www.mypols.de
support@mypols.de
T +49(0)7531 122 965 00

Taq 2x PCR Master Mix

#2001
Store at -20°C

Contents

Taq 2x PCR Master Mix contains all the components necessary for PCR, including wild-type Taq DNA polymerase and an optimized buffer including ultrapure dNTPs.

Description

Taq 2x PCR Master Mix is a ready to use reaction mix. It contains all components necessary for a successful and reliable PCR or primer extension reaction in all standard PCR cyclers. Only primers and template need to be added.
This mix provides robust PCR performance for a wide range of PCR applications. The pre-ready 2x mix ensures reproducible results, significantly reduces set-up times and the risk of pipetting mistakes.

Applications

- Standard PCR
- Realtime-PCR (addition of suitable dye required)
- Primer extension reactions
- TA cloning
- 3'A-tailing of blunt ends
- Screening / High-throughput PCRs

Recommendations for PCR/ Reaction Setup

PCR Mix

Component	Volume	Final concentration
Taq 2x PCR Master Mix	25 µl	1x
Primer forward (10 µM)*	1 µl	0.2 µM (0.05-1 µM)
Primer reverse (10 µM)*	1 µl	0.2 µM (0.05-1 µM)
Template/Sample extract	x µl	<1000 ng** DNA
Nuclease-free water		up to 50µl total vol

* Primers should ideally have a GC content of 40-60% typically

**Suggested template concentration should be about 1 ng - 1 µg (genomic DNA) or 1 ng – 1 pg (plasmid/viral DNA).

Typical 3-step PCR protocol

Initial denaturation	95°C	2 min	
Denaturation	95°C	15 sec	
Annealing*	54–72°C	30 sec	25-40 cycles
Extension	72°C	1 min/1000 bp	
Hold	<10°C		

* Typically, the annealing temperature is about 3-5°C below the calculated melting temperature of the primers used.

Recommendations for sample handling

- Keep all components on ice.
- Spin down and mix all solutions carefully before use.
- Primers should ideally have a GC content of 40-60%.
- Suggested template concentration should be about 1 ng – 1 pg (plasmid/viral DNA) or 1 ng - 1 µg (genomic DNA).
- Minimize the number of freeze-thaw cycles by storing in aliquots. For a day-to-day use, we recommend keeping an aliquot at 4°C.

Quality Control Assays

PCR activity: Taq DNA polymerase Master Mix was tested for successful PCR performance. A 92 bp fragment (beta-actin gene) was amplified from human genomic DNA and analysed by agarose gel electrophoresis.
DNA polymerase activity: Taq DNA polymerase activity has been monitored and adjusted to a specific DNA polymerase activity using an artificial DNA template and a DNA primer.
Enzyme-concentration has been determined by protein-specific staining. Please inquire more information at info@mypols.de for the lot-specific concentration.
No contamination has been detected in standard test reactions.

Safety

This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally recommend the use of gloves, lab coats and eye protection when working with these or any other chemical reagents. myPOLS Biotec takes no liability for damage resulting from handling or contact with this product. Further information can be found in the REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL.

myPOLS Biotec GmbH, Blarerstraße 56, 78462 Konstanz, Germany T +49(0)7531 122 965 00

Troubleshooting

How can I optimize the PCR reaction conditions?

1. The annealing temperature can usually be optimized. Try a temperature gradient and determine the best annealing temperature, which yields in the cleanest product.
2. Add a gradual amount of betaine 0-1M or DMSO 0-7.5% to the reaction mix and select for the cleanest product and the highest yield.
3. Try to shorten the extension and annealing time. Too long and too many cycles may lead to over-amplification and side-products.

References

Isolation, characterization, and expression in Escherichia coli of the DNA polymerase gene from Thermus aquaticus. J. Biol. Chem. 1989; 264 (11):6427–6437. F. C. Lawyer, S. Stoffel, R. K. Saiki, K. Myambo, R. Drummond, and D. H. Gelfand.

Fidelity of DNA synthesis by the Thermus aquaticus DNA polymerase. Biochemistry 1988; 27(16): 6008–6013. K. R. Tindall, T. A. Kunkel.

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Product source: recombinant protein expression in E.coli.