Long Range PCR Master Mix, 2×

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0300401	100 rxn of 50 µl	2 × 1.25 ml Long Range PCR Master Mix 1.5 ml 5× PCR Enhancer
BR0300402	500 rxn of 50 µl	10 × 1.25 ml Long Range PCR Master Mix 1.5 ml 5× PCR Enhancer*

COMPONENT	COMPOSITION
Long Range PCR Master Mix	Optimized 2× Long Range 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 Long Range PCR Master Mix for minimal hands-on and fast setup
- Mix of pure polymerases and highest quality dNTPs for high yield and short cycle times
- Increased fidelity for accurate amplification of GC-rich templates

APPLICATIONS

- High-throughput, long-range PCR up to 40 kb
- Amplification of GC-rich templates

DESCRIPTION

biotechrabbit™ Long Range PCR Master Mix is a perfect choice for fast reaction setup for long-range PCR that reduces the time required for calculation and pipetting and eliminates the need for buffer optimization. It is designed for amplification of targets up to 40 kb in size. The master mix works well with GC-rich templates and amplifies DNA with a higher fidelity than *Taq* DNA polymerase.

The Long Range PCR Master Mix contains a blend of thermophilic polymerases, 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 used to improve results when using templates with GC-rich sequences and complex structures.

Long Range PCR Master Mix produces a mixture of A-tailed and blunt-end PCR products. It is advisable to blunt products before cloning into the blunt-end vector.

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
Long Range 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	30 s/kb	25–35
For fragments longer than 5 kb, use 68°C extension temperature and 1 min/kb timing.			
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 Long Range 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