TaqPlus Precision PCR System

Instruction Manual

Catalog #600210 (100 U), #600211 (500 U), and #600212 (1000 U)

Revision D.0

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600210-12

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TaqPlus Precision PCR System

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MATERIALS PROVIDED

Materials provided	Quantity		
	Catalog #600210	**Catalog #600211**	**Catalog #600212**
TaqPlus Precision polymerase mixture (5 U/µl)	100 U	500 U	1000 U
TaqPlus Precision 10× buffer[a]	1 ml	2 × 1 ml	4 × 1 ml

[a] The *TaqPlus Precision* 10× buffer provided with this system is optimized to produce PCR products with a minimal number of errors. Modifying the *TaqPlus Precision* 10× buffer may adversely affect the fidelity and yield of the PCR product.

STORAGE CONDITIONS

All Components: –20°C

ADDITIONAL MATERIALS REQUIRED

Temperature cycler
PCR tubes
PCR primers
Deoxynucleotides

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Revision D.0

INTRODUCTION

The *TaqPlus Precision* PCR system[1] consists of the *TaqPlus Precision* polymerase mixture and the *TaqPlus Precision* 10× buffer, which are specifically formulated for high-fidelity polymerase chain reaction (PCR) applications. The *TaqPlus Precision* polymerase mixture is an optimized blend of Agilent's cloned *Pfu* DNA polymerase[2] and *Taq2000* DNA polymerase, a highly purified, recombinant version of *Taq* DNA polymerase. The *TaqPlus Precision* PCR system exhibits the highest replication accuracy achieved using DNA polymerase mixtures.[3]

Thermostable DNA polymerases offer distinct advantages for specific PCR applications. *Pfu* DNA polymerase exhibits the lowest error rate of any thermostable DNA polymerase analyzed.[1, 4–6] This proofreading enzyme is also characterized by a relatively slow polymerization rate requiring the use of long extension times (i.e., ≥2 minutes/kb of DNA target) to generate high yields of PCR product.[7] *Taq* DNA polymerase exhibits a fivefold higher polymerization rate than *Pfu* DNA polymerase and is used routinely to generate high yields of PCR product ≤5 kb in length using short extension times (i.e., ~1 minute/kb of DNA target). *Taq* DNA polymerase possesses a significantly higher error rate than *Pfu* DNA polymerase. However, Agilent's *Taq2000* DNA polymerase is superior to other commercial *Taq* DNA polymerases in that this recombinant version of *Taq* DNA polymerase minimizes artifactual smearing in PCR amplification reactions requiring long extension times.[8]

DNA polymerase mixtures, consisting of a nonproofreading DNA polymerase and a proofreading DNA polymerase, are typically employed to amplify longer DNA targets and to generate higher yields of PCR product than can be obtained using either DNA polymerase alone.[9–11] Agilent offers two mixtures of *Taq* and *Pfu* DNA polymerases designed for PCR: the *TaqPlus Long* PCR system and the *TaqPlus Precision* PCR system. The *TaqPlus Long* PCR system typically generates the highest yields of PCR product and can be used to amplify unusually difficult or long targets (up to 35 kb). The *TaqPlus Precision* PCR system also generates relatively high yields of PCR product using short PCR extension times. The error rate, however, is significantly lower than the *TaqPlus Long* PCR system. In addition, the *TaqPlus Precision* PCR system successfully amplifies plasmid and lambda DNA templates up to 15 kb in length and single-copy genomic DNA templates up to 10 kb in length as well as templates that are difficult to amplify using single enzyme formulations.[1]

PROTOCOL CONSIDERATIONS

- The *TaqPlus Precision* PCR system successfully amplifies single-copy genomic DNA templates up to 10 kb in length and plasmid and lambda DNA templates up to 15 kb in length.[1] Amplification of certain longer targets, especially single-copy chromosomal templates ≥5 kb, may require additional optimization of the reaction conditions (see *Troubleshooting*).

- As with all PCR amplification reactions, the following factors are critical for success: template quality, primer design, denaturation conditions, annealing temperature, and extension times.

- If using existing primer pairs, the annealing temperatures may require optimization in the *TaqPlus Precision* 10× buffer provided. If new primers are being designed, design the primers to have similar melting temperatures (typically between 60° and 80°C).

- The *TaqPlus Long* PCR system typically amplifies longer targets with higher yields than can be obtained with the *TaqPlus Precision* PCR system. If high fidelity is not a primary concern, we recommend the use of the *TaqPlus Long* PCR system to generate robust PCR products up to 35 kb in length.

