

Product Information

2X PCR Master Mix, 200 reactions

C/No.	BIO-5180-200
Concentration	60U/ml <i>Taq</i> DNA polymerase (recombinant), 400µM dNTP mix, 3mM MgCl ₂
Packaging	4 x 1.25ml
Storage	-20°C Avoid frequent thawing and freezing.

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Description

2X PCR Master Mix is a premixed, ready to use solution which contains *Taq* DNA polymerase, dNTPs, MgCl₂ and reaction buffer for amplification of DNA templates by PCR. This pre-mixed formulation saves time and reduces contamination by reducing the number of pipetting steps required for usual PCR set up. The mix is optimized for efficient and reproducible PCR.

Source of *Taq* DNA polymerase

An *E.coli* strain that carries a cloned *pol* gene from *Thermus aquaticus*.

Unit Definition

One unit of the enzyme catalyzes the incorporation of 10nmol of deoxyribonucleotides into a polynucleotide fraction in 30min at 74°C.

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Applications

- DNA sequencing • DNA labeling • PCR for cloning
- PCR amplification of DNA fragments up to 5kb

2X PCR Master Mix Composition

Contains reaction buffer, 0.06U/µl of *Taq* DNA polymerase, 3mM MgCl₂ and 400µM of each dNTPs.

Remarks

- Half-life of this enzyme is >40mins @ 95°C.
- *Taq* DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.

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Quality Control

Nuclease Assay

No detectable contaminating endonuclease or exonuclease activity.

Functional Assay

PCR Master Mix was tested for amplification of 1500bp of single copy gene from *E. coli* strain.

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Protocol

The following protocol serves as a starting point and general guideline for any Polymerase Chain Reaction (PCR). Reaction conditions (incubation times and temperatures, concentration of *Taq* DNA polymerase, primers, MgCl₂, and template DNA) vary and need to be optimized. PCR reactions should be prepared in a DNA-free environment, dedicated pipette and aerosol resistant tips are recommended. Always keep the control and template DNA to be amplified isolated from other components.

Recommendations of Template DNA in a 50 µl reaction volume

DNA Type	Amount of DNA
Human genomic DNA	0.1 to 1 µg
Plasmid DNA	0.5 to 5 ng
Phage DNA	0.1 to 10 ng
<i>E.coli</i> genomic DNA	10 to 100 ng

1. Thaw 2X PCR Master Mix at room temperature. Keep the Master Mix on ice after thawing. Vortex briefly and spin down contents quickly.
2. Prepare the following reaction mix in a sterile, nuclease-free PCR tube on ice, overlay with 50µl of mineral or silicone oil.

For a 50µl reaction volume:		
Components	Volume (µl)	Final Conc.
2X PCR Master Mix	25	1X
Forward Primer (10µM)	0.5 – 5	0.1 – 1µM
Reverse Primer (10µM)	0.5 – 5	0.1 – 1µM
DNA template	1 – 5	See above
Nuclease-Free water	Top up to 50	N.A

3. Cap tubes and spin down contents briefly.
4. Place reactions in thermal cycler and incubate at 95°C to completely denature template DNA. Perform about 25 – 35 cycles of PCR amplification (repeat steps 2 to 4 for 25 - 35 cycles). Low amounts of starting template may require 40 cycles.

PCR Amplification as follows:

No.	Step	Temperature	Time
1	Initial Denaturation	95°C	1 – 5 min
2	Denaturation	95°C	0.5 – 1 min
3	Annealing	42 – 65°C	0.5 – 1 min
4	Extension	72 – 75°C	1 min/kb
5	Final Extension	72 – 75°C	5 – 15 min
6	Soak	*4°C	Several hours

*If thermal cycler has a refrigeration or “soak” cycle, cycling reaction can be programmed to end by holding the tubes at 4°C for several hours.

General guidelines for PCR amplifications

Initial denaturation

To ensure efficient utilization of the template during first amplification cycle, it is essential that the template is denatured completely. If GC content of the template is ≤ 50%, an initial 1 – 5 min denaturation at 95°C would be sufficient.

Denaturation

DNA denaturation time of 0.5min/cycle at 95°C is usually sufficient. For GC-rich templates, denaturation could be prolonged to 3 – 4 min.

Annealing

Annealing temperature should be 5°C lower than melting temperature ($T_m - 5^\circ\text{C}$) of primers. 0.5min/cycle is usually sufficient. If non-specific PCR products are observed, the temperature should be optimized stepwise in 1-2°C increments.

Extension

Optimal temperature for extension of *Taq* DNA Polymerase is between 70-75°C. Recommended extension step is 1min/kb at 72°C for PCR products.

Number of cycles

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

Final extension

After the last cycle, incubate PCR mixture at 72°C for an additional 5 – 15 min to allow any possible incomplete amplification to take place.