Taq DNA Polymerase

Cat. Nos.
18038-018 (100 units) **18038-042 (500 units)**
18038-067 (1500 units) **18038-240 (5000 units)**

Conc: 5 U/µl **Store at –20°C (non-frost-free)**

Licensed for PCR

Description

Taq DNA Polymerase is isolated from *Thermus aquaticus* YT1. 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.

	Kit Size			
Component	**100 U**	**500 U**	**1500 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

10X PCR Buffer

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

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

Unit Definition

One unit incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes 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

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 reference 1. PCR reactions should be assembled in a DNA-free environment. Use of "clean" dedicated automatic pipettors and aerosol resistant barrier tips are recommended. **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 Conc.**
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.

Basic PCR Protocol, continued

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 s
   Anneal 55°C for 30 s
   Extend 72°C for 1 min 30 s
6. Incubate for an additional 10 min 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.

Quality Control

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

Reference

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

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