AccuPrime™ GC-Rich DNA Polymerase

Cat. No:	Size:
12337-016	200 reactions
12337-024	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 archaebacterium *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, $MgSO_4$, 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).

	Kit Size	
Component	**200 Rxns**	**1000 Rxns**
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.

Enzyme Storage Buffer

2 U/µl 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 $MgSO_4$ and enhancers. Key components are:

300-mM Tris-HCl (pH 9.2), $MgSO_4$ 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

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

Recommendations and Guidelines

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

Primers: Use ≥50 ng each primer per 25-µl 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: $MgSO_4$ is included in Buffer A at a final concentration of 2 mM and Buffer B at 1.5 mM. For some targets, more Mg^{2+} may be required; use the 50-mM $MgSO_4$ 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 µl
Sense primer (10 µM)	0.5 µl
Anti-sense primer (10 µM)	0.5 µl
5X Buffer A or B	5 µl
AccuPrime™ GC-Rich DNA Polymerase (2 U/µl)*	0.5 µl
Sterile water	to 25 µl

*Up to 2 U of enzyme (1 µl) 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 µl of sample by agarose gel electrophoresis.

Purchaser Notification

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