# AccuPrime™ *Pfx* DNA Polymerase

**Catalog Numbers** 12344-024 and 12344-032

**Doc. Part No.** 12344.pps **Pub. No.** MAN0001079 **Rev.** A.0

**WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

## Product description

The Invitrogen™ AccuPrime™ *Pfx* DNA Polymerase is a proprietary enzyme preparation containing recombinant DNA polymerase from *Thermococcus* species *KOD*. This polymerase possesses a proofreading 3′ to 5′ exonuclease activity that provides higher fidelity than *Pfu* DNA polymerase. The AccuPrime™ *Pfx* DNA Polymerase is a highly processive enzyme and possesses a fast chain extension capability. The 10X AccuPrime™ *Pfx* Reaction Mix contains $MgSO_4$, dNTPs, and thermostable AccuPrime™ proteins; the thermostable proteins enhance specific primer-template hybridization during every cycle of PCR.

The AccuPrime™ *Pfx* DNA Polymerase is provided in an antibody-bound form that is inactive at room temperatures. The enzyme regains activity after the initial denaturation step at 94°C in PCR cycling, providing an automatic "hot start". The hot start increases specificity, sensitivity, and yield, while allowing room-temperature assembly.

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.

## Contents and storage

| Contents | Cat. No. 12344-024 (200 reactions) | Cat. No. 12344-032 (1,000 reactions) | Storage |
|---|---|---|---|
| AccuPrime™ *Pfx* DNA Polymerase (2.5 U/µL) | 100 µL | 500 µL | –20°C |
| 50 mM Magnesium sulfate | 1 mL | 2 × 1 mL | |
| 10X AccuPrime™ *Pfx* Reaction Mix | 1 mL | 5 × 1 mL | |

**Note: Unit (U) definition:** One unit of AccuPrime™ *Pfx* DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-insoluble material in 30 minutes at 74°C.

### Storage buffer

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

## Procedural guidelines

- Take appropriate precautions to avoid cross-contamination.
- $MgSO_4$ 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 0.1–1.0 µL of 50 mM $MgSO_4$ (included in the kit) to the reaction.
- dNTPs are included in the 10X AccuPrime™ *Pfx* Reaction Mix at a final concentration of 0.3 mM.
- Use an annealing temperature that is 5–10°C lower than the $T_m$ of the primers. If needed, gradually increase the annealing temperature by 2–3°C for higher specificity.
- For difficult primer sets, prepare titrations of KCl (not included) at final concentrations of 20–50 mM for further optimization.

## Perform the PCR

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 room temperature or on ice:

| Component | Volume for one 50-µL reaction | Final concentration |
|---|---|---|
| 10X AccuPrime™ *Pfx* Reaction Mix[1] | 5 µL | 1X |
| Primer mix (10 µM each)[1] | 1.5 µL | 0.3 µM each |
| Template DNA (10 pg to 200 ng) | ≥1 µL | as required |
| AccuPrime™ *Pfx* DNA Polymerase[2] | 0.4–1 µL | 1.0–2.5 U |
| Autoclaved, distilled water | to 50 µL | — |

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

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

2. Mix the tube contents; if needed, cover with mineral or silicone oil.

   **Note:** The oil is not needed in thermal cyclers equipped with a heated lid.

3. Cap the tubes, then centrifuge briefly to collect the contents.
4. Perform thermal cycling as follows:

| Step | Three-step cycling | | Two-step cycling | |
|---|---|---|---|---|
| | Temp. | Time | Temp. | Time |
| Initial denaturation | 95°C | 2 minutes | 95°C | 2 minutes |
| **25–35 cycles of:** | | | | |
| Denature | 95°C | 15 seconds | 95°C | 15 seconds |
| Anneal | 55–64°C | 30 seconds | — | — |
| Extend | 68°C | 1 minute per kb | 68°C | 1 minute 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.

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| Revision | Date | Description |
|---|---|---|
| A.0 | 5 May 2016 | Format, style, and legal updates |
| — | 1 June 2010 | Baseline for this revision history |



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