

Product Information

Taq DNA Polymerase, from *Thermus aquaticus* recombinant, expressed in *Escherichia coli*
with 10x reaction buffer without MgCl₂

Catalog Number **D4545**

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 ~94 kDa by SDS-PAGE with no detectable contaminating 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 10x reaction buffer without magnesium chloride. A separate tube of magnesium chloride is included to allow its titration to optimal efficiency.

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
- 10x PCR Buffer without MgCl₂, Catalog No. P2317,
100 mM Tris-HCl, pH 8.3, 500 mM KCl
- Magnesium chloride solution, 25 mM,
Catalog No. M8787

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
- Deoxynucleotide Mix, Catalog No. D7295
containing 10 mM dATP, dCTP, dGTP, TTP
- Water, PCR Reagent, Catalog No. W1754
- Mineral Oil, Catalog No. M8662 (optional)
- Thermal cycler
- Primers
- DNA to be amplified

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 MgCl₂ 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 MgCl₂ concentration. It is recommended the enzyme and the MgCl₂ be titrated to determine the optimal efficiency. PCR Optimization Kit, Catalog No. OPT2, contains a variety of buffers and adjuncts for optimizing the specificity, fidelity and yield of a PCR product.

1. Add the following reagents to a 200 or 500 μL microcentrifuge tube in the following order:

Amount	Component	Final Concentration
- μL	Water	-
5 μL	10x PCR Buffer	1x
- μL	25 mM MgCl ₂	Typically 1.5-3.5 mM
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
- μL	Forward primer (typically 15-30 bases in length)	0.1-0.5 μM
- μL	Reverse primer (typically 15-30 bases in length)	0.1-0.5 μM
0.5 μL	Taq DNA Polymerase	0.05 units/μL
- μL	Template DNA (typically 10 ng)	200 pg/μL
50 μL	Final volume	

*Note: The individual nucleotides (4 μ L total) may be substituted by 1 μ L 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 100 μ L 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:

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

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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