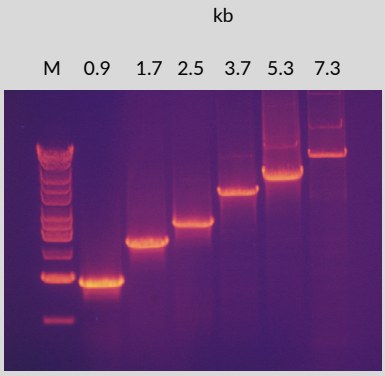
# Pfu DNA Polymerase

*(Pyrococcus sp.)*

| Cat. No. | size |
|---|---|
| E1114-01 | 100 units |
| E1114-02 | 500 units |
| E1114-03 | 2500 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 minutes 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.




**PCR amplification using EURx Pfu DNA Polymerase.**

Lane M: molecular size marker Perfect 1 kb DNA Ladder.

Lanes 0.9-7.3 kb: PCR amplification reactions, using 10 x Pfu Buffer with 0.2-0.25 mM dNTPs and 2.5 U EURx Pfu DNA Polymerase in 50 µl reaction volume.

## References:


1. *Lundberg, K., Shoemaker, D., Adams, M., Short, J., Sorge, J. and Mathur E. (1991) Gene 108, 1.*
2. *Cline, J., Braham, J. and Hogrefe, H. (1996) Nucleic Acids Res. 24, 3546.*


**Extremely thermostable proofreading DNA polymerase, suitable for applications requiring high temperature synthesis of DNA.**

## Description:

- Pfu DNA Polymerase is a thermostable enzyme isolated from hyperthermophilic archaea Pyroccocus sp. (1).
- Ultrapure recombinant enzyme.
- Unmodified enzyme replicates DNA at 74°C and exhibits over 95% activity after 1-hour incubation at 95°C.
- The enzyme catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of magnesium ions.
- The enzyme exhibits the 3'→5' proofreading activity, resulting in over 10-fold higher PCR fidelity than possible with Taq DNA Polymerases (2).
- Pfu DNA Polymerase is recommended for use in high-fidelity PCR, PCR of GC-rich sequences or problematic secondary structures and cloning of blunt-ended PCR products

## Storage Buffer:

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

## 10 x Pfu Buffer:

The buffer contains 15 mM $MgSO_4$.

## 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.

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## Preparation of PCR Reaction:

| Component | Volume/reaction | Final concentration |
|---|---|---|
| 10 x Pfu Buffer contains 15 mM $MgSO_4$ | 5 µl | 1x |
| dNTP mix (5 mM each) | 2.0-2.5 µl | 0.2-0.25 mM of each dNTP |
| Upstream primer | Variable | 0.2-0.5 µM |
| Downstream primer | Variable | 0.2-0.5 µM |
| Pfu DNA Polymerase, 5 U/µl | 0.5 µl | 2.5 U |
| Template DNA | Variable | <0.5 µg/50 µl |
| Sterile double-distilled water | Variable | - |
| Total volume | 50 µl | - |

### Notes:

1. It is recommended to use freshly prepared DNA, primers, dNTPs in Pfu-based PCR reactions in order to avoid the inhibition of Pfu DNA Polymerase caused by degradation products.
2. 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.
3. Prepare reaction mixes on ice. Mix well.
4. Place reactions in a thermal cycler that has been preheated to 94-95°C.
5. Pfu DNA Polymerase should be the last component added to the PCR mixture. In the absence of dNTPs proofreading activity of Pfu DNA Polymerase may degrade primers.
6. For Pfu DNA Polymerase-based PCR, standard recommended concentration of $MgSO_4$ is 1.5 mM (as provided by the 1 x Pfu Buffer). In most cases this concentration will produce satisfactory results. If increased $Mg^{2+}$ concentration is needed use 25 mM $MgSO_4$ provided.
7. Use 0.2-0.25 mM dNTP (each) in PCR reaction (see Table: Preparation of PCR Reaction). The optimal dNTPs concentration may vary between individual reactions.
8. 2.5 U of Pfu 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. For some PCR targets further optimization will be required. Increased amounts of enzyme may generate artifacts like as smearing of bands, etc.
9. The amount of DNA template required depends on the type of DNA being amplified. Generally 50-250 ng of genomic DNA, 1-50 ng of plasmid or phage DNA and 10-100 ng of multicopy chromosomal genes are recommended.

## 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 s | |
| Extension | 72°C | **1 min/1 kb** | |
| Final Extension | 72°C | 7 min | 1 |
| Cooling | 4°C | Indefinite | 1 |

### 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. The recommended extension time is 1 min/1 kb to be amplified.



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