

Taq DNA Polymerase

REF: EG15109S

Storage Condition

-20°C

Components

Component	Amount
Taq DNA Polymerase (5 U/μl)	200 μl
10× Taq Reaction Buffer	5×1 ml

Description

Taq DNA polymerase is a recombinant form of *Thermus aquaticus* DNA polymerase. It is a single polypeptide chain with a molecular weight of approximately 94 kDa. It exhibits 5'→3' polymerase activity and 5'→3' exonuclease activity but lacks 3'→5' exonuclease activity. PCR products amplified with Taq DNA Polymerase have a 3'-terminal adenosine (A), and therefore PCR products can be used directly for cloning into T-vectors (T/A cloning).

Definition of Activity Unit

One unit is defined as the amount of enzyme that can incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 74°C.

Quality Control Assays

Protein Purity

The enzyme is ≥95% pure as determined by SDS-PAGE analysis using Coomassie Blue staining.

Endonuclease Activity

A 50 μl reaction containing 200 ng of supercoiled plasmid and 5 U of Taq DNA Polymerase incubated for 4 hours at 37°C results in <10% conversion to the nicked or linearized form as determined by agarose gel electrophoresis.

Non-specific Nuclease Activity

A 50 μl reaction containing 15 ng of dsDNA fragments and 5 U of Taq DNA Polymerase incubated for 16 hours at 37°C results in no detectable degradation of the dsDNA fragments as determined by agarose gel electrophoresis.

Residual Host DNA

The product was tested by TaqMan qPCR with primers specific for the *E.coli* 16S rDNA, and the results show that the *E.coli* genome residues less than 1 copy.

Protocol

1. Recommended PCR Reaction System

Reagent	Amount	Final Concentration
Taq DNA Polymerase (5 U/μl)	0.25 μl	1.25 U/50 μl
10× Taq Reaction Buffer	5 μl	1×
dNTP (10 mM)	1 μl	0.2 mM
Forward Primer (10 μM) ^a	1 μl	0.2 μM
Reverse Primer (10 μM) ^a	1 μl	0.2 μM
Template DNA ^b	x μl	
ddH ₂ O	To 50 μl	

a. Recommended final concentration for primers is 0.2 μM. Adjustments can be made in the range of 0.1~1 μM. For general amplification of DNA fragments, 18~25 bp and with a GC content of 40%~60% primers are suitable.

b. Optimal reaction concentrations may vary for different templates. For a 50 μl reaction system, the recommended template amounts are approximately 10~400 ng for genomic DNA and 10 pg~20 ng for plasmid or viral DNA.

2. PCR Conditions

Step	Temperature	Time
Initial Denaturation ^a	94°C	3~5 min
Denaturation	94°C	30 s
Annealing	55~65°C	30 s
Extension	72°C	30~60 s/kb
Final Extension	72°C	5 min

30~35 Cycles

a. The initial denaturation condition is suitable for most amplification reactions. For some complex templates, such as bacteria and yeast colon, the initial denaturation time can be extended to 10 minutes to improve denaturation efficiency.

Note: If yeast cell is used as the PCR template, it is recommended that the target fragment length does not exceed 2.5 kb. If it exceeds 2.5 kb, it is advisable to use the lysed cells as template.