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Taq DNA Polymerase, recombinant

Catalog Number: D006-1, D006-2

Table 1. Kit Components and Storage

Kit Component	D006-1 (1,000 units)	D006-2 (5,000 units)	Storage	Stability
Taq DNA Polymerase (5 U/µL)	200 µL	1000 µL	-20 °C, avoid repeated freeze-thaw	The product is stable for one year when stored as directed.
10× Reaction Buffer	2×1.5 mL	15 mL		

Product Description

Taq DNA polymerase is purified from *E. coli.* expressing a cloned *Thermus aquaticus* DNA polymerase gene. *Taq* DNA Polymerase is a thermostable enzyme that synthesizes DNA from single-stranded templates in the presence of dNTPs and a primer. The enzyme consists of a single polypeptide with a molecular weight of 94 kDa. It has a 5'→3' DNA polymerase activity and a 5'→3' exonuclease activity. In PCR, *Taq* DNA polymerase has an extension speed at 1-2 kb/min.

The enzyme is availablein 1,000 and 5,000 unit sizes at a concentration of 5 U/µL. The enzyme is supplied with a 10× Reaction Buffer.

Special Features

- Robust performance
- Leaves 3'A overhang
- Stable at storage temperature

Applications

- General PCR.
- Genomic analysis.
- TA cloning.

Product Specifications

- **Storage Buffer:** 20 mM Tris-HCl (pH 8.0 at 25°C), 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 0.5% (v/v) NP-40, and 50% (v/v) glycerol.
- **Unit Definition:** One unit is defined as the amount of enzyme required to incorporate 10 nmoles of deoxyribonucleotide into DNA in 30 min at 74°C.
- **Unit Assay Conditions:** 25 mM TAPS (pH 9.3), 50 mM KCl, 2 mM $MgCl_2$, 1 mM DTT, 0.5 mg/ml activated salmon sperm DNA, 0.2 mM dATP, dCTP, dGTP, dTTP.
- **10× Reaction Buffer:** 200 mM Tris-HCl (pH 8.8), 100 mM KCl, 25 mM $MgCl_2$, and other components.

General Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation times and temperatures, concentration of *Taq* DNA polymerase, primers, Mg^{2+}, and template DNA) vary and need to be optimized. Critical parameters and troubleshooting information are documented in reference 1.

Assemble PCR reactions in a nuclease-free environment. Use of “clean” dedicated pipettes and aerosol resistant barrier tips are recommended.

1. Thaw template DNA and all reagents on ice. Mix each solution by vortexing, and centrifuge briefly to collect residual liquid from the sides of the tubes. (End user supplies Primers, and dNTP.)
2. Prepare the following reaction mixture in a PCR tube on ice:

Component	Volume	Final Concentration
Template DNA	x µL	1-500 ng
10× Reaction Buffer	5 µL	1×
10 mM dNTP Mix	1 µL	0.2 mM each
Taq DNA Polymerase (5 U/µL)	0.2 µL	1-2.5 U/rxn
Forward Primer (10 µM)	2.5 µL	500 nM
Reverse Primer (10 µM)	2.5 µL	500 nM
Nuclease-free H_2O	to 50 µL	-

3. Mix, and then briefly centrifuge the contents.
4. Program the thermal cycler for PCR amplification.

Steps	Temperature	Duration	Cycle
Initial Denaturation	94°C	3 min	1
Denaturation	94°C	45 sec	25-35
Annealing*	55°C (depending on the primer T_m)	30 sec	
Extension	72°C	1 kb/min	
Final Extension	72°C	10 min	1
Holding	4°C	-	1

Note: The thermal cycling program listed above is optimized for primers with an annealing temperature at 55°C.

5. Analyze the amplification products by agarose gel electrophoresis.

Reference

Innis, M.A., Gelfand, D.H., Sninsky, J.J., and White, eds. (1990) PCR Protocols: A Guide to Methods and Applications, Academic Press, San Diego, CA.