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

AccuPrime™ *Pfx* DNA Polymerase is functionally tested in an amplification reaction using 100 ng of K562 genomic DNA. A DNA polymerization activity assay measures percent of DNA polymerase inhibition versus an uninhibited control. AccuPrime™ proteins are tested for absence of double- and single-strand endonuclease activity and absence of 5′ and 3′ exonuclease activity.

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

1. Takagi, M., Nishioka, M., Kakihara, H., Kitabayashi, M., Inoue, H., Kawakami, B., Oka, M., and Imanaka, T. (1997) *Appli. Environ. Microbiol.*, *63*, 4504-4510.
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.
4. Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., Daiss, J.L. (1994) *BioTechnology*, *12*, 506.
5. Rapley, R. (1994) *Mol.* Biotechnol., *2*, 295–298.

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