# Cloned *Pfu* DNA Polymerase AD

**Catalog** #600353, 600355, and 600357
600353-12, Revision C.0

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## MATERIALS PROVIDED

| Materials provided | Quantity | | |
|---|---|---|---|
| | **Catalog #600353** | **Catalog #600355** | **Catalog #600357** |
| Cloned *Pfu* DNA Polymerase AD | 100 U | 500 U | 1000 U |
| 10× Cloned *Pfu* Reaction Buffer AD | 1 ml | 2 × 1 ml | 4 × 1 ml |

**Storage:** Store at –20°C upon receipt.

## INTRODUCTION

*Pfu* DNA polymerase AD* features a reformulated buffer system for increased economy with the same high performance as our original *Pfu* DNA polymerase, a proofreading DNA polymerase isolated from *Pyrococcus furiosus*.[1–3] This enzyme is an ideal choice for a variety of applications requiring high-fidelity DNA synthesis by the polymerase chain reaction (PCR), including cloning, gene expression, and site-directed mutagenesis. Successful PCR using *Pfu* DNA polymerase is readily performed requiring only slight modifications from PCR protocols optimized with *Taq* DNA polymerase.

## OPTIMIZATION PARAMETERS (50 µL REACTION VOLUME)

| Parameter | Genomic or Vector DNA Targets ≤2 kb | cDNA Targets ≤2 kb |
|---|---|---|
| Extension time | 1–2 minutes per kb | 2 minutes per kb |
| Cloned *Pfu* DNA polymerase AD | 1.25–2.5 U | 1.25–2.5 U |
| Input template | 25–100 ng genomic DNA;<br>0.5–50 ng vector DNA | 0.5–1 µl cDNA (from cDNA synthesis reaction) |
| Primers (each) | 50–125 ng (0.1–0.5 µM) | 50–125 ng (0.1–0.5 µM) |
| dNTP concentration | 100–250 µM each dNTP | 100–250 µM each dNTP |
| $Mg^{+2}$ concentration | 2 mM (obtained in 1.0× dilution of provided 10× reaction buffer) | 3 mM (supplement reaction with 1mM $MgSO_4$) |
| Denaturing temperature | 95°C | 95°C |
| Extension temperature | 72°C | 68°C |

## PCR PROTOCOL

The reaction conditions given here are for amplification of a typical single-copy chromosomal target of ≤2 kb. See the *Optimization Parameters* section for guidelines on amplifying vector DNA or cDNA targets. The reaction conditions are for one reaction and must be adjusted for multiple samples. The final volume of each reaction is 50 µl.

1. Add the components *in order* into sterile thin-walled PCR tubes while mixing gently.

**Reaction Mixture for a Typical Single-Copy Chromosomal Locus PCR Amplification (≤2 kb)**

| Component | Amount per reaction |
|---|---|
| Distilled water ($dH_2O$) | 40.1 µl |
| 10× Cloned *Pfu* Reaction Buffer AD [a] | 5.0 µl |
| dNTP mix (25 mM each dNTP) | 0.4 µl |
| DNA template (100 ng/µl) | 1.0 µl |
| Primer #1 (100 ng/µl) | 1.25 µl[b] |
| Primer #2 (100 ng/µl) | 1.25 µl[b] |
| Cloned *Pfu* DNA Polymerase AD (2.5 U/µl) | 1.0 µl[c] |
| Total reaction volume | 50 µl |

[a] The 10× buffer provides a final 1× $Mg^{2+}$ concentration of 2 mM. When amplifying cDNA, add $Mg^{2+}$ to a final 1× concentration of 3 mM. (For example, $Mg^{2+}$ concentration may be adjusted to 3 mM in the final 50-µl reaction volume by adding 2 µl of a PCR-grade 25 mM $MgSO_4$ solution and reducing the amount of $dH_2O$ to 38.1 µl.)

[b] The recommended primer concentration of 0.1–0.5 µM corresponds to 50–125 ng for a typical 18- to 25-mer oligonucleotide primer in a 50-µl reaction volume.

[c] The amount of *Pfu* DNA polymerase AD varies depending on the length of the PCR target. The standard amount for PCR targets up to 2 kb in length is 1 µl (2.5 U).

2. Perform PCR using optimized cycling conditions. Suggested cycling parameters are provided below. (Optimized cycling parameters are not necessarily transferable between thermal cyclers. Consult the instrument manufacturer's recommendations if further optimization of cycling parameters is required.) Analyze the PCR amplification products on a 0.7–1.0% (w/v) agarose gel.

**PCR Cycling Program for Cloned *Pfu* DNA Polymerase AD**

| Segment | Number of cycles | Temperature | Duration |
|---|---|---|---|
| 1 | 1 | 94–98°C[a] | 1 minute |
| 2 | 25–30 | 94–98°C[a] | 30 seconds |
| | | Primer $T_m$ – 5°C | 30 seconds |
| | | 72°C[b] | 1–2 minute per kb |
| 3 | 1 | 72°C[b] | 10 minutes |

[a] Denaturing temperatures above 95°C are recommended only for GC-rich templates.
[b] An extension temperature of 68°C is recommended for cDNA target amplification.

## Troubleshooting and Application Notes

- **Low yield:** If PCR product yields are lower than expected, optimize the extension time, amount of DNA template (excess DNA can inhibit PCR), and amount of *Pfu* DNA polymerase AD. Optimize the PCR program denaturation time (typically 30–60 seconds at 95°C is sufficient; prolonged denaturation steps may damage the template DNA) and the annealing temperature. Extraneous salts contributed by primer or template DNA solutions may inhibit the PCR reaction. Use high-quality, gel purified primers and highly-purified template DNA.
- **Multiple bands:** The annealing temperature may require optimization. Typically annealing temperatures will range between 55°C and 72°C. Try adding Perfect Match PCR Enhancer (Catalog #600129) to improve specificity. Redesign primers.
- **PCR adjuncts and cosolvents**: Including a cosolvent, such as 1–10% (v/v) DMSO or 5–20% glycerol, or an adjunct, such as 1.25–10% formamide or 0.01–1 U of Perfect Match PCR Enhancer (Catalog #600129), in the PCR reaction may increase performance for some targets/cycling programs.
- **Amplification of targets >2 kb:** For amplification of PCR targets >2 kb in length, we recommend using *PfuTurbo* DNA Polymerase AD (Catalog #600255, #600257, and #600259). This enzyme features equivalent fidelity with increased target-length capability compared to *Pfu* DNA polymerase AD.
- **PCR cloning:** If generating PCR fragments for cloning applications, use the StrataClone Blunt PCR Cloning Kit (Catalog #240207) or another blunt PCR cloning strategy. *Pfu* DNA polymerase does **not** exhibit terminal deoxynucleotidyltransferase (TdT) activity, which is characterized by the addition of nontemplate-directed nucleotide(s) at the 3´ end of PCR-generated fragments.

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## References


1. Cha, R. S. and Tilly, W. G. (1995). In *PCR Primer*, C. W. Dieffenbach and G. S. Dveksler (Eds.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
2. Flaman, J. M., Frebourg, T., Moreau, V., Charbonnier, F., Martin, C. et al. (1994) *Nucleic Acids Res* 22(15):3259-60.
3. Lundberg, K. S., Shoemaker, D. D., Adams, M. W., Short, J. M., Sorge, J. A. et al. (1991) *Gene* 108(1):1-6.


## Endnotes

* U.S. Patent Nos. 5,948,663 and 5,545,552.

## MSDS Information

Material Safety Data Sheets (MSDSs) are provided online at *http://www.genomics.agilent.com*. MSDS documents are not included with product shipments.

 