Thermo SCIENTIFIC

PRODUCT INFORMATION

PCR Master Mix (2X)

#_____ for __ rxns

Lot: __ **Expiry Date:** __

Store at -20°C

www.thermoscientific.com/onebio

Ordering Information

Component	#K0171 200 rxns of 50 µL	#K0172 1000 rxns of 50 µL
PCR Master Mix (2X)	4 × 1.25 mL	20 × 1.25 mL
Water, nuclease-free	4 × 1.25 mL	20 × 1.25 mL

Description

PCR Master Mix is a 2X concentrated solution of *Taq* DNA polymerase, dNTPs and all other components required for PCR, except DNA template and primers. This pre-mixed formulation saves time and reduces contamination due to a reduced number of pipetting steps required for PCR set up. The mix is optimized for efficient and reproducible PCR.

Applications

- High throughput PCR.
- Routine PCR with high reproducibility.
- Generation of PCR products for TA. cloning.
- RT-PCR.

Composition of the PCR Master Mix (2X)

0.05 U/µL *Taq* DNA polymerase, reaction buffer, 4 mM $MgCl_2$, 0.4 mM of each dNTP (dATP, dCTP, dGTP and dTTP).

PROTOCOL

1. Gently vortex and briefly centrifuge PCR Master Mix (2X) after thawing.
2. Place a thin-walled PCR tube on ice and add the following components for each 50 µL reaction:

PCR Master Mix (2X)	25 µL
Forward primer	0.1-1.0 µM
Reverse primer	0.1-1.0 µM
Template DNA	10 pg - 1 µg
Water, nuclease-free	to 50 µL
Total volume	50 µL

3. Gently vortex the samples and spin down.
4. When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µL of mineral oil.
5. Perform PCR using the recommended thermal cycling conditions outlined below:

Step	Temperature, °C	Time	Number of cycles
Initial denaturation	95	1-3 min	1
Denaturation	95	30 s	25-40
Annealing	Tm-5	30 s	
Extension	72	1 min/kb	
Final Extension	72	5-15 min	1

GUIDELINES FOR PREVENTING CONTAMINATION OF PCR REACTION

During PCR more than 10 million copies of template DNA are generated. Therefore, care must be taken to avoid contamination with other templates and amplicons that may be present in the laboratory environment. General recommendations to lower the risk of contamination are as follows:

- Prepare your DNA sample, set up the PCR mixture, perform thermal cycling and analyze PCR products in separate areas.
- Set up PCR mixtures in a laminar flow cabinet equipped with an UV lamp.
- Wear fresh gloves for DNA purification and reaction set up.
- Use reagent containers dedicated for PCR. Use positive displacement pipettes, or pipette tips with aerosol filters to prepare DNA samples and perform PCR set up.
- Always perform “no template control” (NTC) reactions to check for contamination.

GUIDELINES FOR PRIMER DESIGN

Use the Thermo Scientific REviewer primer design software at www.thermoscientific.com/reviewer or follow the general recommendations for PCR primer design as outlined below:

- PCR primers are generally 15-30 nucleotides long.
- Differences in melting temperatures (Tm) between the two primers should not exceed 5°C.
- Optimal GC content of the primer is 40-60%. Ideally, C and G nucleotides should be distributed uniformly along the primer.
- Avoid placing more than three G or C nucleotides at the 3’-end to lower the risk of non-specific priming.
- If possible, the primer should terminate with a G or C at the 3’-end.
- Avoid self-complementary primer regions, complementarities between the primers and direct primer repeats to prevent hairpin formation and primer dimerization.
- Check for possible sites of undesired complementary between primers and template DNA.
- When designing degenerate primers, place at least 3 conservated nucleotides at the 3’-end.
- When introducing restriction enzyme sites into primers, refer to the table “Cleavage efficiency close to the termini of PCR fragments” located on www.thermoscientific.com/onebio to determine the number of extra bases required for efficient cleavage.

Estimation of primer melting temperature

For primers containing less than 25 nucleotides, the approx. melting temperature (Tm) can be calculated using the following equation:

$$Tm = 4 (G + C) + 2 (A + T),$$

where G, C, A, T represent the number of respective nucleotides in the primer.

