— PROTOCOL —

# *Taq* DNA Polymerase

| Catalog Number | Unit Size | Reactions |
|---|---|---|
| MB101-0500 | 500 units | Conc. 5 units/µl |

**Storage** : Store at -20°C

## Description

*Taq* DNA Polymerase is purified from E. coli. expressing a Thermus aquaticus DNA polymerase gene. This enzyme has a 5' → 3' DNA polymerase and a 5' → 3' exonuclease activity but lacks a 3' → 5' exonuclease activity. The enzyme consists of a single polypeptide with a molecular weight of approximately 94 kDa. *Taq* DNA polymerase is heat-stable and will synthesize DNA at elevated temperatures from single-stranded templates in the presence of a primer.

*Taq* Polymerase is recommended for use in routine PCR reactions. The buffer system is optimized for high specificity and guarantees minimal by-product formation. We supplied *Taq* Polymerase with appropriate buffers. Usually 1-1.5 u of *Taq* DNA Polymerase are used in 50 µl of reaction mix. Higher *Taq* DNA Polymerase concentrations may cause synthesis of nonspecific products. However, if inhibitors are present in the reaction mix (e.g., if the template DNA used is not highly purified), higher amounts of *Taq* DNA Polymerase (2-3 u) may be necessary to obtain a better yield of amplification products.

## Storage Buffer

The enzyme is supplied in a storage buffer consisting of 20 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 50% (v/v) glycerol, and 1% Triton X-100.

## Kit content

| | |
|---|---|
| *Taq* DNA Polymerase (5 units/ul) | 500 units |
| 10X PCR buffer | 1.25 ml X 2 vials |

## Unit Definition

One unit is defined as the amount of enzyme that will catalyze the incorporation of 10 nmol of dNTP into acid-insoluble form in 30 min at 74°C in a reaction containing 25 mM TAPS (tris-[hydroxymethyl]-methyl-amino-propane-sulfonic acid, sodium salt), pH 9.3 at 25°C, 50 mM KCl, 2 mM MgCl2, 1 mM β-mercaptoethanol, 0.2 mM dATP, dGTP, and dTTP, 0.1 µM [α-32P] dCTP, and activated salmon sperm DNA.

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## Standard PCR with *Taq* DNA Polymerase

1. For each 50 µl reaction, assemble the following in a 0.5 ml PCR tube on ice just prior to use:

| Volume | Final Conc. | |
|---|---|---|
| add to 50µl | - | PCR Grade Water |
| 1 µl | 200 µM | dNTP Mix (10 mM each dATP, dCTP, dGTP |
| 1 µl | 0.1-0.2 µM | Forward primer, 5-10 µM |
| 1 µl | 0.1-0.2 µM | Reverse primer, 5-10 µM |
| 5 µl | 2 mM $MgCl_2$ | 10X PCR Buffer |
| 0.25 µl | 1.25 units | Taq DNA Polymerase (5 units/ul) |
| xul | 10 ng | DNA template |
| 50 µl | | Total volume |

2. Mix gently. If necessary, centrifuge briefly. Cap tubes and place in thermal cycler.
3. Process in thermal cycler for 25-35 cycles as follows:

| | | |
|---|---|---|
| Initial Denaturation | 2-5 minutes at 94°C | |
| Denaturation | 20-40 seconds | |
| Annealing | 1 min at the proper annealing temperature | 30 cycles |
| Extention | 2 min at 72°C | |
| Final extension | 5 min at 72°C | |

**Note:** Optimal conditions for amplification will vary depending on the primers and thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.