

## YourTaq™ Hot Start PCR Mix, lyophilized, 2×

LOT: See product label

EXPIRY DATE: See product label

## ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0202403	200 rxn of 50 µl	4 × Lyo YourTaq Hot Start PCR Mix 4 × 1.25 ml PCR Mix Reconstitution Buffer

COMPONENT	COMPOSITION
Lyo YourTaq Hot Start PCR Mix	Cake of lyophilized 2× YourTaq Hot Start PCR Mix
PCR Mix Reconstitution Buffer	Optimized PCR buffer for reconstituting Lyo YourTaq Hot Start Mix

**LYO HOT START PCR  
MASTER MIX  
RECONSTITUTION**

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

**STORAGE**

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

## FEATURES

- Stable enzyme and mix for ambient shipment and room-temperature storage
- Exceptionally pure YourTaq Hot Start DNA Polymerase
- Optimized Master Mix for increased yield of amplification
- Resistant to PCR inhibitor carry-over
- Excellent PCR specificity and sensitivity for a broad range of amplicons

## APPLICATIONS

- Routine and demanding PCR amplification up to 3 kb
- Suitable for amplification of low target copy number
- TA cloning

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## DESCRIPTION

biotechrabbit™ lyophilized YourTaq Hot Start PCR 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 optimized for high yield of amplification of 0.1–3 kb DNA targets, even from low copy number. YourTaq Hot Start PCR Mix shows excellent PCR specificity and sensitivity for a broad range of amplicons. The mix is resistant to PCR inhibitors, such as blood (up to 20%), Ethanol or humic acid enabling PCR amplification from DNA templates with carry-over of PCR-inhibitors.

The 2× YourTaq Hot Start PCR Mix contains pure biotechrabbit YourTaq Hot Start DNA Polymerase, extremely high-quality dNTPs and optimized PCR buffer; thus, only template, PCR primers and PCR-grade water are added. In addition, the mix is suitable for amplification of GC-rich templates (up to 70%) pairing with 5× PCR Enhancer (BR1900201).

*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
- 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.
- 5× PCR Enhancer (BR1900201) can be used to apply the mix for GC-rich target amplification (up to 70% GC).
- 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
YourTaq Hot Start PCR Mix, 2× (reconstituted lyophilisate)	25 µl	1×
5× PCR Enhance (optional, see BR1900201)	10 µl	1×
Forward primer	Variable	0.2–1 µM
Reverse primer	Variable	0.2–1 µM
Template DNA	Variable	10 pg–1 µg
<i>Use 0.01–1 ng for plasmid or phage DNA and 0.05–1 µg for genomic DNA</i>		
Nuclease free water	Variable	
Total volume	50 µl	

- For total reaction volumes other than 50 µl, scale reagents proportionally.
- 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*	(55–68°C)	15–30 s	25–35
<i>*Recommended annealing temperature is 2°C above T<sub>m</sub> of primers. 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 cycler	4°C	Indefinitely	1

- Add loading dye solution (see DNA Loading Dye, 6×, BR0800301) to the reactions to analyze PCR products on a gel or store them at –20°C.

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## CERTIFICATE OF ANALYSIS

### Quality Control

### Functional assay

Human genomic DNA was amplified using the reconstituted YourTaq Hot Start PCR 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.biotechrabb.it.com/support/documentation.html>.

## USEFUL HINTS

- Visit Applications at [www.biotechrabb.it.com](http://www.biotechrabb.it.com) for more products and product selection guides.
- Most biotechrabb.it products are available in custom formulations and bulk amounts.

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