Taq II DNA Polymerase (recombinant) - $MgCl_2$ separate

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

Concentration: 5U/µl

Supplied with: 10X Taq II buffer
25mM $MgCl_2$

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
Stabilizer
50% (v/v) Glycerol
0.1mM EDTA
1mM DTT
100mM KCl

10X *Taq* II buffer
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 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, $MgCl_2$ 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 II DNA polymerase, 10X Taq II 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.

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 $MgCl_2$	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 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°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.