Paq5000 Hotstart PCR Master Mix

Catalog #600870, 600872

Research Use Only. Not for Use in Diagnostic Procedures.

Technical Services

Telephone: To find worldwide support telephone numbers visit www.agilent.com/genomics/contactus.

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MATERIALS PROVIDED

Materials provided	Quantity	
	Catalog #600870[a]	**Catalog #600872**[b]
Paq5000 Hotstart PCR Master Mix[c]	2.5 ml	4 × 2.5 ml

[a] Catalog #600870 provides enough PCR reagents for 100 × 50-µl PCR reactions.

[b] Catalog #600872 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× Paq5000 hotstart PCR master mix is 2.25 mM. The total dNTP concentration present in the final 1× dilution is 1 mM (250 µM of each dNTP).

Storage: The Paq5000 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.

INTRODUCTION

The Paq5000 Hotstart PCR Master Mix is a 2× formulation containing Paq5000 hotstart DNA polymerase, optimized PCR reaction buffer, magnesium, and dNTPs. Paq5000 hotstart DNA polymerase*, an alternative to hot start *Taq* DNA polymerase, provides amplification of longer targets, faster extension times, greater economy, and excellent PCR yields. The novel hot start mechanism allows room temperature PCR assembly, reduces background, and improves detection sensitivity. Paq5000 hotstart PCR master mix is ideal for routine endpoint PCR for up to 6 kb genomic targets. It is not recommended for high-fidelity cloning or 5´ nuclease assays.

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)	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
Paq5000 Hotstart PCR 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 oligonucleotides in a 50-µl reaction volume).

[b] The amount of DNA template required varies depending on the type of DNA being amplified. Generally 100–200 ng of genomic DNA template is recommended. Less DNA template (1–15 ng) may be used for the amplification of low-complexity targets, such as lambda DNA or cloned DNA.

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

4. Perform PCR using optimized cycling conditions. The table below lists a suggested cycling protocol.

Cycling Protocol [a,b]

Number of cycles	Temperature	Duration
1	95°C	2 minutes
30	95°C	20 seconds
	Primer T_m – 5°C [c]	20 seconds
	72°C	30 seconds/kb of PCR target
1	72°C	5 minutes

[a] The provided cycling protocol has been optimized for the Agilent SureCycler 8800. 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] Thin-wall PCR tubes are highly recommended (Agilent Catalog #410082 [tube strips] and #410086 [tube cap strips]).

[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.

- U.S. Patent Nos. 6,734,293; 6,444,428; 6,183,997; 5,489,523.

Troubleshooting

Observation	Suggestion
No product or low yield	Increase extension time to 40 seconds per kb of PCR target.
	If using the Agilent SureCycler 8800 to run PCR in plates sealed with film and a compression mat, decrease the denaturation time during cycling to 3–10 seconds whenever the reaction volume is <50 µl.
	Increase the number of cycles up to a maximum of 40 cycles.
	Lower the annealing temperature in 5°C increments.
	Use intact and highly purified DNA templates.
	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. 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.

