PfuTurbo Hotstart PCR Master Mix

Instruction Manual

Catalog #600600 (100 reactions)
#600602 (400 reactions)

Revision C

Research Use Only. Not for Use in Diagnostic Procedures.

600600-12

Agilent Technologies

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Agilent. Agilent shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

Email

techservices@agilent.com

World Wide Web

www.genomics.agilent.com

Telephone

Location	Telephone
United States and Canada	800 227 9770
Austria	01 25125 6800
Benelux	02 404 92 22
Denmark	45 70 13 00 30
Finland	010 802 220
France	0810 446 446
Germany	0800 603 1000
Italy	800 012575
Netherlands	020 547 2600
Spain	901 11 68 90
Sweden	08 506 4 8960
Switzerland	0848 8035 60
UK/Ireland	0845 712 5292
All Other Countries	Please visit *www.genomics.agilent.com* and click **Contact Us**

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 Mg^{2+} 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.

NOTICE TO PURCHASER: LIMITED LICENSE

Purchase of this product includes an immunity from suit under patents specified in the product insert to use only the amount purchased for the purchaser's own internal research. No other patent rights (such as 5' Nuclease Process patent rights) are conveyed expressly, by implication, or by estoppel. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

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_2O)	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. 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.
4. 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, 5,948,663, and 5,545,552.

Revision C

TABLE II PCR Cycling Parameters for a Typical PCR Amplification[a]

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

[a] 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.

[b] 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.
Multiple bands	Increase the annealing temperature in 5°C increments.
Artifactual 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

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