

# Taq DNA Polymerase

Cat. No. HBB101-0500 Size: 500 x 2 units
Cat. No. HBB101-0050 Size: 50 units

Store at -20°C

#### Description

Taq DNA Polymerase is a simple, specificity PCR reaction mixture. Simply add primers, template, dNTPs, and PCR grade water, the reagent will execute primer extensions and other molecular biology applications. Taq DNA Polymerase contains two components, include the Taq DNA polymerase and 10X PCR buffer. The Taq DNA Polymerase is purified from E coll, expressing a Thermus aquaticus DNA polymerase gene. This enzyme has a  $5' \rightarrow 3'$  DNA polymerase and a  $5' \rightarrow 3'$  exonuclease activity but lacks a  $3' \rightarrow 5'$  exonuclease activity. The enzyme consists of a single polypeptide with a molecular weight of approximately 94 kilo Dalton. Taq DNA polymerase is heat-stable and will synthesize DNA at elevated temperatures from single-stranded templates in the presence of a primer. The Taq DNA Polymerase is recommended for use in routine PCR reactions. The 10X PCR buffer is optimized for high specificity and guarantees minimal by product formation. Usually 1-1.5 unit of Taq DNA polymerase is used in 50  $\mu$ 1 of reaction mix. Higher Taq DNA polymerase concentrations may cause synthesis of nonspecific products. However, if inhibitors exist in the reaction mixture (e.g., if the template DNA used is not highly purified), higher amounts of Taq DNA polymerase (2-3 units) may be necessary to obtain a better yield of amplification products.

#### **Features**

- > Ideal for routine PCR applications.
- Kit includes optimized reagents and enables flexible experiments.

#### **Applications**

➤ Microarray analysis.
➤ Colony PCR.
➤ PCR Amplification

#### Kit contents

Contents	HBB101-0500	HBB101-0050
Taq DNA Polymerase (5 units/μl)	500 units X 2 vials	50 units X 1 vial
10X PCR buffer	1.25 ml X 4 vials	250 µl X 1 vial

### **Quality Control**

The quality of the Taq DNA Polymerase is tested on a lot-to-lot basis to ensure consistent product quality

### **Required Materials**

➤ 10 mM dNTP mix ➤ PCR microcentrifuge tubes

PCR grade water
PCR thermal cycler

# Storage Buffer

The enzyme is supplied in a storage buffer consisting of 50 mM Tris-HCl (pH 8.0), 100 mM NaCl, 0.1 mM EDTA, 5 mM DTT, 50% glycerol and 1.0% Triton®X-100.

#### **Unit Definition**

One unit is defined as the enzyme catalyzes the incorporation of 10 nanomoles of deoxyribonucleotides into a polynucleotide fraction in 30 minutes at 70°C.

#### Taq DNA Polymerase Protocol

1. For each 50 µl reaction, assemble the following in a 0.2 ml PCR tube on ice just prior to use:

Components	50 µl Reaction	Final Concentration.
10 mM dNTP Mix	1 μΙ	200 μΜ
Forward primer, 5-10 µM	1 µl	0.1-0.2 µM
Reverse primer, 5-10 µM	1 µl	0.1-0.2 µM
10X PCR Buffer	5 µI	2 mM MgCl <sub>2</sub>
Template DNA	Variable	10 ng
Taq DNA Polymerase (5 units/μl)	0.25 µl	1.25 units
Add PCR grade water to	50	-

- 2. Mix gently. If necessary, centrifuge briefly. Cap tubes and place in thermal cycler.
- 3. Perform PCR reactions using the following cycling program:

Program	Temperature	Time
Initial Denaturation	94°C	2-5 minutes
Denaturation	94°C	20-40 seconds
Annealing	at the proper annealing temperature	1 minute
Extension	72°C	2 minutes
Final extension	72°C	5 minutes

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.

- 4. Analyze the amplified DNA using agarose gel electrophoresis.
- 5. Use the PCR reaction products for down-stream applications, or store it at -20°C.

# Troubleshooting

Problem	Cause	Solution
No amplicon	Error in set up	Repeat the experiment, checking all reagents are added in correct volumes. Use master mix to ensure all components added correctly.
	Primer design error	Check primer parameters on primer design software. Redesign primers.
Low yield	Insufficient template	Check the concentration of template, if necessary increase the template concentration.
	GC rich or difficult template	Use GDP-HiFi DNA Polymerase (Cat. No. MB601-0100) with high performance buffer.

Problem	Cause	Solution
Non-specific amplification – smeared product	Template degraded	Minimize freeze thawing of DNA. Run template on agarose gel to check integrity.
Wrong size band amplified	Contamination	Check no template control for bands.
	Wrong primers or template added	Check primers and template vials have been labeled correctly and selected correctly during setup.

# Caution

- > Check buffers before use for precipitation.
- > Aliquot reagents to avoid contamination and repeated freeze-thaw cycles.
- > During operation, always wear a lab coat, disposable gloves, and protective equipment.
- > All products are for research use only.