Product Information

Taq DNA Polymerase (recombinant) - $MgCl_2$ separate, 500U

C/No.	BIO-5110-500U
Concentration	5U/µl
Packaging	1 X 500U
Supplied with	10X *Taq* buffer 25mM $MgCl_2$
Storage	-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.

Applications

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

Buffer Composition

Storage buffer

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

10X *Taq* buffer without $MgCl_2$

750mM Tris-HCl (pH 8.8 at 25°C)
200mM KCl
50mM $(NH_4)_2SO_4$
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.

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.

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, $MgCl_2$, 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
E.coli genomic DNA	10 to 100 ng

1. Thaw *Taq* DNA polymerase, 10X *Taq* buffer and 25mM $MgCl_2$ 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 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* Buffer	5	1X
dNTP Mix (10mM of each)	1	0.2mM
25mM $MgCl_2$	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°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.