master Mix

LYO PCR MASTER MIX RECONSTITUTION

1) Transfer the whole content of one vial PCR Reconstitution Buffer to one vial Lyo PCR Master Mix
2) Mix well – the lyophilisate will dissolve within seconds
3) Store the reconstituted PCR Master Mix, 2× at -20°C

STORAGE

Lyophilisate and Buffer:
Room temperature for up to 6 months, or 4°C for up to 12 months
Reconstituted Master Mix:
–20°C (until expiry date – see product label)

FEATURES

- Room-temperature stable enzymes and mixes
- Exceptionally pure *Taq* DNA Polymerase
- Optimized Master Mix for fast setup

APPLICATIONS

- Ambient shipment and room-temperature storage
- Routine and demanding PCR applications
- PCR amplification up to 5 kb
- TA cloning

DESCRIPTION

biotechrabbit™ Lyo PCR Master Mix is a freeze dried version of the well-established liquid equivalent. The stabilized format allows shipment and storage without cooling. The Master Mix 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 of 0.2–5 kb DNA targets.

The 2x PCR Master Mix contains pure biotechrabbit *Taq* DNA Polymerase, extremely high-quality dNTPs and optimized PCR buffer; thus, only template, PCR primers and PCR-grade water are 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
PCR Master Mix, 2× (reconstituted lyophilisate)	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°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

- 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

Quality Control

Functional assay

Human genomic DNA was amplified using the reconstituted PCR Master Mix 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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