Instructions for use

2x HotStart PCR RED MasterMix

1 ml, 100 reactions

2x HotStart PCR RED MasterMix, antibody blocked, for all standard PCR amplifications followed by direct gel-loading

1. Description

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

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

2. Applications

2x HotStart PCR RED MasterMix is recommended for use in all PCR applications, particularly if PCR is to be followed directly by gel electrophoresis. PCR assays with ***2x HotStart PCR RED MasterMix*** not only reduces contamination risks, but is also timesaving, highly reproducible and very easy to prepare. ***2x HotStart PCR RED MasterMix*** is superior for use in all standard Taq-based cycling protocols when big sample numbers shall be amplified with high specificity as well as high reproducibility. ***2x HotStart PCR RED MasterMix*** is, therefore, the optimal choice for high throughput PCR assays in colony screening, prior to sequencing, in cycle sequencing, and in similar assays. The antibody-mediated blocking of the DNA polymerase is released only at the initial denaturation step, hence resulting in highly specific amplification of the target sequence without production of unwanted side products caused by unspecific primer annealing. Antibody concentration is adjusted for the effective inhibition of polymerase activity at temperatures up to 60°C. The polymerase is activated during normal cycling conditions, allowing for a convenient assembly of PCR reactions at room-temperature.

2x HotStart PCR RED MasterMix is able to amplify PCR products up to 3 kb with genomic DNA and up to 5 kb with Lambda DNA, and is appropriate for use with pure DNA solutions, cDNA, and bacterial colonies as templates. The Hot Start 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.

2x HotStart PCR RED MasterMix contains components for direct gel loading after PCR, which do not hinder PCR reactions in any way. In 1 % agarose gels, the included red dye migrates approx. as fast as a 1 kb DNA fragment. During denaturation in Southern blotting, the dye turns yellow at an acidic pH.

3. Contents

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

Reagent	Amount	Lid colour
2x HotStart PCR RED MasterMix (100 reactions)	1 tube, 1 ml	red

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.

Q-bios GmbH, Treidlerstr. 3, D-68535 Edingen-Neckarhausen, Germany; Phone +49-6203-92579-22 /-23

5. Suggested pipetting scheme

Reactions can be conveniently set up at room temperature:

Components	Apply for PCR reaction of 20 µl	Final concentration (recommended)
2x HotStart PCR RED 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	

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.

Q-bios GmbH, Treidlerstr. 3, D-68535 Edingen-Neckarhausen, Germany; Phone +49-6203-92579-22 /-23