- Cycling parameters require optimization specific for the primer–template sets and thermal cycler used. These optimized cycling parameters are not necessarily transferable between thermal cyclers designed by different manufacturers.

1. Prepare a reaction mixture on ice for the appropriate number of samples to be amplified. Add the components *in order* and mix gently. Table I provides an example of a reaction mixture for the amplification of a typical single-copy chromosomal target. The recipe listed in Table I is for one reaction and must be adjusted for multiple samples. The final volume of each sample reaction is 50 µl.

2. Immediately before thermal cycling, aliquot 50 µl of the reaction mixture into the appropriate number of sterile thin-wall PCR tubes or standard 0.5-ml microcentrifuge tubes and place the tubes on ice.

TABLE I

Reaction Mixture for a Typical Single-Copy Chromosomal Locus PCR Amplification

Component	Amount per reaction
Distilled water (dH_2O)	41.1 µl
TaqPlus Precision 10× buffer[a,b]	5.0 µl
100 mM dNTP mix (25 mM of each dNTP)	0.4 µl
DNA template (250 ng/µl)[c]	1.0 µl[c]
Primer #1 (100 ng/µl)[d,e]	1.0 µl
Primer #2 (100 ng/µl)[d,e]	1.0 µl
TaqPlus Precision polymerase mixture (5 U/µl)[f]	0.5 µl (2.5 U)
Total reaction volume	50 µl

[a] The *TaqPlus Precision* 10× buffer provided with this system is optimized to produce PCR products with a minimal number of errors. Modifying the reaction buffer may adversely affect the fidelity and yield of the PCR product.

[b] If the *TaqPlus Precision* 10× buffer appears cloudy, heat the buffer for ~5 minutes at 42°C until clear.

[c] The amount of DNA template required will vary depending on the type of DNA being amplified. Generally 50–1000 ng of genomic DNA template is recommended; however, less DNA template can be used for amplification of lambda or plasmid PCR targets or for amplification of multicopy chromosomal genes. Use of higher amounts of DNA template reduces the number of PCR cycles required and therefore reduces the percentage of PCR products exhibiting mutations.

[d] Primer concentrations between 0.3 and 0.5 µM are recommended (generally 100–250 ng for typical 20- to 30-mer oligonucleotide primers in a 50-µl reaction volume).

[e] Design primers to have similar melting temperatures (typically between 60°C and 80°C).

[f] The amount of *TaqPlus Precision* polymerase mixture will vary depending on the complexity and length of the template to be amplified. A typical starting point is 2.5 U per 50-µl reaction. The standard range for most plasmid and lambda DNA templates is 0.5–1.0 µl (2.5–5.0 U) and for most single-copy genomic DNA templates is 0.5–1.5 µl (2.5–7.5 U) (see *Troubleshooting*).

TABLE II

PCR Cycling Parameters[a,b]

Segment	Number 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/kb PCR target
3	1	72°C	10 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] 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.

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 (Sigma, St. Louis, Missouri).

4. Perform PCR using optimized cycling conditions. Suggested cycling parameters for the *TaqPlus Precision* PCR system are indicated in Table II.

5. Analyze the PCR amplification products by electrophoresis on a 1.0–4.0% (w/v) agarose gel.

TROUBLESHOOTING

Observations	Suggestions
No product or low yield	Increase the amount of *TaqPlus Precision* polymerase mixture. This is especially important for plasmid and lambda targets >10 kb in length or for single-copy, genomic DNA targets >5 kb in length.
	Increase the amount of full-length intact DNA template and/or increase the number of cycles up to a maximum of 40 cycles.
	Use intact and highly purified DNA template.
	Use minimum primer concentrations of 0.3 µM (i.e., at least 100 ng for typical 20-mer oligonucleotide primers in a 50-µl reaction volume).
	Lower the annealing temperature in 5°C increments.
	Allow at least 60 seconds of extension time for each kilobase to be amplified (90 seconds of extension time per kilobase may also be helpful for difficult templates).
	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.
	Primer pairs with matched primer melting temperatures (T_m) and complete complementarity between the primer and template are recommended.
	Gel-purified primers ≥18 nucleotides in length are desired for successful PCR.
	Adjust the ratio of primer versus template to optimize yield of the desired product.
Artifactual smears	Decrease the amount of *TaqPlus Precision* polymerase mixture.
	Reduce the extension time.
	Optimize the cycling parameters specifically for the primer–template set and the thermal cycler used.
Multiple bands	Increase the annealing temperature in 5°C increments and/or use a hot start.[12, 13]
	Use Perfect Match PCR enhancer to improve PCR product specificity or increase the annealing temperature as indicated previously.

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MSDS INFORMATION

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