Taq DNA Polymerase (recombinant)

For sensitive and reproducible PCR and RT-PCR

Product Description

Taq DNA Polymerase is purified from *E. coli.* expressing a cloned Thermus aquaticus DNA polymerase gene. This enzyme has both 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.

Storage Conditions

Taq DNA Polymerase and Buffers should be stored immediately upon receipt at –20°C. It can be stored for at least 12 months without showing any reduction in PCR performance.

Unit Definition

One unit incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Quality Control

This product has passed the following quality control assays: functional absence of double- and single- stranded endonuclease activity; >90% homogeneous by SDS gel electrophoresis; functional absence of contaminating 5'- and 3'-exonuclease activity. Each lot of Taq DNA Polymerase is assayed for amplification of b-actin gene from 10 ng of human genomic DNA isolated from the K562 cells.

Storage Buffer

20 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 50% (v/v) glycerol, Stabilizers

10X PCR Buffer

100 mM Tris-HCl (pH 8.4), 500 mM KCl, 1% Triton x-100, 15mmol MgCl2

Kit Contents

Cat.No.	TQ2100-00	TQ2100-01	TQ2100-02	TQ2100-03
Taq DNA Polymerase	500 units	2 x 500 units	10 x 500 units	20 x 500 units
10 x PCR Buffer	1.5 ml	2 x 1.5 ml	10 x 1.5 ml	30 ml

Basic PCR 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, MgCl2, and template DNA) vary and need to be optimized.

1. Add the following components to a sterile 0.2 ml tube sitting on ice:

Components	**Volume**	**Final Concentration**
10X PCR buffer	10 ul	1X
10 mM dNTP mixture	2ul	0.2 mM each
Primer mix (10 uM each)	5ul	0.5 uM each
Template DNA	1–20ul	n/a
Taq DNA Polymerase(5U/ul)	0.2–0.5ul	1.0–2.5 units
Autoclaved distilled water to	100ul	

   We recommend preparing a master mix for multiple reactions, to minimize reagent loss and enable accurate pipetting.

2. Mix well and (optional)overlay with 50ul of mineral oil.
3. Cap tubes and spin briefly to collect liquid to the bottom.
4. Set program as follows and place tubes in a thermal cycler.
   Denature the template at 94°C for 3min;
   (**25-35cycles:** Denature 94°C for 45 s, Anneal 55°C for 30 s, Extend 72°C for 1 min 30 s);
   Additional 10 min at 72°C, then 4°C.
5. **Analyze the amplification products** by agarose gel (1.0-3.0%) electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.