

Product Information

Taq II PCR Master Mix (2X), 20 reactions (SAMPLE)

| | |
|--------------------------------|---|
| C/No. | BIO-5181-20 |
| Lot No. | - |
| Expiry Date | - |
| Concentration | 60U/ml Taq II DNA polymerase (recombinant), 400µM dNTP mix, 3mM MgCl ₂ |
| Packaging Supplied with | 1 X 500µl |
| Storage | -20°C Avoid frequent thawing and freezing. |



Applications

- DNA sequencing • DNA labeling • PCR for cloning
- PCR amplification of DNA fragments up to 6kb
- Routine PCR

Taq II PCR Master Mix (2X) Composition

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

Remarks

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

Description

Taq II PCR Master Mix (2X) is a premixed, ready to use solution which contain Taq II 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 II 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 an acid-insoluble material in 30mins at 74°C by using activated salmon sperm DNA as a template / primer.

Quality Control

Nuclease Assay

No detectable contaminating endonuclease or exonuclease activity.

Functional Assay

Taq II PCR Master Mix (2X) was tested for amplification of a 3500bp of human genomic DNA.

Certified by:

Ng Xue Qi

Laboratory Officer

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 II 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 Taq II PCR Master Mix (2X) at room temperature.
Keep the tubes 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.

| For a 50µl reaction volume: | | |
|-----------------------------|--------------|-------------|
| Components | Volume (µl) | Final Conc. |
| Taq II PCR Master Mix(2X) | 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 25 - 35 cycles of PCR amplification by repeating steps 2 to 4 in the PCR reaction.
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 | 50- 68°C | 0.5 – 1 min |
| 4 | Extension | 72 - 75°C | *0.5 - 1 min/kb |
| 5 | Final Extension | 72 - 75°C | 5 – 15 min |
| 6 | Soak | 4°C | ∞ |

*For amplification of >3kb, it is recommended to use 1min/kb as extension rate.

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 mins 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 3-4mins.

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 II DNA Polymerase is between 70-75°C. Recommended extension step is 30sec/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 5-15mins to allow any possible incomplete amplification to take place.