PfuTurbo DNA Polymerase AD

Catalog #600255, 600257, and 600259
600255-12, Revision C.0

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Materials Provided

Materials provided	Quantity		
	Catalog #600255	**Catalog #600257**	**Catalog #600259**
PfuTurbo 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

PfuTurbo DNA polymerase AD* features a reformulated buffer system for increased economy with the same high performance as the original *PfuTurbo* DNA polymerase for robust, high-fidelity PCR. As with the original *PfuTurbo* DNA polymerase, *PfuTurbo* DNA polymerase AD is a mixture of cloned *Pfu* DNA polymerase and the exclusive thermostable ArchaeMaxx polymerase-enhancing factor[1] that enhances PCR product yields and increases target length capability without altering DNA replication fidelity. *PfuTurbo* DNA polymerase AD can be used to amplify complex genomic DNA targets up to 19 kb and vector targets up to 15 kb in length. *PfuTurbo* DNA polymerase AD exhibits the same low error rate as *Pfu* DNA polymerase of 1–3 errors per 10^6 bases, while *Taq* DNA polymerase exhibits an error rate of 8–9 errors per 10^6 bases in the same assay.[2]

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
PfuTurbo 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	100–250 µM each dNTP	500 µM each dNTP	100–250 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 mixture given here is 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× 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)	1.0 µl[b]
Primer #2 (100 ng/µl)	1.0 µl[b]
PfuTurbo 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 *PfuTurbo* 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. Perform PCR using optimized cycling conditions. Suggested cycling parameters for *PfuTurbo* 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 *PfuTurbo* DNA Polymerase AD

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

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

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

Segment	Number of cycles	Temperature	Duration
1	1	92°C[b]	2 minutes
2	10	92°C[b]	10 seconds
		Primer T_m – 5°C	30 seconds
		68°C	2 minutes per kb
3	20	92°C	10 seconds
		Primer T_m – 5°C	30 seconds
		68°C	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 *PfuTurbo* 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 Agilent 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 1–5% formamide or 0.01–1 U of Agilent 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. *PfuTurbo* 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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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. 6,734,293, 6,444,428, 6,183,997, 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.

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