# Opti*Taq* DNA Polymerase

*Taq DNA Polymerase*
*(Thermus aquaticus)*
*Pfu DNA Polymerase*
*(Pyrococcus furiosus)*

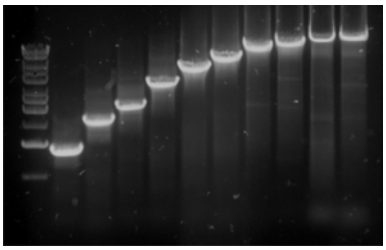
**Mixture of thermostable DNA polymerases capable of generating PCR products up to 20 kb with high fidelity; suitable for applications requiring high temperature synthesis of DNA.**

| Cat. No. | Size |
|---|---|
| E2600-01 | 200 units |
| E2600-04 | 500 units |
| E2600-02 | 1000 units |
| E2600-03 | 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 $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, dTTP (a mix of unlabeled and [$^3$H]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.

KB
M 0.9 1.7 2.5 3.7 5.3 6.9 9.3 12 15 20



**PCR amplification using EURx Opti*Taq* DNA Polymerase.** Lane M: molecular size marker- Perfect 1 kb DNA Ladder. Lanes 0.9 to 20 kb: PCR amplification reactions, using Pol Buffer B with 0.2 mM dNTPs and 1.25 U EURx Opti*Taq* DNA Polymerase in 50 µl reaction volume.

**Description:**

- Opti*Taq* DNA Polymerase is a modified and optimized thermostable enzymes blend containing *Thermus aquaticus* DNA polymerase, *Pyrococcus furiosus* DNA polymerase and enhancing factors.
- Ultrapure, recombinant enzymes are used to prepare Opti*Taq* DNA Polymerase.
- The enzymes blend exhibits 3´→5´ proofreading activity, resulting in considerably higher PCR fidelity than possible with unmodified *Taq* DNA polymerase (1).
- Enables increased amplification product yield in comparison with *Taq* DNA polymerase over a wide range of PCR products.
- Maintains the 5'→3' exonuclease activity.
- Adds extra A at the 3' ends. Both, TA- and Blunt End cloning are possible.
- Excellent for multiplex PCR as it exhibits wider tolerance for $Mg^{2+}$ and salts concentration and pH (2,3).
- Improves PCR results with critical templates, such as templates containing GC-rich regions, palindromes or multiple repeats.
- Increased amplification product yields and purity. Less PCR cycles are needed due to reduced content of prematurely terminated amplification products.
- Ideal for genomic sequencing and mapping by greatly simplifying contig assembly from large amplification products.
- Opti*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 over 20 kb.

**Storage Buffer:**

20 mM Tris-HCl (pH 8.0 at 22˚C), 100 mM KCl, 0.5% Tween 20, 0,5% Igepal CA-630, 0.1 mM EDTA, 50% glycerol.

**10 x Reaction Buffer:**

**10 x Pol Buffer A (optimalization buffer without $MgCl_2$):**
The buffer allows to optimize $MgCl_2$ concentration.

**10 x Pol Buffer B (general application, up to 8-10 kb):**
The buffer contains 15 mM $MgCl_2$ 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. *Cline, J., Braham, J. and Hogrefe, H. (1996) Nucleic Acids Res. 24, 3546.*
2. *Chien, A., Edgar, D.B. and Trela, J.M. (1976) J. Bacteriol. 127, 1550.*
3. *Kaledin, A.S., Sliusarenko, A.G. and Gorodetskii, S.I. (1980) Biokhimiya 45, 644.*



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## PCR PROTOCOL

**Preparation of PCR Reaction:**

| Component | Volume/reaction | Final concentration |
|---|---|---|
| 10 x Pol Buffer A or<br>10 x Pol Buffer B or<br>10 x Pol Buffer C | 5 µl | 1x |
| 25 mM $MgCl_2$ | 2 - 10 µl when using 10 x Pol Buffer A or<br>0 - 7 µl when using 10 x Pol Buffer B or 10 x Pol Buffer C | 1 – 5 mM<br>1.5 – 5 mM |
| dNTP mix (5mM each) | 2 µl | 0.2 mM of each dNTP |
| Upstream primer | Variable | 0.3-0.5 µM |
| Downstream primer | Variable | 0.3-0.5 µM |
| Opti*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 | - |

