

Taq DNA Polymerase

Recombinant

Taq DNA Polymerase (*Thermus aquaticus*)

Thermostable DNA polymerase, suitable for applications requiring high temperature synthesis of DNA.

Cat. No.	Size
E2500-01	200 units
E2500-04	500 units
E2500-02	1000 units
E2500-03	5000 units

Unit Definition:

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmoles of dNTP into acid-insoluble material in 30 min at 74°C. The reaction conditions are: 50 mM Tris-HCl (pH 9.0 at 25°C), 50 mM NaCl, 5 mM MgCl₂, 200 µM each of dATP, dCTP, dGTP, dTTP (a mix of unlabeled and [³H]dTTP), 10 µg activated calf thymus DNA and 0.1 mg/ml BSA in a final volume of 50 µl.

Storage Conditions: Store at -20°C



PCR amplification using EURx Taq DNA Polymerase. Lane M: molecular size marker- Perfect 1 kb DNA Ladder. Lanes 0.5 to 9.3 kb: PCR amplification reactions, using Pol Buffer B with 0.2 mM dNTPs and 1.25 U EURx Taq DNA Polymerase in 50 µl reaction volume.

Description:

- Taq DNA Polymerase is a thermostable enzyme of approximately 94 kDa from *Thermus aquaticus*.
- Ultra pure, recombinant protein.
- The enzyme replicates DNA in a broad range of temperatures with optimal activity at 72°C – 74°C. It exhibits a half-life of 40 min at 95°C (1,2).
- Catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions.
- Maintains the 5'→3' exonuclease activity.
- Lacks the 3'→5' exonuclease activity ("proofreading"). Cannot extend a DNA molecule upon incorporation of a mismatching nucleotide.
- Adds extra A at the 3' ends (approx. 5 % of amplicons). 95 % of amplicons are blunt ended molecules.
- Taq DNA Polymerase is recommended for use in PCR and primer extension reactions at elevated temperatures to obtain a wide range of DNA products from several hundred bp to 10 kb.

Storage Buffer:

20 mM Tris-HCl (pH 8.0 at 22°C), 100 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 50 % glycerol.

10 x Reaction Buffer:

10 x Pol Buffer A (optimization buffer without MgCl₂):

The buffer allows to optimize MgCl₂ concentration.

10 x Pol Buffer B (general application, up to 10 kb):

The buffer contains 15 mM MgCl₂ and is optimized for use with 0.2 mM of each dNTP.

10 x Pol Buffer C (coloured):

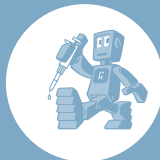
Similar to 10 x buffer B, but additionally enriched with two gel tracking dyes and a gel loading reagent. The buffer enables direct loading of PCR products to agarose gels.

Quality Control:

All preparations are assayed for contaminating endonuclease, 3'-exonuclease, and nonspecific single- and double-stranded DNase activities. Typical preparations are greater than 95 % pure, as judged by SDS polyacrylamide gel electrophoresis.

References:

1. Chien, A., Edgar, D.B. and Trela, J.M. (1976) *J. Bacteriol.* 127, 1550.
2. Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I. (1980) *Biokhimiya* 45, 644.



Taq DNA Polymerase PCR PROTOCOL

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
10 x Pol Buffer A or 10 x Pol Buffer B or 10 x Pol Buffer C	5 µl	1x
25 mM MgCl ₂	2-10 µl when using 10 x Pol Buffer A or 0 - 7 µl when using 10 x Pol Buffers B or C	1 - 5mM 1.5 - 5 mM
dNTP mix (5mM each)	2 µl	0.2 mM of each dNTP
Upstream primer	Variable	0.1-0.5 µM
Downstream primer	Variable	0.1-0.5 µM
Taq DNA Polymerase, 5 U/µl	0.25µl	1.25 U
Template DNA	Variable	0.5 µg/50 µl
Sterile double- distilled water	Variable	-
Total volume	50 µl	-

