YourTaq™ Direct-Load Hot Start PCR Mix, 2×

LOT: See product label

EXPIRY DATE: See product label

ORDERING INFORMATION

CAT. NO.	SIZE	PACKAGE CONTENT
BR0202301	200 rxn of 50 µl	4 × 1.25 ml YourTaq Direct-Load Hot Start PCR Mix
BR0202302	1000 rxn of 50 µl	20 × 1.25 ml YourTaq Direct-Load Hot Start PCR Mix

COMPONENT	COMPOSITION
YourTaq Direct-Load Hot Start PCR Mix	Optimized 2× YourTaq Hot Start PCR Master Mix containing electrophoresis tracking dyes (yellow and blue) and density reagent for direct gel loading
STORAGE	–20°C (until expiry date – see product label)

FEATURES

- Exceptionally pure YourTaq Hot Start DNA Polymerase
- Optimized Master Mix for increased yield of amplification and direct loading on the gel
- 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

DESCRIPTION

biotechrabbit™ YourTaq Direct-Load Hot Start PCR 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 Direct-Load Hot Start PCR Mix contains pure biotechrabbit YourTaq Hot Start DNA Polymerase, extremely high-quality dNTPs, two dyes (blue and yellow) that separate during electrophoresis, allowing migration progress to be monitored, and sufficient buffer density for direct loading onto agarose gels. 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 Tm (use gradient PCR to check).

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 Direct-Load Hot Start PCR Mix, 2×	25 µl	1×
5× PCR Enhancer (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
Use 0.01–1 ng for plasmid or phage DNA and 0.05–1 µg for genomic DNA		
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
	**Recommended annealing temperature is 2°Cabove Tm of primers. Use gradient PCR to optimize the annealing temperature.*		
Extension	72°C	30–60 s/kb	25–35
Final extension	72°C	5 min	1
	To extend all incomplete PCR products		
Storage in the cycler	4°C	Indefinitely	1

- Directly load the reactions on a gel to analyze PCR products or store them at –20°C.

CERTIFICATE OF ANALYSIS

Quality Control

Functional assay

Human genomic DNA was amplified using the YourTaq Direct-Load 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.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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