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Taq DNA Polymerase, recombinant

Cat. nos.	Size	Conc. 5 U/µL
10342-053	100 units	**Store at –30°C to –10°C**
10342-020	500 units	
10342-046	1,500 units	
10342-178	5,000 units	

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Description

Taq DNA Polymerase is purified from *E. coli.* expressing a cloned *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 synthesizes DNA at elevated temperatures from single-stranded templates in the presence of a primer.

Contents

	Kit Size			
Component	**100 U**	**500 U**	**1,500 U**	**5,000 U**
Taq DNA Polymerase	20 µL	100 µL	300 µL	1000 µL
10X PCR Buffer, Minus Mg^{++}	1.25 mL	2.5 mL	7.5 mL	20 mL
50 mM Magnesium Chloride	1 mL	1 mL	3 mL	10 mL

Storage Buffer

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

Product Use: For research use only.
Not intended for any animal or human therapeutic or diagnostic use.

10X PCR Buffer

200 mM Tris-HCl (pH 8.4), 500 mM KCl.

The supplied PCR Buffer is a 10X concentrate and should be diluted for use.

Unit Definition

One unit incorporates 10 nmol of deoxyribonucleotide into DNA in 30 minutes at 74°C.

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, $MgCl_2$, and template DNA) vary and need to be optimized.

Critical parameters and troubleshooting information are documented in Innis et al., 1988. Assemble PCR reactions in a DNA-free environment. We recommend the use of clean dedicated automatic pipettors and aerosol resistant barrier tips. **Always** keep the control DNA and other templates to be amplified isolated from the other components.

1. Add the following components to a sterile 0.5-ml microcentrifuge tube sitting on ice:

Components	**Volume**	**Final Concentration**
10X PCR buffer minus Mg^{++}	10 µL	1X
10 mM dNTP mixture	2 µL	0.2 mM each
50 mM $MgCl_2$	3 µL	1.5 mM
Primer mix (10 µM each)	5 µL	0.5 µM each
Template DNA	1–20 µL	n/a
Taq DNA Polymerase (5 U/µL)	0.2–0.5 µL	1.0–2.5 units
Autoclaved distilled water	to 100 µL	n/a

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

2. Mix contents of tube and overlay with 50 µL of mineral or silicone oil.
3. Cap tubes and centrifuge briefly to collect the contents to the bottom.

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4. Incubate tubes in a thermal cycler at 94°C for 3 minutes to completely denature the template.
5. Perform 25–35 cycles of PCR amplification as follows:
   Denature 94°C for 45 seconds
   Anneal 55°C for 30 seconds
   Extend 72°C for 1 min 30 seconds
6. Incubate for an additional 10 minutes at 72°C and maintain the reaction at 4°C. The samples can be stored at –20°C until use.
7. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.

"Hot Start" Protocol

In the "hot-start" method, the addition of *Taq* DNA Polymerase is withheld until the reaction temperature is at 80°C, to ensure high specificity of the products being synthesized.

1. Add all components as in the Basic PCR Protocol, except for the *Taq* DNA Polymerase.
2. Mix contents of tube and overlay with 50 µL of mineral or silicone oil.
3. Cap tubes and centrifuge briefly to collect the contents to the bottom.
4. Incubate tubes in a thermal cycler at 94°C for 3 minutes to completely denature the template.
5. After denaturation at 94°C, maintain the reaction at 80°C.
6. Add 0.2–0.5 µL of *Taq* DNA Polymerase (1.0–2.5 U) to each reaction. Be certain to add the enzyme beneath the layer of oil.
7. Continue with 25–35 cycles of denaturation, annealing and extension as in the Basic PCR Protocol.

Reference

Innis, M.A., Myambo, K.B., Gelfand, D.H. and Brow, M.A.D. (1988) *Proc. Natl. Acad. Sci. USA 85*, 9436.

Product Qualification and SDS

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