**Thermal Cycling Conditions for Products 0.1-10 kb in Size:**

| Step | Temperature | Time | Number of Cycles |
|---|---|---|---|
| Initial Denaturation | 93-95˚C | 2-5 min | 1 |
| Denaturation | 93-95˚C | 15-30 s | 25-35 |
| Annealing | 50-68˚C | 30 s | |
| Extension | 72˚C or 68˚C | 1 min/1 kb | |
| Final Extension | 72˚C or 68˚C | 7 min | 1 |
| Cooling | 4˚C | Indefinite | 1 |

**Thermal Cycling Conditions for Products Larger Than 10 kb in Size:**

| Step | Temperature | Time | Number of Cycles |
|---|---|---|---|
| Initial Denaturation | 92-94˚C | 2 min | 1 |
| Denaturation | 92-94˚C | 10-15 s | 10 |
| Annealing | 60-68˚C | 30 s | |
| Extension | 68˚C | 1 min/1 kb | |
| Denaturation | 92-94˚C | 10-15 s | 15-25 |
| Annealing | 60-68˚C | 30 s | |
| Extension | 68˚C | 1 min/1 kb +20 s per additional cycle | |
| Final Extension | 68˚C | 7 min | 1 |
| Cooling | 4˚C | Indefinite | 1 |

**Notes:**

1. 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 gradient when frozen.
2. Prepare reaction mixes on ice, mix well.
3. Place reactions in a thermal cycler that has been preheated to denaturation temperature.
4. Standard concentrations of $MgCl_2$ in PCR reaction are: 1.5 mM (as provided in the 1 x Pol Buffers B or C) when using 0.2 mM dNTP (each). In most cases these concentrations will produce satisfactory results. However, in some cases, reaction may be improved by determining the optimal concentration of $MgCl_2$.
5. 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. 1.25 U of Opti*Taq* DNA Polymerase is the recommended concentration of the enzyme per 50 µl amplification reaction. For most applications, enzyme will be in excess and will produce satisfactory results. In some cases it may be necessary to optimize the enzyme concentration.
6. 1.25 U of Taq DNA Polymerase is the recommended concentration of 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.
7. As a general guide for how much template DNA to use, start with a minimum $10^4$ copies of the target sequence to obtain a signal in 25-35 cycles (i.e. 1 µg of 1 kb ds DNA equals 9.1 x $10^{11}$ molecules, 1 µg of *E. coli* genomic DNA equals 2 x $10^8$ molecules, 1 µg of human genomic DNA equals 3 x $10^5$ molecules).
8. For long range PCR use: 50-500 ng of human genomic DNA, 0.1-10 ng of bacterial DNA, phage DNA or plasmid DNA.
9. Ensure that template DNA is of sufficiently high quality. Use only high-molecular-weight DNA, when amplifying long PCR targets (over 20-50 kb, depending on the amplicon length).
10. Complex genomic DNA should be stored at 2-8˚C. Avoid vortexing the genomic DNA.
11. Use only thin-walled 0.2 ml tubes performing long PCR amplification.

**Notes:**

1. 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 $T_m$.
2. Typical primers for long PCR amplification have a length of 22-34 bp and should have annealing temperatures above 60˚C to enhance reaction specificity.
3. When amplifying long PCR products, keep denaturation steps as short as possible and denaturation temperature as low as possible (try not to exceed 2 min at 94˚C during initial denaturation and 10-15 s at 94˚C during cycle denaturation step). Sometimes fragments with high GC-content need higher denaturation temperatures, but keep in mind that the yield increases when denaturation temperature / duration is decreased.
4. For PCR products over 5 kb an elongation temperature of 68˚C is strongly recommended.
5. For PCR products exceeding 10 kb in length, an elongation of the extension step (+20 s in each additional cycle, starting from the 11$^{th}$ cycle) is strongly recommended due to loss of processivity of the enzymes blend.

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