Pfu PCR Master Mix, 2×

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0300201	100 rxn of 50 µl	2 × 1.25 ml *Pfu* PCR Master Mix 1.5 ml 5× PCR Enhancer
BR0300202	500 rxn of 50 µl	10 × 1.25 ml *Pfu* PCR Master Mix 1.5 ml 5× PCR Enhancer*

COMPONENT	COMPOSITION
Pfu PCR Master Mix	Optimized 2× *Pfu* PCR Master Mix
5× PCR Enhancer*	Proprietary PCR enhancer mix

* For reaction optimization. Sufficient PCR Enhancer for all reactions to be ordered separately (BR1900201).

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

FEATURES

- Optimized *Pfu* PCR Master Mix for minimal hands-on and fast setup
- Pure *Pfu* DNA Polymerase and highest quality dNTPs
- Approximately ten times higher accuracy than *Taq* DNA Polymerase for accurate PCR in demanding applications

APPLICATIONS

- High-throughput, high-fidelity PCR
- Generation of PCR products for blunt cloning

DESCRIPTION

biotechrabbit™ *Pfu* PCR Master Mix is a perfect choice for fast, high-fidelity PCR setup that reduces the time required for calculation and pipetting and eliminates the need for buffer optimization. It is designed for routine high-throughput, high-fidelity amplification of targets up to 3–4 kb in size.

The 2× *Pfu* PCR Master Mix contains *Pfu* DNA Polymerase, extremely high-quality dNTPs and optimized PCR buffer; thus, only template, PCR primers and PCR-grade water are added. For the most demanding applications, the supplied 5× PCR Enhancer can be optionally be used to improve results when using templates with GC-rich sequences and complex structures.

Pfu DNA Polymerase exhibits approximately 10 times higher accuracy compared to *Taq* DNA polymerase. *Pfu* DNA Polymerase produces blunt-end PCR products suitable for blunt cloning.

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
- Using a PCR Enhancer (i.e. BR1900201) for low amounts of template, impure or GC-rich templates
- 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.
- Optionally, use the supplied 5× PCR Enhancer to increase the yield and to lower the background in more complicated PCR reactions (low amounts of template, impure or GC-rich template).
- 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
Pfu PCR Master Mix, 2×	25 µl	1×
5× PCR Enhancer (optional)	10 µl	1×
* For reaction optimization. Sufficient PCR Enhancer for all reactions to be ordered separately (BR1900201).		
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	30–45 s	25–35
	Approximately 5°C below T_m of primers		
Extension	72°C	2 min/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.
- For cloning, always purify the PCR product from a gel (see BR0700401 GenUP™ Gel Extraction Kit).

CERTIFICATE OF ANALYSIS

Quality Control

Functional assay

Human genomic DNA was amplified using the *Pfu* 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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Certified Quality-System mdc ISO 13485 ISO 9001

valid from 24.01.2017