PfuUltra II Hotstart PCR Master Mix

Catalog #600850 and 600852

For Research Use Only. Not for use in diagnostic procedures.

Technical Services

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MATERIALS PROVIDED

Materials Provided	Quantity	
	Catalog #600850	Catalog #600852
PfuUltra II Hotstart 2× Master Mix	100 reactions	400 reactions

Storage: Store at –20°C upon receipt. After thawing, store at 4°C. Once thawed, full activity is guaranteed for 6 months.

INTRODUCTION

PfuUltra II Hotstart PCR Master Mix* is a 2× formulation of *PfuUltra* II Fusion HS DNA polymerase,* an optimized PCR reaction buffer, magnesium, and dNTPs. *PfuUltra* II Fusion HS DNA polymerase couples fusion polymerase technology with our engineered *PfuUltra* DNA polymerase,* and proprietary ArchaeMaxx PCR enhancing factor to achieve extreme accuracy, high specificity, and long target-length capability while dramatically reducing overall PCR extension times.

OPTIMIZATION PARAMETERS (50 µL REACTION VOLUME)

Parameter	Targets: ≤10 kb (vector or genomic DNA)	Targets: >10 kb (vector or genomic DNA)	cDNA Targets
Extension time	15 seconds for targets ≤1 kb; 15 seconds per kb for targets >1 kb	30 seconds per kb	30 seconds for targets ≤1 kb; 30 seconds per kb for targets >1 kb
Input template	100 ng genomic DNA; 5–30 ng vector DNA	200–250 ng genomic DNA; 5–30 ng vector DNA	1–2 µl cDNA from RT-PCR reaction (50–500 ng starting total RNA template)
Primer concentration	0.2–0.4 µM each primer	0.4 µM each primer	0.2 µM each primer
Denaturing temperature and time	95°C/20 seconds per cycle	92°C/20–40 seconds per cycle	95°C/20 seconds per cycle
Extension temperature	72°C	68°C	72°C

PCR PROTOCOL

The reaction conditions given here are for amplification of a typical single-copy chromosomal target of ≤10 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. Add the components in order into sterile thin-walled PCR tubes while mixing gently.

Important *Before dispensing the* PfuUltra *II Hotstart 2× Master Mix, invert the container several times, then vortex briefly to ensure homogeneity.*

Reaction Mixture for a Typical Single-Copy Chromosomal Locus PCR Amplification (≤10 kb)

Component	Amount per reaction
PfuUltra II Hotstart 2× Master Mix[a]	25.0 µl
Primer #1 (10 µM)[b]	1.0 µl
Primer #2 (10 µM)[b]	1.0 µl
DNA template (100 ng/µl)[c]	1.0 µl
Distilled water (dH_2O)	22.0 µl
Total reaction volume	50 µl

[a] The final concentration of Mg^{2+} in the 1× reaction mixture is 2 mM.

[b] Generally final primer concentrations of 0.2 µM (each primer) are optimal. For longer targets (7–10 kb), increasing the concentration to 0.4 µM (each primer) may substantially increase product yield.

[c] The amount of DNA template required varies depending on the type of DNA being amplified. Generally 100 ng of genomic DNA template is recommended. Less DNA template should be used for amplification of lambda or plasmid (5–30 ng) PCR targets.

Perform PCR using optimized cycling conditions. Suggested cycling parameters for PCR are indicated below. The PCR cycling parameters have been tested on the following: the MJ Research® DNA Engine® PTC-200, the Applied Biosystems® GeneAmp® PCR systems 9600 and 9700, the Bio-Rad iCycler, and the Agilent Mx3000P QPCR system. Optimized cycling parameters are not necessarily transferable between thermal cyclers designed by different manufacturers.

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

* U.S. Patent Nos. 6,734,293, 6,444,428, 6,183,997, and 5,948,663.

PCR Cycling Parameters for *PfuUltra* II Hotstart PCR Master Mix[a]

Targets ≤10 kb (vector DNA, genomic DNA, and cDNA)

Segment	Number of cycles	Temperature	Duration (vector or genomic DNA)	Duration (cDNA)
1	1	95°C[b]	2 minutes	1 minute
2	30 cycles for vector or genomic DNA; 40 cycles for cDNA	95°C	20 seconds	20 seconds
		Primer T_m – 5°C[c]	20 seconds	20 seconds
		72°C	15 seconds for targets ≤1 kb 15 seconds per kb for targets >1 kb	30 seconds for targets ≤1 kb 30 seconds per kb for targets >1 kb
3	1	72°C	3 minutes	3 minutes

Targets >10 kb (vector or genomic DNA)

Segment	Number of cycles	Temperature	Duration
1	1	92°C	2 minutes
2	30	92°C	20 seconds[d]
		Primer T_m – 5°C[c]	20 seconds
		68°C	30 seconds per kb
3	1	68°C	5 minutes

[a] Thin-wall PCR tubes are highly recommended. These PCR tubes are optimized to ensure more efficient heat transfer and to maximize thermal-cycling performance.

[b] Denaturing temperatures above 95°C are recommended only for GC-rich templates.

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

[d] For long targets (10–19 kb), increasing the duration of the denaturing step to 40 seconds per cycle may increase PCR product yield.

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