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AccuPrime™ *Pfx* DNA Polymerase

Cat. No. 12344-024 **Size: 200 Reactions**
12344-032 **1000 Reactions**

Conc. 2.5 U/µl **Store at -20°C**

Description

AccuPrime™ *Pfx* DNA Polymerase is a proprietary enzyme preparation containing recombinant DNA polymerase from *Thermococcus* species strain KOD (1,2). This polymerase possesses a proofreading 3′ to 5′ exonuclease activity that provides higher fidelity than *Pfu* DNA polymerase (3). AccuPrime™ *Pfx* DNA Polymerase is a highly processive enzyme and possesses a fast chain extension capability. It is provided in an antibody-bound form that is inactive at ambient temperatures. The enzyme regains activity after the initial denaturation step at 94°C in PCR cycling, providing an automatic "hot start" that increases specificity, sensitivity, and yield, while allowing room temperature assembly (4).

10X AccuPrime™ *Pfx* Reaction Mix contains thermostable AccuPrime™ proteins, $MgSO_4$, and dNTPs. Thermostable AccuPrime™ proteins enhance specific primer-template hybridization during every cycle of PCR (5). The high specificity, fidelity, and yield offered by AccuPrime™ *Pfx* DNA Polymerase make it ideal for demanding PCR applications such as site-directed mutagenesis and PCR expression cloning.

Reagents are provided for 200 or 1000 amplification reactions of 50 µl each.

Component	200-Rxn kit	1000-Rxn kit
AccuPrime™ *Pfx* DNA Polymerase (2.5 U/µl)	100 µl	500 µl
50-mM Magnesium Sulfate	1 ml	2 × 1 ml
10X AccuPrime™ *Pfx* Reaction Mix	1 ml	5 × 1 ml

Unit Definition

One unit of AccuPrime™ *Pfx* DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-insoluble material in 30 min at 74°C.

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AccuPrime™ *Pfx* DNA Polymerase Storage Buffer

50-mM Tris-HCl (pH 8.0), 50-mM KCl, 1-mM DTT, 0.1-mM EDTA, stabilizers, and 50% (v/v) glycerol

Quality Control

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

General Recommendations and Guidelines for PCR

PCR is a powerful technique capable of amplifying trace amounts of DNA. All appropriate precautions should be taken to avoid cross-contamination.

$MgSO_4$: $MgSO_4$ is included in the 10X AccuPrime™ *Pfx* Reaction Mix at a final concentration of 1 mM, which is sufficient for most templates. For further optimization, add 0.1 µl to 1.0 µl of 50-mM $MgSO_4$ (included in the kit) to the reaction.

dNTPs: dNTPs are included in the 10X AccuPrime™ *Pfx* Reaction Mix at a final concentration of 0.3 mM.

Annealing Temperature: The optimal annealing temperature should be 5–10°C lower than the T_m of the primers used; if necessary, gradually increase the annealing temperature by 2–3°C for higher specificity.

KCl: For difficult primer sets, prepare titrations of KCl (not included) at final concentrations of 20–50 mM for further optimization.

PCR Protocol

The following general procedure is suggested as a starting point when using AccuPrime™ *Pfx* DNA Polymerase in any PCR amplification.

1. Add the following components to an autoclaved microcentrifuge tube at either room temperature or on ice:

Component	Volume	Final Conc.
10X AccuPrime™ *Pfx* Reaction mix*	5 µl	1X
Primer mix (10 µM each)*	1.5 µl	0.3 µM each
Template DNA (10 pg–200 ng)	≥1 µl	As required
AccuPrime™ *Pfx* DNA Polymerase**	0.4–1 µl	1.0–2.5 units
Autoclaved, distilled water	to 50 µl	

   *AccuPrime™ *Pfx* DNA Polymerase will not function in reactions that contain dUTP either in the primers or in the dNTP mix.

   **For most targets, 1 unit is optimal. Higher concentrations may be inhibitory. More enzyme may be required for longer targets (>3 kb).

2. Mix contents of the tubes and overlay with mineral or silicone oil, if necessary. (Note: The oil overlay is unnecessary in thermal cyclers equipped with a heated lid.)
3. Cap the tubes and centrifuge briefly to collect the contents.
4. Denature the template for 2 min at 95°C. Perform 25–35 cycles of PCR amplification as follows:

Three-step cycling	Two-step cycling
Denature: 95°C for 15 s	Denature: 95°C for 15 s
Anneal: 55–64°C for 30 s	Extend: 68°C for 1 min per kb
Extend: 68°C for 1 min per kb	

   Note: Two-step cycling can be used for long primers with high T_m.

5. Maintain the reaction at 4°C after cycling. The samples can be stored at -20°C until use.
6. Analyze the products by agarose gel electrophoresis and visualize by ethidium bromide staining.

References

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2. Nishioka M, Mizuguchi H, Fujiwara S, Komatsubara S, Kitabayashi M, Uemura H, Takagi M, Imanaka T. (2001) *J. Biotechnol., 88*, 141–9.
3. Cline, J., Braman., and Hogrefe, H. H. (1996) *Nucleic Acid Res*., *24*, 3546.
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