

## Instructions for use

### 2x ProofRead MasterMix

1 ml, 100 reactions

2x PCR MasterMix for all standard PCR amplifications

#### 1. Description

Our **2x ProofRead MasterMix** is an optimized ready-to-use mixture for all standard PCR amplifications. It contains the recombinant Pfu DNA Polymerase, dNTPs and MgSO<sub>4</sub> 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 ProofRead MasterMix** is recommended for use in all high-fidelity PCR applications. PCR assays with **2x ProofRead MasterMix** not only reduces contamination risks, but is also time-saving, highly reproducible and very easy to prepare. **2x ProofRead MasterMix** can be applied to all standard cycling protocols. Pfu polymerase delivers sequence identical PCR amplicates of good yield with a wide range of PCR templates. Additionally, Pfu polymerase is well suited for amplification of target sequences of high GC content, or with complex secondary structures.

Pfu polymerase is able to amplify PCR products up to 3 kb with genomic DNA and is appropriate for use in the amplification of a broad variety of template DNAs. Pfu DNA polymerase included in the set replicates DNA 5' → 3' at 72 °C to 75 °C under presence of magnesium ions. Furthermore, the Pfu DNA polymerase possesses a 5' → 3' (proof reading) exonuclease activity, rapidly substituting misincorporated bases during polymerization, and thus being responsible for the high sequence fidelity. Pfu DNA polymerase generated DNA fragments are blunt-ended.

#### 3. Contents

**2x ProofRead MasterMix** in **2x reaction buffer** containing Pfu polymerase, 0,4 mM each dNTP, and 4 mM MgSO<sub>4</sub>.

The use of the colourless PCR master mix is adequate for all PCR applications.

Reagent	Amount	Lid colour
<b>2x ProofRead MasterMix</b> (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)
<b>2x ProofRead MasterMix</b>	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	

## 6. Basic amplification protocol

Step	Time	Temperature
Initial denaturation	2 minutes	92-95 °C
<b>25-35 cycles</b>		
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 40 seconds 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 MgSO<sub>4</sub> concentrations, primer concentrations and PCR cycle parameters depending on your DNA source and quality of your primers.

## 8. Recommended MgSO<sub>4</sub> concentration

2 mM (final)

In case the MgSO<sub>4</sub> concentration has to be adjusted, use a separate MgSO<sub>4</sub> solution (10 mM) in PCR quality and add in appropriate amounts according to the scheme below. We recommend doing PCR with a MgSO<sub>4</sub> gradient in order to find the optimal concentration.

### Pipetting scheme for additional MgSO<sub>4</sub>

Final MgSO <sub>4</sub> conc. in mM	2.5	3	3.5	4
Add 10 mM MgSO <sub>4</sub> 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 MasterMix 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.*