# YourTaq™ Hot Start DNA Polymerase, 5 U/µl

LOT: See product label

EXPIRY DATE: See product label

## ORDERING INFORMATION

| CAT. NO. | SIZE | PACKAGE CONTENT |
|---|---|---|
| BR0202101 | 250 Units | 50 µl YourTaq Hot Start DNA Polymerase<br>1.8 ml 5 PCR Reaction Buffer<br>1.5 ml 50 mM $MgCl_2$ |
| BR0202102 | 1250 Units | 250 µl YourTaq Hot Start DNA Polymerase<br>3 × 1.8 ml 5 PCR Reaction Buffer<br>1.5 ml 50 mM $MgCl_2$ |

| COMPONENT | COMPOSITION |
|---|---|
| YourTaq Hot Start DNA Polymerase | YourTaq Hot Start DNA Polymerase, 5 U/µl, in storage buffer containing 50% (v/v) glycerol |
| 5 PCR Reaction Buffer | Optimized PCR buffer without magnesium ions |
| 50 mM $MgCl_2$ | 50 mM $MgCl_2$ in water |
| STORAGE | –20°C (until expiry date – see product label) |

## FEATURES

- Exceptionally pure YourTaq Hot Start DNA Polymerase
- Optimized buffer composition 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
- RT-PCR and TA cloning

## DESCRIPTION

biotechrabbit™ YourTaq Hot Start DNA Polymerase is optimized for high yield of amplification of 0.1–3 kb DNA targets, even from low copy number. The enzyme shows excellent PCR specificity and sensitivity for a broad range of amplicons. YourTaq Hot Start DNA Polymerase 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.

YourTaq Hot Start DNA Polymerase is a highly pure enzyme. Together with the optimized buffer and high-quality dNTPs (BR0600202) a mix is achieved for the most demanding PCR applications. In addition, the enzyme is suitable for amplification of GC-rich templates (up to 70%) pairing with 5X 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 by choosing the optimal quantities of template and primers and optimizing cycling conditions.

### Optimizing magnesium concentration

Many applications use the standard concentration of 1.5–2 mM $MgCl_2$. However, reactions with increased amounts of template (e.g., genomic DNA), primer and nucleotides might require higher $MgCl_2$ concentrations (2–3 mM). A separate 50 mM $MgCl_2$ solution is supplied with the enzyme and can be used to adjust the $MgCl_2$ concentration according to the table below:

| Final concentration of $MgCl_2$ in a 50 µl reaction, mM | 2.00 | 2.25 | 2.5 | 2.75 | 3.0 |
|---|---|---|---|---|---|
| Volume of 50 mM $MgCl_2$ solution to add, µl | 2.00 | 2.25 | 2.5 | 2.75 | 3.0 |

## BASIC PROTOCOL

- Thaw on ice and mix all reagents well, especially the $MgCl_2$ solution and dNTPs.
- Keep all reagents and reactions on ice.
- Use 5× PCR Enhancer (BR1900201) for GC-rich target amplification (up to 70% GC).
- When setting up multiple reactions, prepare a master mix of water, buffer, dNTPs and

polymerase. Prepare enough master mix for one more than the actual number reactions. Alternatively, use biotechrabbit 2X YourTaq Hot Start PCR Mix (cat. no. BR0202201)

- 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 |
|---|---|---|
| 5× PCR Reaction Buffer | 10 µl | 1× |
| 50 mM $MgCl_2$ | Variable (standard 2 µl) | 2 mM |
| | *Higher than 2 mM MgCl2 might increase yield but reduce fidelity* | |
| 5X PCR Enhancer (optional, see BR1900201) | 10 µl | 1× |
| 10 mM dNTP (BR0600202) | 1 µl | 200 µM |
| 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* | |
| YourTaq Hot Start DNA Polymerase, 5 U/µl | 0.5 µl | 2.5 U |
| 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 |

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

## CERTIFICATE OF ANALYSIS

### Unit Definition

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol of dNTP into acid-insoluble form in 30 minutes at 72°C in the presence of the reaction buffer.

### Quality Control

#### Functional assay

Human genomic DNA was amplified using the DNA Polymerase and specific primers to produce a distinct band of 750 bp.

#### Self-priming activity

Standard PCR is carried out without primers, using the DNA Polymerase and human genomic DNA. No products were amplified.

#### Exonuclease assay

Linearized lambda/HindII fragments are incubated with the DNA Polymerase in a 50 µl reaction mixture for 4 h at 37°C. No degradation of DNA was observed.

#### Endonuclease assay

lambda DNA is incubated with the DNA Polymerase in a 50 µl reaction mixture for 4 h at 37°C. No degradation of DNA was observed.

#### Nick Activity

Supercoiled plasmid DNA is incubated with the DNA Polymerase in a 50 µl reaction mixture for 4 h at 37°C. No conversion of covalently closed circular DNA to nicked DNA was detected.

#### *E. coli* DNA contamination assay

A sample of the denatured DNA Polymerase is analyzed with specific primers targeting the 16S rRNA gene in qPCR for the presence of contaminating *E. coli* DNA. No *E. coli* DNA was detectable.

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

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*valid from 11.12.2020*