

## Taq II DNA Polymerase (recombinant) - MgCl<sub>2</sub> separate

**Cat. No.:** BIO-5111-50U  
BIO-5111-500U  
BIO-5111-2500U

**Concentration:** 5U/μl

**Supplied with:** 10X Taq II buffer  
25mM MgCl<sub>2</sub>

**Storage:** -20°C.  
Avoid frequent thawing and freezing.

### Quality Control

**Nuclease Assay:** No detectable contaminating endonuclease or exonuclease activity.

**Functional Assay:** Taq II DNA Polymerase was tested for amplification of a 3500bp of human genomic DNA. 1500bp of single copy gene from E. coli strain.

### 1.0 DESCRIPTION

Taq II DNA Polymerase is a thermostable DNA polymerase that catalyzes a 5'→3" polymerase activity and a 5' flap endonuclease activity. In addition, Taq II DNA Polymerase exhibits deoxynucleotidyl transferase activity, which frequently results in the addition of extra adenines at the 3'-end of PCR products. Recombinant Taq II DNA Polymerase is ideal for standard PCR of templates up to 6kb.

### Source

An *E.coli* strain that carries a cloned 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.

### Applications

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

## Buffer Composition

### Storage buffer

20mM Tris-HCl	0.1mM EDTA
Stabilizer	1mM DTT
50% (v/v) Glycerol	100mM KCl

### 10X Taq II buffer

750mM Tris-HCl (pH 8.8 at 25°C)
200mM KCl
50mM (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>
0.5% (v/v) Nonidet P40

### 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.

## 2.0 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, MgCl<sub>2</sub> 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 DNA polymerase, 10X Taq II buffer and 25mM MgCl<sub>2</sub> 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.

**Table 1. For a 50µl reaction volume:**

Components	Volume (µl)	Final Concentration
Taq II DNA Polymerase (recombinant)	0.2 – 0.5	1 – 2.5U
Forward Primer (10µM)	0.5 – 5	0.1 – 1µM
Reverse Primer (10µM)	0.5 – 5	0.1 – 1µM
10X Taq II Buffer	5	1X
dNTP Mix (10mM of each)	1	0.2mM
25mM MgCl <sub>2</sub>	2-6	1 - 3mM
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.

**Table 2. PCR Amplification as follows:**

Step	Temperature	Time
Initial Denaturation	95°C	1 – 5 min
Denaturation	95°C	0.5 – 1 min
Annealing	42 - 65°C	0.5 – 1 min
Extension	72 - 75°C	*0.5 – 1 min/kb
Final Extension	72 - 75°C	5 – 15 min
Soak	4°C	∞

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

### 3.0 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 3-4mins.

#### 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 a 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.