PfuUltra Hotstart DNA Polymerase AD

Catalog #600396
600396-12, Revision D.0

Technical Services

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MATERIALS PROVIDED

Materials provided	Quantity[a]
PfuUltra Hotstart DNA Polymerase AD	1000 U
10× *PfuUltra* HS Reaction Buffer AD	4 × 1 ml

[a] Sufficient reagents are provided for four hundred 50-µl reactions.

Storage: Store all components at –20°C upon receipt.

INTRODUCTION

PfuUltra Hotstart DNA Polymerase AD* features a reformulated buffer system and novel hot start mechanism for increased economy with the same high performance as the original *PfuUltra* Hotstart DNA polymerase. Formulated from a genetically engineered mutant of *Pfu* DNA polymerase**, *PfuUltra* hotstart DNA polymerase AD provides robust, high-fidelity amplification of long, complex genomic targets of up to 19 kb. The hot start capability allows room temperature PCR assembly, provides reduced background, and improves detection sensitivity.

PCR PROTOCOL

1. The table below provides an example reaction mixture for the amplification of a typical single-copy chromosomal target. Add the PCR reaction components in order while mixing gently. The recipe listed is for one reaction and must be adjusted for multiple samples.

Reaction Mixture for a Typical PCR Amplification

Component	Amount per reaction
Distilled water (dH_2O)	40.6 µl
10× *PfuUltra* HS reaction buffer AD	5 µl
dNTP mix (100 mM)	0.4 µl
Primer #1 (100 ng/µl)[a]	1 µl
Primer #2 (100 ng/µl)[a]	1 µl
DNA template (100–200 ng/µl)[b]	1 µl
PfuUltra hotstart DNA polymerase AD	1 µl
Total reaction volume	50 µl

[a] Primer concentrations between 0.3 and 0.4 µM are recommended (this corresponds to 100–150 ng for typical 18- to 25-mer oligonucleotides in a 50-µl reaction volume).

[b] The amount of DNA template required varies depending on the type of DNA and the length of the target being amplified. Generally 100 ng of genomic DNA template is recommended for targets less than 10 kb. For genomic targets longer than 10 kb, use 200 ng of DNA. Less DNA template (1–15 ng) may be used for low-complexity targets such as plasmid DNA or cloned DNA.

2. Aliquot 50 µl of the reaction mixture into the appropriate number of sterile thin-wall PCR tubes.

* U.S. Patent Nos 6,734,293, 6,444,428, 6,183,997, 5,948,663, and 5,545,552.
** U.S. Patent No. 5,948,663, and 5,545,552.

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3. Perform PCR using optimized cycling conditions. See the tables below for suggested cycling protocols.

Cycling Protocol: Targets <10 kb (Genomic DNA, plasmid DNA, and cloned DNA templates)

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

[a] The annealing temperature may require optimization. Typically annealing temperatures will range between 55° and 72°C.

Cycling Protocol: cDNA Targets and Targets >10 kb

Number of cycles	Temperature	Duration
1	92°C[a]	2 minutes
30[b]	92°C[a]	30 seconds
	Primer T_m – 5°C[c]	30 seconds
	68°C	2 minutes/kb
1	68°C	5–10 minutes

[a] For cDNA targets <5 kb, use a denaturation temperature of 95°C.

[b] For cDNA targets, increase the number of cycles to 40.

[c] The annealing temperature may require optimization. Typically annealing temperatures will range between 55° and 72°C.

5. Analyze the PCR amplification products on a 0.7–1.0% (w/v) agarose gel.

TROUBLESHOOTING

Observation	Solution(s)
No product or low yield	Increase the quantity of DNA template. For genomic targets >10 kb use 200 ng of DNA template.
	Increase the number of cycles up to a maximum of 40 cycles.
	Lower the annealing temperature in 5°C increments.
	If amplifying a cDNA target, try supplementing the reaction with 1 mM $MgS0_4$.
	Use intact and highly purified DNA templates.
	Use the recommended primer concentration of 0.3 µM (corresponding to 100 ng for typical 18- to 25-mer oligonucleotide primers in a 50-µl reaction volume).
	Check the melting temperature, purity, GC content, and length of the primers.
	Use thin-walled PCR tubes. These PCR tubes are designed to permit more efficient heat transfer and to maximize thermal-cycling performance.
Multiple bands	Increase the annealing temperature in 5°C increments.
Artifactual smears	Reduce the extension time.

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MSDS INFORMATION

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