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Product Information

Taq* DNA Polymerase, from *Thermus aquaticus
recombinant, expressed in *Escherichia coli*

Catalog Number **D1806**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Introduction
Taq DNA Polymerase is a thermostable enzyme derived from the thermophilic bacterium *Thermus aquaticus*. The enzyme is in a recombinant form, expressed in *E. coli*. It is able to withstand repeated heating to 95 °C without significant loss of activity. The enzyme is approximately 94 kDa by SDS-PAGE with no detectable endonuclease or exonuclease activity. It has 5′→3′ DNA polymerase activity and 5′→3′ exonuclease activity. Each lot of *Taq* DNA Polymerase is tested for PCR amplification and double-stranded sequencing. The enzyme is supplied at 5 units/µL and comes with an optimized 10× reaction buffer.

Unit Definition: One unit incorporates 10 nmol of total deoxyribonucleoside triphosphates into acid precipitable DNA in 30 minutes at 74 °C.

Reagents Provided
- *Taq* DNA Polymerase, Catalog No. D6677
  5 units/µL in 20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, stabilizers, 50% glycerol
- 10× PCR Buffer, Catalog No. P2192
  100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$ and 0.01% gelatin

Reagents required, but not provided
- 10 mM dATP, Catalog No. D6920
- 10 mM dCTP, Catalog No. D7045
- 10 mM dGTP, Catalog No. D7170
- 10 mM TTP, Catalog No. T7791
  or, in place of individual nucleotides
- Deoxynucleotide Mix, Catalog No. D7295 containing 10 mM dATP, 10 mM dCTP, 10 mM dGTP, 10 mM TTP
- Water, PCR Reagent, Catalog No. W1754
- Mineral Oil, Catalog No. M8662 (optional)
- Thermal cycler
- Primers
- DNA to be amplified
- 0.2 ml or 0.5 ml Thin-Walled PCR Tubes, Catalog Nos. P3114 and P3364

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage
Store at –20 °C

Amplification Procedure
The optimal conditions for the concentration of *Taq* DNA polymerase, template DNA, primers, and $MgCl_2$ will depend on the system being utilized. It may be necessary to determine the optimal conditions for each individual component. This is especially true for the *Taq* DNA polymerase, cycling parameters, and the $MgCl_2$ concentration. It is recommended the enzyme and the $MgCl_2$ be titrated to determine the optimal efficiency.

1. Add the following reagents to a 0.2 or 0.5 ml microcentrifuge tube in the following order:

Amount	Component	Final Concentration
w µL	Water	
5 µL	10× PCR Buffer	1×
1 µL*	10 mM dATP	200 µM
1 µL*	10 mM dCTP	200 µM
1 µL*	10 mM dGTP	200 µM
1 µL*	10 mM TTP	200 µM
x µL	Forward primer (typically 15-30 bases in length)	0.1-0.5 µM
y µL	Reverse primer (typically 15-30 bases in length)	0.1-0.5 µM
0.5 µL	*Taq* DNA Polymerase	0.05 units/µL
z µL	Template DNA (typically 10 ng)	200 pg/µL
50 µL	Total reaction volume	

*Note: The individual nucleotides (4 µL total) may be substituted by 1 µL of Deoxynucleotide Mix, Catalog No. D7295.

2. Mix gently by vortex and briefly centrifuge to collect all components to the bottom of the tube.

3. Add 50 µL of mineral oil to the top of each tube to prevent evaporation if using a thermal cycler without a heated lid.

4. The amplification parameters will vary depending on the primers and the thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

   Typical cycling parameters:

25-30 cycles of amplification are recommended		
Denature template	94 °C	1 min
Anneal primers	55 °C	2 min
Extension	72 °C	3 min

5. The amplified DNA can be evaluated by agarose gel electrophoresis and subsequent ethidium bromide staining. Mineral oil overlay may be removed by a single chloroform extraction (1:1), recovering the aqueous phase.

References

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3. Innis, M.A., et al. (Eds.) *PCR Strategies*, Academic Press, New York (1995).
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10. Sambrook, J, et al. *Molecular Cloning: A Laboratory Manual,* Third Edition, Cold Spring Harbor Laboratory Press, New York (2000) . Catalog No. M8265.
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Related Products

Reagents

- Lambda DNA *Hind* III Digest, Catalog No. D9780
- Enhanced Avian HS RT-PCR kits, Catalog No HSRT100 (100 reactions).

Equipment

- PCR Multiwell Plate, 96-well, Catalog No. Z374903
- PCR Multiwell Plate, 384-well, Catalog No. Z374911
- PCR Microtubes, 0.2 ml, attached caps, Catalog No. Z374873
- PCR Microtubes, 0.2 ml strip tubes with strip caps, Catalog No. Z374962
- Sealing accessory for PCR vessels, Micro Mats, Catalog No. Z374938
- PCR Workstation, 120V, Catalog No. Z376213
- PCR Workstation, 240V, Catalog No. Z376221

Trademarks

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