PicoMaxx High Fidelity PCR Master Mix

Instruction Manual

Catalog ##600650 (100 reactions)

Revision B

Research Use Only. Not for Use in Diagnostic Procedures.

600650-12

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PicoMaxx High Fidelity PCR Master Mix

MATERIALS PROVIDED

Materials provided	Quantity
PicoMaxx 2× master mix[a]	2.5 ml (100, 50-µl reactions)

[a] The total Mg^{2+} concentration present in the final 1× dilution of the 2× PicoMaxx master mix is 2 mM. The total dNTP concentration present in the final 1× dilution is 800 µM (200 µM each dNTP).

Storage: The PicoMaxx 2× master mix should be stored at –20°C upon receipt. After thawing, store at 4°C. Once thawed, full activity is guaranteed for 6 months.

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INTRODUCTION

The PicoMaxx High Fidelity PCR Master mix* is a 2× formulation of the PicoMaxx high fidelity PCR enzyme blend, an optimized PCR reaction buffer, magnesium, and dNTPs. The master mix includes a blend of cloned *Taq* and *Pfu* DNA polymerases** and Agilent's ArchaeMaxx polymerase-enhancing factor. Together with the optimized buffer in the master mix, this enzyme blend provides maximum PCR sensitivity and efficiency. The PicoMaxx high fidelity PCR master mix successfully amplifies even where starting material is limited, and reliably produces high PCR product yields on a wide variety of templates up to 10 kb.

The PicoMaxx high fidelity PCR master mix is formulated with antibodies that inhibit polymerase activity until cycling begins, promoting high specificity and reducing background.

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
PicoMaxx 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 100–200 ng of genomic DNA template is recommended. To amplify low-complexity targets (for example, lambda DNA or cloned DNA), we recommend using 5–20 ng of template. Excess template DNA can inhibit the PCR reaction.

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 are indicated in Table II.
4. Analyze the PCR amplification products on a 0.7–1.0% (w/v) agarose gel.

*,** See *Endnotes*.

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

Segment	# of Cycles	Temperature	Duration
1	1	95°C	2 minutes
2	30–35	95°C	40 seconds
		Primer T_m – 5°C[c]	30 seconds
		72°C	1 minute per kb PCR target
3	1	72°C	10 minutes

[a] Thin-wall PCR tubes are highly recommended.

[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]

TROUBLESHOOTING

Observation	Suggestion(s)
No product or low yield	Increase extension time to 90 seconds per kb of PCR target.
	Use intact and highly purified DNA templates.
	Increase the amount of full-length intact DNA template, adjust the ratio of primer versus template, and/or increase the number of cycles up to a maximum of 40 cycles to optimize yield of the desired product.
	Remove extraneous salts from the PCR primers and DNA preparations.
	For some targets, increasing the dNTP concentration by supplementing with additional dNTPs may be beneficial.
	Check the melting temperature, purity, GC content, and length of the primers.
	Use the recommended primer concentrations between 0.3 and 0.5 µM (generally 100–250 ng for typical 20- to 30-mer oligonucleotide primers in a 50-µl reaction volume).
	Primer pairs with matching primer melting temperatures (T_m) and complete complementarity between the primer and template are recommended.
	If using existing primer pairs, the annealing temperatures may require optimization.
	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.
	Use thin-wall PCR tubes. These PCR tubes are optimized to ensure ideal contact with the block to permit more efficient heat transfer and to maximize thermal-cycling performance.
Artifactual smears or multiple bands	Increase the annealing temperature in 4°C increments.
	Optimize the cycling parameters specifically for the primer–template set and the thermal cycler used.

REFERENCES

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.

ENDNOTES

* U.S. Patent Nos. 6,734,293, 6,444,428, 6,183,997, 5,948,663, 5,556,772, 5,545,552.
** U.S. Patent Nos. 5,545,552 and 5,948,663.

MSDS INFORMATION

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