

Hot Start PCR Master Mix, lyophilized, 2×

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0201103	200 rxn of 50 µl	4 × Lyo Hot Start PCR Master Mix 4 × 1.25 ml PCR Mix Reconstitution Buffer

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

**LYO HOT START PCR
MASTER MIX
RECONSTITUTION**

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

STORAGE

Store at room temperature or below (until expiry date – see product label)
 Reconstituted lyophilisate: store at -20°C for up to 12 months

FEATURES

- Room-temperature stable enzymes and mixes
- Exceptionally pure Taq DNA Polymerase
- Hot-start for high PCR specificity and sensitivity
- Optimized Master Mix for fast setup

APPLICATIONS

- Ambient shipment and room-temperature storage
- Routine and demanding PCR applications
- PCR amplification up to 3 kb
- TA cloning

Hot Start PCR Master Mix, lyophilized, 2×

DESCRIPTION

biotechrabbit™ Lyo Hot Start 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 0.2–3 kb DNA targets.

The 2x Hot Start PCR Master Mix contains pure biotechrabbit Hot Start *Taq* DNA Polymerase, extremely high-quality dNTPs and optimized PCR buffer; thus, only template, PCR primers and PCR-grade water are added.

The 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. There is no need for prolonged heating or denaturation steps. The hot start minimizes primer–dimers and mispriming.

Info: Recommended annealing temperature is 2°C above primer T_m (use gradient PCR to optimize the annealing temperature).

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, 5× PCR Enhancer (BR1900201) can be used 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
Hot Start PCR Master Mix, 2× (reconstituted lyophilisate)	25 μ l	1×
5× PCR Enhancer (optional)	10 μ l	1×
Forward primer	Variable	0.2–1 μ M
Reverse primer	Variable	0.2–1 μ M
Template DNA	Variable	10 pg–1 μ g
<i>Use 0.01–1 ng for plasmid or phage DNA and 0.1–1 μg for genomic DNA</i>		
Nuclease free water	Variable	
Total volume	50 μ l	

- Mix and centrifuge briefly to collect the liquid in the bottom of the tube.
- Place in the PCR cyclor.

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
<i>*Recommended annealing temperature is 2°C above T_m of primers, or use gradient PCR to optimize the annealing temperature</i>			
Extension	72°C	30–60 s/kb	25–35
Final extension	72°C	5 min	1
<i>To extend all incomplete PCR products</i>			
Storage in the cyclor	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.

Hot Start PCR Master Mix, lyophilized, 2×

CERTIFICATE OF ANALYSIS

Quality Control

Functional assay

Human genomic DNA was amplified using the reconstituted Hot Start 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.

CONTACT BIOTECHRABBIT

biotechrabbit GmbH

Volmerstr. 9a

12489 Berlin,

Germany

info@biotechrabbit.com

support@biotechrabbit.com

www.biotechrabbit.com

Phone: +49 30 555 7821-10

Fax: +49 30 555 7821-99



Legal Disclaimer and Product Use Limitation

Purchase of product does not include a license to perform any patented applications; therefore it is the sole responsibility of users to determine whether they may be required to engage a license agreement depending upon the particular application in which the product is used. This product was developed, manufactured, and sold for in vitro use only. It is not suitable for administration to humans or animals.

Trademarks: biotechrabbit™ (biotechrabbit GmbH).

valid from 13.01.2020