# 2x AbTaq Master Mix

| Catalogue No. | Description | Pack Size |
|---|---|---|
| **OP-MM-001-100** | **100 reactions of 20 µL** | **1 x 1 mL** |
| **OP-MM-001-500** | **500 reactions of 20 µL** | **5 x 1 mL** |

## Product Description

2x AbTaq Master Mix is an **efficient, fast and convenient** way to perform routine PCR. Our engineered AbTaq polymerase supports robust PCR amplification over wide range of samples and even in the presence of commonly known PCR inhibitors. The ready-to-use 2x Master Mix is designed to minimize handling, yet provide optimal AbTaq polymerase activity and PCR performance. The generated PCR product can be directly loaded into the gel without addition of DNA loading buffer.

## Product Application

The 2x AbTaq Master Mix is suitable for PCR amplification from the following templates:

- cDNA
- purified plasmid, genomic DNA
- crude/impure DNA sample preparations

and is ideal for

- PCR-based fingerprinting including but not limited to genotyping, colony PCR etc.

| Features | Advantages |
|---|---|
| AbTaq 2.0 Polymerase | • Amplification of up to 4 kbp long fragment from a plasmid template<br>• Fast and reliable extension speed of 15 sec / kbp<br>Resistant to high concentrations of common PCR inhibitors e.g.:<br>- up to 1 mM EDTA<br>- 200 mM urea<br>- >10% fetal bovine serum<br>- up to 5% EtOH<br>thus reducing the need to obtain cleaner DNA samples |
| 2x Master Mix | The 2x concentrate contains all the components required for a PCR reaction, except water, DNA template and primers. It is formulated to enable (i) optimal amplification with our AbTaq polymerase and (ii) direct loading on the gel. With less pipetting steps required for PCR reaction setup and analysis, the 2x AbTaq Master Mix saves time, materials and increases assay reproducibility. |

## Product Protocol

### PCR reaction

The following PCR reaction conditions are suggested as a starting point for further optimization.

| Component | Final concentration | PCR reaction-volume 20 µL | 50 µL |
|---|---|---|---|
| 2x Master Mix | 1x | 10 µL | 25 µL |
| Forward primer (10 µM) | 400 nM | 0.8 µL | 2 µL |
| Reverse primer (10 µM) | 400 nM | 0.8 µL | 2 µL |
| Template | variable | variable | variable |
| dd$H_2O$ | | to 20 µL | to 50 µL |

### PCR amplification conditions

| Step in PCR cycle | Temp. °C | Duration | Cycle(s) |
|---|---|---|---|
| Step 1 : Initial denaturation | 95 | 2 min | 1 |
| Step 2: Denaturation | 95 | 15 sec | 33 |
| Step 3: Annealing | 50-65 | 15-30 sec | |
| Step 4: Extension | 72 | 15 sec / kbp | |
| Step 5: Final extension | 72 | 2 min | 1 |
| Step 6: Hold | 10 | ∞ | 1 |

## Important considerations

***PCR Reaction***

Our suggested reaction **volume** of 20 µL is sufficient for the vast majority of assays but can be scaled up or down if necessary. For multiplex PCR, we do not recommend using < 20 µL reaction volume.

**Primers** should be designed according to standard PCR guidelines and are typically used in the concentration range of 100-400 nM.

For the amount of **template** to use, a wide range, from 0.1 pg to 1 µg can be used successfully for a PCR amplification. The optimal amount will vary depending on the quality and complexity of the DNA sample preparation. In a 50 µL PCR reaction, our recommended amount is (i) 10 pg - 50 ng for plasmids, and viral genomes, (ii) 10 ng - 200 ng for complex prokaryotic and eukaryotic genomes, and (iii) 2 µL from a standard 20 µL cDNA reverse transcription reaction converting up to 2 µg of RNA.

For problematic **templates with high GC** content, up to 10% of DMSO is recommend to be added to a PCR reaction to relax the DNA secondary structures.

***PCR Amplification Conditions***

**Initial denaturation** of the DNA strands is performed at 95°C for 1 min for simple DNA templates (cDNA, plasmid) and can be increased up to 3 min for complex eukaryotic genomes or templates rich in GC. For colony PCR assays, 2 - 4 min is suggested to ensure complete bacteria lysis. For cycling, we recommend that DNA **denaturation** is set at 95°C for 15 s.

The **annealing temperature** will depend on the user's primer design and is usually 3 - 5 °C below the lowest primer melting temperature (Tm). Ideally, the optimal annealing temperature should be empirically determined by performing a temperature gradient PCR in the range +/- 5 °C of the calculated Tm of the primers. If DMSO is added to the PCR reaction, the annealing temperature should be reduced by 0.75°C for every 1% of DMSO added (by volume).

The **extension** step is performed at 72°C and the duration will be dependent on the length of the PCR product and the template complexity. For low complexity templates (e.g. cDNA, plasmid), the recommended extension time is 1 kbp / 15s. The duration should at least be doubled for genomic DNA.

Typically, a PCR reaction will consist of 25-35 **cycles.** Up to 40 cycles may be necessary to enable sufficient amplification of a low-abundance template.

The **final extension** step is set to fill any incomplete ends of the newly synthesized strands and is performed at 72°C. Duration of this step will dependent on the length and composition of the PCR fragment but 2 - 10 min is usually sufficient.

**Storage Condition and Stability**

Stored at -20°C. Avoid repeated freezing / thawing cycles. For handling smaller sample sizes, we recommend aliquoting the 2x Master Mix before storage.

**Technical support**

If you are experiencing difficulties with setting up your PCR reaction and/or PCR amplification conditions, please contact our team with any relevant information at **infoab@abasiabiolabs.com**