# 2X AmPelify™ PCR Master Mix

LOT: See product label

EXPIRY DATE: See product label

## ORDERING INFORMATION

| CAT. NO. | SIZE | PACKAGE CONTENT |
|---|---|---|
| BR0101001 | 100 rxns of 50 µl | 2 × 1.25 ml AmPelify PCR Master Mix |
| BR0101002 | 500 rxns of 50 µl | 10 × 1.25 ml AmPelify PCR Master Mix |
| BR0101004 | 2,000 rxns of 50 µl | 40 × 1.25 ml AmPelify PCR Master Mix |

| COMPONENT | COMPOSITION |
|---|---|
| AmPelify PCR Master Mix | Optimized 2X AmPelify PCR Master Mix |
| STORAGE | –20°C until expiry date |

## FEATURES

- Optimized PCR Master Mix for minimal hands-on and fast setup
- Exceptionally pure *Taq* DNA Polymerase and highest quality dNTPs
- High product yields and robustness in a wide application range

## APPLICATIONS

- Routine and high-throughput PCR up to 3 kb
- TA cloning

## DESCRIPTION

biotechrabbit™ 2X AmPelify PCR 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 high yield of the products in routine, high-throughput PCR amplification of up to 3 kb DNA targets. The 2X AmPelify PCR Master Mix contains highly purified recombinant biotechrabbit Taq DNA Polymerase, extremely high-quality dNTPs and optimized PCR buffer; thus, only template, PCR primers and PCR Grade Water need to be added.

biotechrabbit Taq DNA polymerase is a thermostable, highly processive 5'→3' DNA polymerase that has 5'→3' exonuclease activity and lacks 3'→5' exonuclease (proofreading) activity. The latter allows incorporation of modified nucleotides.

The enzyme also exhibits deoxynucleotidyltransferase activity that results in the addition of extra A overhang at the 3' ends of PCR products, allowing easy cloning of PCR products into vectors with T overhangs.

## HANDLING

### Prevention of contamination

Contamination with undesired DNA is a concern when assembling the amplification reactions. To eliminate the possibility of contamination with undesired DNA, follow the guidelines below:

- Wear disposable gloves when handling the solutions.
- Dedicate separate sterile areas for the preparation of samples and reaction mixtures.
- Use molecular-grade nuclease-free water and reagents.
- Include a non-template control reaction in every PCR assay.
- Avoid carryover contamination.

### 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.
- 5X 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 |
|---|---|---|
| 2X AmPelify PCR Master Mix | 25 µl | 1X |
| 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 the reaction into 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 | 15–30 s | 25–35 |
| | *Approximately 5°C below $T_m$ of primers* | | |
| 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, cat. no. BR0800301) to the reactions to analyze PCR products on a gel or store them at –20°C.

## QUALITY CONTROL ASSAYS

### Functional assay

Human genomic DNA was amplified using the 2X Ampelify PCR Master Mix and specific primers to produce a distinct band.

## 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.
- In case any customization is required, please contact biotechrabbit via oem@biotechrabbit.com.

## CONTACT BIOTECHRABBIT

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