

# Product Information

## Taq DNA Polymerase (recombinant) - MgCl<sub>2</sub> separate, 2500U

<b>C/No.</b>	BIO-5110-2500U
<b>Concentration</b>	5U/μl
<b>Packaging</b>	5 X 500U
<b>Supplied with</b>	10X Taq buffer 25mM MgCl <sub>2</sub>
<b>Storage</b>	-20°C Avoid frequent thawing and freezing.

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## Description

Taq DNA Polymerase is a thermostable DNA polymerase that catalyzes 5'→3' synthesis of DNA, has zero detectable 3'→5' exonuclease (proofreading) activity and possesses minimal 5'→3' exonuclease activity. In addition, Taq DNA Polymerase exhibits deoxynucleotidyl transferase activity, which frequently results in the addition of extra adenines at the 3'-end of PCR products. Recombinant Taq DNA Polymerase is ideal for standard PCR of templates 5kb or shorter.

## Source

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

## Buffer Composition

<b>Storage buffer</b>	
20mM Tris-HCl	0.1mM EDTA
0.5% (v/v) Nonidet P40	1mM DTT
0.5% (v/v) Tween 20	100mM KCl
50% (v/v) Glycerol	

### 10X Taq buffer without MgCl<sub>2</sub>

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 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

Taq DNA Polymerase 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, 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* DNA polymerase, 10X *Taq* 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.

For a 50µl reaction volume:		
Components	Volume (µl)	Final Conc.
<i>Taq</i> 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 <i>Taq</i> 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 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 – 5min 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 – 4min.

### 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 – 15min to allow any possible incomplete amplification to take place.