PfuUltra High-Fidelity DNA Polymerase AD

Catalog #600385, 600387, and 600389
600385-12, Revision A.01

MATERIALS PROVIDED

Materials provided	Quantity		
	Catalog #600385	**Catalog #600387**	**Catalog #600389**
PfuUltra High-Fidelity DNA Polymerase AD	100 U	500 U	1000 U
10× *PfuUltra* HF Reaction Buffer AD	1 ml	2 × 1 ml	4 × 1 ml

Storage: Store at –20°C upon receipt.

INTRODUCTION

PfuUltra High-Fidelity DNA Polymerase AD* features a reformulated buffer system for increased economy with the same high performance as the original Stratagene *PfuUltra* DNA polymerase for robust, ultra-high-fidelity PCR. As with the original *PfuUltra* DNA polymerase, *PfuUltra* high-fidelity DNA polymerase AD is a mixture of a genetically-engineered higher-fidelity mutant of *Pfu* DNA polymerase and the exclusive thermostable ArchaeMaxx polymerase-enhancing factor,[1] which enhances PCR product yields and increases target length capability. *PfuUltra* high-fidelity DNA polymerase AD is an ideal choice for PCR cloning and other applications that require the highest-accuracy amplification. *PfuUltra* DNA polymerase AD exhibits an extremely low error rate of only 1 error per 2,500,000 bases, while *Pfu* DNA polymerase produces approximately 1 error per 770,000 bases and *Taq* DNA polymerase produces approximately 1 error every 125,000 bases.

OPTIMIZATION PARAMETERS (50 µL REACTION VOLUME)

Parameter	Genomic DNA Targets ≤6 kb or Vector DNA Targets ≤10 kb	Genomic DNA Targets >6 kb or Vector DNA Targets >10 kb	cDNA Targets
Extension time	1 minute per kb	2 minutes per kb	2 minutes per kb
PfuUltra High-Fidelity DNA polymerase AD	2.5 U	5.0 U	5.0 U
Input template	50–100 ng genomic DNA; 100 pg–30 ng vector DNA	200–250 ng genomic DNA; 100 pg–30 ng vector DNA	1–2 µl cDNA (from cDNA synthesis reaction)
Primers (each)	100–200 ng (0.2–0.5 µM)	200 ng (0.5 µM)	100–200 ng (0.2–0.5 µM)
dNTP concentration	200–250 µM each dNTP	500 µM each dNTP	200–250 µM each dNTP
Final reaction buffer conc.	1.0×	1.5× for genomic DNA or 1.0× for vector DNA	1.0× (supplemented with 1 mM $MgSO_4$)
Denaturing temperature	95°C	92°C	95°C
Extension temperature	72°C	68°C	68°C

PCR PROTOCOL

The reaction conditions given here are for amplification of a typical single-copy chromosomal target of ≤6 kb. See the *Optimization Parameters* section for guidelines on amplifying longer targets, vector DNA targets, 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 (≤6 kb)

Component	Amount per reaction
Distilled water (dH_2O)	40.6 µl
10× *PfuUltra* HF 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)	1.0 µl[b]
Primer #2 (100 ng/µl)	1.0 µl[b]
PfuUltra High-Fidelity 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 38.6 µl.)

[b] The recommended primer concentration of 0.2–0.5 µM corresponds to 100–200 ng for a typical 18- to 25-mer oligonucleotide primer in a 50-µl reaction volume.

[c] The amount of *PfuUltra* DNA polymerase AD varies depending on the length of the PCR target. The standard amount for vector targets up to 10 kb and genomic targets up to 6 kb in length is 1 µl (2.5 U).

2. If the extension time is >30 minutes, overlay each reaction with ~50 µl of DNase-, RNase-, and protease-free mineral oil (Sigma, St. Louis, Missouri).

3. Perform PCR using optimized cycling conditions. Suggested cycling parameters for *PfuUltra* high-fidelity DNA polymerase AD-based PCR 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 *PfuUltra* High-Fidelity DNA Polymerase AD

Genomic DNA Targets ≤6 kb and Vector DNA Targets ≤10 kb

Segment	Number of cycles	Temperature	Duration–Single Block Thermocycler	Duration–Stratagene RoboCycler Thermocycler
1	1	95°C[a]	1 minute	1 minute
2	30	95°C	30 seconds	1 minute
		Primer T_m – 5°C	30 seconds	1 minute
		72°C	1 minute per kb	1 minute per kb
3	1	72°C	10 minutes	10 minutes

Genomic DNA Targets >6 kb, Vector DNA Targets >10 kb, and cDNA Targets

Segment	Number of cycles	Temperature	Duration–Single Block Thermocycler	Duration–Stratagene RoboCycler Thermocycler
1	1	92°C[b]	2 minutes	1 minute
2	10	92°C[b]	10 seconds	1 minute
		Primer T_m – 5°C	30 seconds	1 minute
		68°C	2 minutes per kb	2 minutes per kb
3	20	92°C	10 seconds	1 minute
		Primer T_m – 5°C	30 seconds	1 minute
		68°C	2 minutes per kb plus 10 seconds per cycle	2 minutes per kb plus 10 seconds per cycle

[a] Denaturing temperatures above 95°C are recommended only for GC-rich templates.
[b] For cDNA targets, use a denaturation temperature of 95°C.

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 *PfuUltra* high-fidelity 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 Stratagene Perfect Match PCR Enhancer 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 Stratagene Perfect Match PCR Enhancer, in the PCR reaction may increase performance for some targets/cycling programs.
- **PCR cloning:** If generating PCR fragments for cloning applications, use the StrataClone Blunt PCR Cloning Kit (Catalog #240207) or another blunt PCR cloning strategy. *PfuUltra* high-fidelity DNA polymerase AD 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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Limited Label License for Pfu-Containing DNA Polymerase Products

This product is covered by the claims of one or more of the following U.S. Patents: 5,545,552; 5,556,772; 5,866,395; 5,948,663; 6,183,997; 6,444,428, 6,489,150; 6,734,293; 7,045,328, and patents pending. Purchase of this product conveys to the purchaser only the non-transferable right under these patents to use the product ***for research use only by the purchaser***. No rights are granted to the purchaser hereunder to sell, modify for resale or otherwise transfer this product, either alone or as a component of another product, to any third party. Agilent reserves all other rights, and this product may not be used in any manner other than as provided herein. For information on obtaining a license to use this product for purposes other than research, please contact the Stratagene Products Division, Business Development, 11011 North Torrey Pines Road, La Jolla, California 92037. Phone (858) 535-5400.

References

1. Hogrefe, H. H., Hansen, C. J., Scott, B. R. and Nielson, K. B. (2002) *Proc Natl Acad Sci U S A* 99(2):596–601.
2. Cline, J., Braman, J. C. and Hogrefe, H. H. (1996) *Nucleic Acids Res* 24(18):3546-51.

Endnotes

* U.S. Patent Nos. 7,045,328; 6,734,293; 6,489,150; 6,444,428; 6,183,997; 5,948,663; 5,866,395; 5,545,552 and patents pending.

MSDS Information

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at http://www.stratagene.com/MSDS/. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

