Multiplex PCR Master Mix, 2×

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0200801	50 rxn of 50 µl	1.25 ml Multiplex PCR Master Mix
BR0200802	250 rxn of 50 µl	5 × 1.25 ml Multiplex PCR Master Mix
BR0200804	1000 rxn of 50 µl	20 × 1.25 ml Multiplex PCR Master Mix

COMPONENT	COMPOSITION
Multiplex PCR Master Mix	Optimized 2× Multiplex PCR Master Mix
STORAGE	–20°C (until expiry date – see product label)

FEATURES

- Excellent performance and robustness in multiplex PCR
- Optimized Master Mix for minimal hands-on and fast setup
- Hot-start for highest sensitivity and specificity
- Exceptionally pure Hot Start *Taq* DNA Polymerase and highest quality dNTPs

APPLICATIONS

- Fast and high-throughput multiplex PCR
- Parallel detection of multiple targets in a single assay
- Gene expression analysis, diagnostic and forensic genotyping
- Amplification of 50 bp to 2 kb targets

DESCRIPTION

biotechrabbit™ Multiplex PCR Master Mix is a perfect choice for endpoint multiplex PCR. The unique buffer composition is optimized for robust simultaneous amplification of 10 or more targets from 50 bp – 2 kb in a single reaction.

The Master Mix contains pure biotechrabbit Hot Start *Taq* DNA Polymerase, extremely high-quality dNTPs and optimized Multiplex PCR buffer; thus, only template, PCR primers and PCR-grade water are added. The enhancers included in the mix enable efficient amplification of low abundant or GC-rich templates.

Hot Start *Taq* DNA Polymerase is inactive during reaction setup due to the bound antibody, which is quickly released at elevated temperatures, ensuring the enzyme is active only during PCR. The hot-start efficiently minimizes primer–dimers and mispriming.

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
Multiplex PCR Master Mix, 2×	25 µl	1×
Forward primers	Variable	0.2–1 µM
Reverse primers	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	30-40
Annealing	(55-68°C)*	45 s	
	**Recommended annealing temperature is 5°C below Tm of primers. Use gradient PCR to optimize the annealing temperature.*		
Extension	72°C	30–60 s/kb	
Final extension	72°C	5 min	1
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 Multiplex PCR Master Mix and specific primers to produce distinct bands.

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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Certified Quality-System mdc ISO 13485 ISO 9001

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