If the primer contains more than 25 nucleotides we recommend using specialized computer programs e.g., REviewer™ (www.thermoscientific.com/reviewer) to account for interactions of adjacent bases, effect of salt concentration, etc.

COMPONENTS OF THE REACTION MIXTURE

Template DNA

Optimal amounts of template DNA for a 50 µL reaction volume are 0.01-1 ng for both plasmid and phage DNA, and 0.1-1 µg for genomic DNA. Higher amounts of template increase the risk of generation of non-specific PCR products. Lower amounts of template reduce the accuracy of the amplification.

All routine DNA purification methods can be used to prepare the template, e.g. Thermo Scientific GeneJET Genomic DNA Purification Kit (#K0721) or GeneJET™ Plasmid Miniprep Kit (#K0502). Trace amounts of certain agents used for DNA purification, such as phenol, EDTA and proteinase K, may inhibit DNA polymerase. Ethanol precipitation and repeated washes of the DNA pellet with 70% ethanol usually removes trace contaminants from DNA samples.

Primers

The recommended concentration range of the PCR primers is 0.1-1 µM. Excessive primer concentrations increase the probability of mispriming and generation of non-specific PCR products.

For degenerate primers and primers used for long PCR we recommend higher primer concentrations in the range of 0.3-1 µM.

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CYCLING PARAMETERS

Initial DNA denaturation and enzyme activation

It is essential to completely denature the template DNA at the beginning of PCR to ensure efficient utilization of the template during the first amplification cycle. If the GC content of the template is 50% or less, an initial 1-3 min denaturation at 95°C is sufficient.

Denaturation

A DNA denaturation time of 30 seconds per cycle at 95°C is normally sufficient. For GC-rich DNA templates, this step can be prolonged to 3-4 min.

Primer annealing

The annealing temperature should be 5°C lower than the melting temperature (Tm) of the primers. Annealing for 30 seconds is normally sufficient. If non-specific PCR products appear, the annealing temperature should be optimized stepwise in 1-2°C increments.

Extension

The optimal extension temperature for *Taq* DNA polymerase is 70-75°C. The recommended extension step is 1 min/kb at 72° for PCR products up to 2 kb. For larger products, the extension time should be prolonged by 1 min/kb.

Number of cycles

If less than 10 copies of the template are present in the reaction, about 40 cycles are required. For higher template amounts, 25-35 cycles are sufficient.

Final extension

After the last cycle, it is recommended to incubate the PCR mixture at 72°C for an additional 5-15 min to fill-in any possible incomplete reaction products. If the PCR product has to be cloned into a TA vector, e.g. using Thermo Scientific InsTAclone PCR Cloning Kit (#K1213), the final extension step may be prolonged to 30 min to ensure the highest efficiency of 3'-dA tailing of the PCR product. If the PCR product has to be used for cloning using Thermo Scientific CloneJET PCR Cloning Kit (#K1231), the final extension step can be omitted.

Troubleshooting

For troubleshooting please visit
www.thermoscientific.com/onebio

CERTIFICATE OF ANALYSIS

Endodeoxyribonuclease Assay

No detectable degradation of DNA was observed after incubation of 1.2 µg of pUC19 DNA with 10 µL of PCR Master Mix (2X) in 20 µL of reaction mixture for 4 hours at 37°C.

Exodeoxyribonuclease Assay

No detectable degradation of DNA was observed after incubation of 1 µg of lambda DNA/HindIII fragments with 25 µL of PCR Master Mix (2X) in 50 µL of reaction mixture for 4 hours at 37°C.

Ribonuclease Assay

No contaminating RNase activity was detected after incubation of 1 µg of [^{3}H]-RNA with 25 µL PCR Master Mix (2X) in 50 µL of reaction mixture for 4 hours at 37°C.

Functional Assay

PCR Master Mix (2X) was tested for amplification of 956 bp single copy gene from human genomic DNA.

Quality authorized by:  Jurgita Zilinskiene