

***PfuTurbo* Hotstart PCR Master Mix**

INSTRUCTION MANUAL

Catalog #600600 (100 reactions)

#600602 (400 reactions)

Revision B

For In Vitro Use Only

600600-12

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PfuTurbo Hotstart PCR Master Mix

MATERIALS PROVIDED

Materials provided	Quantity	
	Catalog #600600 ^a	Catalog #600602 ^b
PfuTurbo hotstart 2× master mix (0.1 U/μl) ^c	2.5 ml (250 U)	4 × 2.5 ml (1000 U)

^a Catalog #600600 provides enough PCR reagents for 100, 50-μl PCR reactions.

^b Catalog #600602 provides enough PCR reagents for 400, 50-μl PCR reactions.

^c The total Mg²⁺ concentration present in the final 1× dilution of the 2× PfuTurbo hotstart master mix is 2 mM.

The total dNTP concentration present in the final 1× dilution is 800 μM (200 μM of each dNTP).

Storage: The PfuTurbo hotstart PCR master mix should be stored at –20°C upon receipt. Store the PCR master mix at 4°C after thawing. Once thawed, full activity is guaranteed for 6 months.

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INTRODUCTION

The PfuTurbo hotstart PCR master mix* is a 2× formulation of PfuTurbo hotstart DNA polymerase,* an optimized PCR reaction buffer, magnesium, and dNTPs. The PfuTurbo DNA polymerase has a significantly lower error rate than most other proofreading enzymes or DNA polymerase mixtures, and its enhanced performance allows the use of shorter extension times, fewer PCR cycles, and lower DNA template concentrations. The exclusive ArchaeMaxx polymerase-enhancing factor eliminates dUTP, a PCR inhibitor, thus promoting higher yield and greater target length capabilities. The antibody-based hotstart feature allows room temperature PCR assembly, provides reduced background, and improves detection sensitivity.

PCR PROTOCOL

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

TABLE I Reaction Mixture for a Typical PCR Amplification

Component	Amount per reaction
Distilled water (dH ₂ O)	22 μl
Primer #1 (100 ng/μl) ^a	1 μl
Primer #2 (100 ng/μl) ^a	1 μl
DNA template (100 ng/μl) ^b	1 μl
PfuTurbo hotstart 2× master mix	25 μl
Total reaction volume	50 μl

^a Primer concentrations between 0.3 and 0.5 μM are recommended (this corresponds to 100–200 ng for typical 18- to 25-mer oligonucleotide primers in a 50-μl reaction volume).

^b The amount of DNA template required varies depending on the type of DNA being amplified. Generally 50–100 ng of genomic DNA template is recommended. Less DNA template can be used for amplification of lambda (1–30 ng) and vector (0.1–10 ng) PCR targets or for amplification of multicopy chromosomal genes (10–100 ng).

2. Aliquot 50 μl of the reaction mixture into the appropriate number of sterile thin-wall PCR tubes or standard 0.5-ml microcentrifuge tubes.
3. If the temperature cycler is not equipped with a heated cover, overlay each reaction with ~50 μl of DNase-, RNase-, and protease-free mineral oil.
4. Perform PCR using optimized cycling conditions. Suggested cycling parameters for PfuTurbo hotstart PCR master mix-based PCR using single-block temperature cyclers and the Stratagene RoboCycler temperature cyclers are indicated in Table II.
5. Analyze the PCR amplification products on a 0.7–1.0% (w/v) agarose gel.

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

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TABLE II PCR Cycling Parameters for a Typical PCR Amplification^{a,b}

Segment	Number of cycles	Temperature	Duration	
			Single-block temperature cycler	RoboCycler temperature cycler
1	1	95°C	2 minutes	1 minute
2	30	95°C	30 seconds	1 minute
		Primer T _m – 5°C ^c	30 seconds	1 minute
		72°C	1 minute for targets ≤ 1 kb 1 minute/kb for targets > 1 kb 2 min/kb for genomic targets > 6 kb 2 min/kb for vector targets > 10 kb	1 minute for targets ≤ 1 kb 1 minute/kb for targets > 1 kb 2 min/kb for genomic targets > 6 kb 2 min/kb for vector targets > 10 kb
3	1	72°C	10 minutes	10 minutes

^a Thin-wall PCR tubes are highly recommended. These PCR tubes are optimized to ensure ideal contact with the multiblock design to permit more efficient heat transfer and to maximize thermal-cycling performance.

^b Optimized cycling parameters are not necessarily transferable between thermal cyclers designed by different manufacturers; therefore, each manufacturer's recommendations for optimal cycling parameters should be consulted.

^c The annealing temperature may require optimization. Typically annealing temperatures will range between 55° and 72°C.¹ Optimal PCR annealing temperatures may be determined quickly using Stratagene's RoboCycler gradient temperature cyclers.

TROUBLESHOOTING

Observation	Solution(s)
No product or low yield	Increase extension time to 2 minutes per kb of PCR target.
	Use cosolvents such as DMSO in a 1–10% (v/v) final concentration for GC-rich templates.
	Lower the annealing temperature in 5°C increments.
	Use higher denaturing temperatures (94–98°C) if the template DNA contains a high GC content or secondary structures.
	Denaturation times of 30–60 seconds at 94–95°C are usually sufficient while longer denaturation times may damage the DNA template; use the shortest denaturation time compatible with successful PCR on the thermal cycler.
	Remove extraneous salts from the PCR primers and DNA preparations.
	Excessive template DNA can be inhibitory. Follow the recommendations given for template amount.
	Use the recommended primer concentrations between 0.3 and 0.5 μM (corresponding to 100–200 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 for the Stratagene RoboCycler thermal cyclers. These PCR tubes are optimized to ensure ideal contact with the multiblock design to permit more efficient heat transfer and to maximize thermal-cycling performance.
Multiple bands	Increase the annealing temperature in 5°C increments.
Artificial smears	Reduce the extension time.

REFERENCE

1. Innis, M. A., Gelfand, D. H., Sninsky, J. J. and White, T. J. (1990). PCR Protocols: A Guide to Methods and Applications. Academic Press, New York.

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.