# Instructions for use

## 2x PCR MasterMix

### 1 ml, 100 reactions

### 2x PCR MasterMix for all standard PCR amplifications

#### 1. Description

Our ***2x PCR MasterMix*** is an optimized ready-to-use mixture for all standard PCR amplifications. It contains a fast Taq DNA Polymerase, dNTPs and $MgCl_2$ and all other components required for PCR except primers and template DNA.

For research use only. Not approved for use in clinical or *in vitro* diagnostics.

#### 2. Applications

***2x PCR MasterMix*** is recommended for use in all standard PCR applications. PCR assays with ***2x PCR MasterMix*** not only reduces contamination risks, but is also time saving, highly reproducible and very easy to prepare. ***2x PCR MasterMix*** can be applied to all standard Taq-based cycling protocols and is, therefore, the optimal choice for high throughput PCR as being performed in, for instance, analysis of cloning efficiency or colony screening, for routine screening processes, and for student's courses.
Due to the optimized composition of the ***2x PCR MasterMix***, the Taq polymerase delivers specific PCR amplification of good yield with a wide range of PCR templates. ***2x PCR MasterMix*** is able to amplify PCR products up to 3 kb with genomic DNA, and is appropriate for use with pure DNA solutions, cDNA, and bacterial colonies as templates. The Taq polymerase included in the master mix possesses a 5´→ 3´ polymerase- as well as a 5´-flap endonuclease activity and generates a 3'dA (adenine)-overhang which may well be used for TA-cloning purposes.

#### 3. Contents

***2x PCR MasterMix*** in 2x reaction buffer containing the Taq polymerase, 0.4 mM each dNTP and an optimized buffer system containing 4mM $MgCl_2$.

| Reagent | Amount | Lid colour |
|---|---|---|
| ***2x PCR MasterMix*** (100 reactions) | 1 tube, 1 ml | white |

#### 4. Reaction volume

The ready-to-use 2x MasterMix has been optimised for 20 µl reaction volumes. Use 10 µl of the 2x MasterMix solution and add up to 20 µl with primers, target DNA and water as described below.

#### 5. Suggested pipetting scheme

At best prepare on ice:

| Components | Apply for PCR reaction of 20 µl | Final concentration (recommended) |
|---|---|---|
| ***2x PCR MasterMix*** | 10 µl | 1x |
| Forward primer (e.g. 5 pmol/µl) | variable (e.g. 1 µl) | 0.1 – 0.5 µM |
| Reverse primer (e.g. 5 pmol/µl) | variable (e.g. 1 µl) | 0.1 – 0.5 µM |
| Template DNA | variable | 0.01 – 10 ng |
| Sterile dest. water | Adjust to 20 µl final volume | |



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## 6. Basic amplification protocol

| Step | Time | Temperature |
|---|---|---|
| Initial denaturation | 2 minutes | 92-95 °C |
| **25-35 cycles** | | |
| Denaturation | 2-10 seconds | 92-95 °C |
| Annealing | 2-10 seconds | 55-68 °C |
| Extension | variable, depends on the length of product | 72 °C |

## 7. Notes

For maximum yield and specificity, annealing temperatures and annealing time as well as extension time and cycle numbers should be optimized for each template target and primer pair. Usually the optimal annealing temperature is 2-5 °C below the melting temperature of the primers. Elongation times of 30 secs. per 1 kb may be sufficient but longer elongation times may be necessary depending on the complexity of the template DNA.

Further optimization may still be necessary by increasing $MgCl_2$ concentrations, primer concentrations and PCR cycle parameters depending on your DNA source and quality or your primers.

## 8. Recommended $MgCl_2$ concentration

2 mM (final)

In case the $MgCl_2$ concentration has to be adjusted, use a separate $MgCl_2$ solution (10 mM) in PCR quality and add in appropriate amounts according to the scheme below. We recommend doing PCR with a $MgCl_2$ gradient in order to find the optimal concentration.

**Pipetting scheme for additional $MgCl_2$**

| **Final $MgCl_2$ conc. in mM** | 2.5 | 3 | 3.5 | 4 |
|---|---|---|---|---|
| Add 10 mM $MgCl_2$ solution in following amounts to 20 µl reaction volume | 1 µl | 2 µl | 3 µl | 4 µl |

## 9. Storage conditions

Store at -20 °C. Avoid extensive freeze/thaw cycles or prepare and store working aliquots. However, the master mix is stable for at least 8 freeze/thaw cycles.

Infrequent short term storage (few hours) of the master mix may be done at +4 °C.

***Product is not covered by pending or issued patents or may have certain limitations. To our best knowledge, this product does not provide any conflict with pending or issued patents.***



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