

AccuPrime™ GC-Rich DNA Polymerase

Cat. No:
12337-016
12337-024

Size:
200 reactions
1000 reactions

Conc: 2 U/μl

Store at -20°C in a non-frost-free freezer

Description

AccuPrime™ GC-Rich DNA Polymerase is a robust enzyme formulation designed for high-specificity, high-yield PCR amplification of difficult GC-rich templates (>65% GC content). This extremely thermostable DNA polymerase, from the archaeobacterium *Pyrolobus fumarius*, retains full activity after incubation at 95°C for 4 hours and has five-fold better processivity than *Taq* DNA polymerase.

The enzyme is supplied with two separate 5X AccuPrime™ GC-Rich Buffer mixtures (A and B) containing thermostable AccuPrime™ proteins, MgSO₄, and dNTPs. Thermostable AccuPrime™ proteins enhance primer-template hybridization during every cycle of PCR, greatly increasing the specificity and robustness of the reaction. Buffer A is optimized for GC-rich genomic DNA targets, while Buffer B is optimized for non-GC-rich genomic DNA, cDNA, and plasmids.

Sufficient reagents are provided for 200 or 1000 amplification reactions of 25 μl each (at 1 unit of enzyme per reaction).

<u>Component</u>	<u>Kit Size</u>	
	<u>200 Rxns</u>	<u>1000 Rxns</u>
AccuPrime™ GC-Rich DNA Polymerase	100 μl	500 μl
5X AccuPrime™ GC-Rich Buffer A	1 ml	5 ml
5X AccuPrime™ GC-Rich Buffer B	1 ml	5 ml
50-mM MgSO ₄	1 ml	1 ml

Unit Definition

One unit of enzyme is the amount of enzyme required to incorporate 10 nmoles of dNTPs into acid insoluble material in 30 minutes at 74°C.

Part no. 12337.pps

Rev. Date: 26 Mar 2004

This product is distributed for laboratory research only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

Enzyme Storage Buffer

2 U/ μ l in 50-mM Tris-HCl (pH 8.0), 100-mM KCl, 1-mM Dithiothreitol (DTT), 0.1-mM EDTA, 50% Glycerol, and 0.1% Triton[®] X-100

5X AccuPrime™ GC-Rich Buffer

Buffer A and B differ in their concentration of MgSO₄ and enhancers. Key components are:

300-mM Tris-HCl (pH 9.2), MgSO₄ at 10 mM (Buffer A) or 7.5 mM (Buffer B), 150-mM NaCl, 1-mM dGTP, 1-mM dATP, 1-mM dTTP, 1-mM dCTP, thermostable AccuPrime™ proteins, and enhancers

Product Qualification

AccuPrime™ GC-Rich DNA Polymerase is evaluated in a PCR functional assay. The polymerase and AccuPrime™ proteins are tested for the absence of double- and single-stranded endonuclease activity as well as the absence of contaminating 5'- and 3'-exonuclease activity.

Recommendations and Guidelines

Template: Use 5–100 ng genomic DNA or plasmid DNA, or 10–100 ng cDNA or bacteriophage lambda DNA

Primers: Use \geq 50 ng each primer per 25- μ l reaction. A T_m of 65–70°C is optimal for most applications. Primer design is one of the most important factors in successful PCR. We recommend using the OligoPerfect™ Designer, available at www.invitrogen.com/oligos.

Buffers: In general, we recommend using Buffer A for GC-rich genomic DNA targets and Buffer B for non-GC-rich genomic DNA, cDNA, and plasmids. Also use Buffer B if you find that Buffer A is inhibitory with your genomic targets.

Magnesium: MgSO₄ is included in Buffer A at a final concentration of 2 mM and Buffer B at 1.5 mM. For some targets, more Mg²⁺ may be required; use the 50-mM MgSO₄ provided in the kit to prepare a titration from 2 mM to 4 mM (final concentration) in 0.25-mM increments.

Reaction: Take appropriate precautions to avoid cross-contamination of DNA between reactions. Ideally, amplification reactions should be assembled in a DNA-free environment. Use of aerosol-resistant barrier tips is recommended.

Protocol

The following protocol is recommended as a starting point. Optimal reaction conditions (incubation times and temperatures; concentrations of enzyme, primers, and template) may vary. After preparation of the samples, transfer them immediately to a preheated thermal cycler and start the amplification program.

1. Add components in the following order to each reaction vessel. Prepare a master mix for multiple reactions to enable accurate pipetting.

DNA template (see previous page)	x μ l
Sense primer (10 μ M)	0.5 μ l
Anti-sense primer (10 μ M)	0.5 μ l
5X Buffer A or B	5 μ l
AccuPrime™ GC-Rich DNA Polymerase (2 U/ μ l)*	0.5 μ l
Sterile water	to 25 μ l

*Up to 2 U of enzyme (1 μ l) may be added for difficult templates.

2. Cap/seal the reaction vessels and flick with your finger for several seconds to mix.
3. Program the thermal cycler as follows. Note that the annealing temperature will vary depending on the T_m of your primers. The optimal annealing temperature is typically 5°C below the T_m of the primers.

Step	Temp (GC-rich template)	Time	Cycle
Denaturation	95°C	3 min	1
Denaturation	95°C	30 sec	25–30
Annealing	55–65°C (5°C < T_m)	30 sec	
Extension	72°C	1 min/kb	
Final Extension	72°C	10 min	1

4. Maintain the reaction at 4°C after cycling. The samples can be stored at -20°C until use. Analyze 5–10 μ l of sample by agarose gel electrophoresis.

References

1. Barnes, W. M. (1992) "The Fidelity of *Taq* Polymerase Catalyzing PCR is Improved by an N-terminal Deletion." *Gene* 112: 29–35.
2. Barnes, W. M. (1994) "PCR Amplification of Up to 35-kb DNA with High Fidelity and High Yield from Lambda Bacteriophage Templates." *Proc. Natl. Acad. Sci. USA* 91: 2216–2220.
3. Rapley, R. (1994) "Enhancing PCR amplification and sequencing using DNA-binding proteins." *Mol. Biotechnol.*, 2, 295–298.

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