General formula for calculating total gene / genome copy numbers from the total amount of template DNA:

$$\text{Template DNA copy number [molecules]} =$$

$$\frac{\text{DNA amount [ng]} \cdot 6.022 \times 10^{23} [\text{molecules mol}^{-1}]}{\text{Genomic DNA length [kb]} \cdot 616 [\text{g mol}^{-1} \text{bp}^{-1}]} \cdot \frac{10^{-3} [\text{kb bp}^{-1}]}{10^9 [\text{ng g}^{-1}]}$$

Optimum: 10⁴ template DNA copies
Maximum: 0.5 µg template DNA or less

Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	94-95°C	2-5 min	1
Denaturation	94-95°C	15-60 s	25-35
Annealing	50-68°C	30-60 s	
Extension	72°C	1 min/1 kb	
Final Extension	72°C	7 min	1
Cooling	4°C	Indefinite	1

Notes:

- First Law of PCR:** PCR is a sort of homeopathic process, which works best, when all components are present only in homeopathic doses.
- Concentration Differences:** Completely thaw and mix thoroughly all components of PCR reaction before use to avoid localized differences in salt concentration. It is especially important for magnesium solutions, because they form concentration gradients when frozen.
- On Ice:** Prepare reaction mixes on ice, mix well
- Preheat Cycler:** Place reactions in a thermal cycler that has been preheated to 94-95°C.
- MgCl₂:** Standard concentration of MgCl₂ in PCR reaction is 1.5 mM (as provided in the 1 x Pol Buffers B or C) when using 0.2 mM dNTP (each). In most cases this concentration will produce satisfactory results. However, in some cases, reaction may be improved by determining optimal concentration of MgCl₂.
Adding 1 µl of a 25 mM MgCl₂ solution to a total reaction volume of 50 µl will add 25 nmol MgCl₂ and thus increase total MgCl₂ reaction concentration in 0.5 mM.
Increasing the MgCl₂ concentration enhances PCR yield but decreases reaction specificity (amplification of more bands, but also of non-specific bands). Decreasing the MgCl₂ concentration decreases PCR yield but enhances reaction specificity (less bands, but specific PCR products).
- Colored Loading Buffer:** The 10 x Pol Buffer C allows PCR reactions to be directly loaded onto an agarose gel without prior addition of a gel loading buffer. The buffer contains a gel loading reagent as well as two gel tracking dyes (a red and a yellow dye) that separate during electrophoresis. In a 1 % [w/v] agarose gel the red dye migrates at the same rate as a 600 bp DNA fragment and the yellow dye migrates faster than 20 bp. The dyes do not interfere with most downstream enzymatic applications. However, it is recommended to purify PCR products prior to enzymatic manipulation.
- Amount of Enzyme:** 1.25 U of Taq DNA Polymerase is recommended concentration of the enzyme per 50 µl amplification reaction. For most applications, enzyme will be in excess and will produce satisfactory results. Increased amounts of enzyme may generate artifacts like as smearing of bands, etc.
- Template Copies:** As a general guide for how much template DNA to use, start with a minimum 10⁴ copies of the target sequence to obtain a signal in 25-35 cycles (i.e. 1 µg of 1 kb ds DNA equals 9.8 x 10¹¹ molecules, 1 µg of *E. coli* genomic DNA equals 2 x 10⁹ molecules, 1 µg of *E. coli* genomic DNA equals 3 x 10⁵ molecules). Increased amounts of template DNA (0.5 µg) may negatively affect or inhibit the PCR reaction.

Notes:

- Annealing:** Annealing temperature should be optimized for each primer set based on the primer T_m. Optimal annealing temperatures may be above or below the estimated T_m. As a starting point, use an annealing temperature 5°C below T_m.
- Long PCR:** When amplifying long PCR products (over 5 kb):
 - initial denaturation should be 2 min at 94°C
 - cycle denaturation should be 15-20 s at 94°C
 - use an elongation temperature of 68°C instead of 72°C.