# AccuPrime™ *Pfx* SuperMix

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**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* SuperMix provides qualified reagents for the high-fidelity PCR amplification of DNA templates. It includes recombinant DNA polymerase from *Thermococcus* species *KOD*, anti-*KOD* antibodies, thermostable AccuPrime™ proteins, $MgSO_4$, dNTPs, and stabilizers in a convenient and highly optimized SuperMix formulation for ease of reaction setup.

The AccuPrime™ *Pfx* DNA Polymerase possesses a proofreading 3′ to 5′ exonuclease activity that provides higher fidelity than *Pfu* DNA polymerase. This highly processive enzyme 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 thermostable AccuPrime™ proteins enhance specific primer-template hybridization during every cycle of PCR.

The high specificity, fidelity, and yield offered by AccuPrime™ *Pfx* SuperMix make it ideal for demanding PCR applications such as site-directed mutagenesis and PCR expression cloning.

The AccuPrime™ *Pfx* SuperMix is suitable for targets up to 15 kb in length. It is supplied at 1.1X concentration to allow ~10% of the final reaction volume to be used for the addition of primer and template solutions.

## Contents and storage

| Contents | Number of reactions | Amount | Storage |
|---|---|---|---|
| AccuPrime™ *Pfx* SuperMix[1] | 200 | 4 × 1.125 mL | –20°C in a non-frost-free freezer |

[1] The AccuPrime™ *Pfx* SuperMix contains 22 U/mL of *Thermococcus* species *KOD* thermostable polymerase complexed with anti-*KOD* antibodies, 66 mM Tris-$SO_4$ (pH 8.4), 30.8 mM $(NH4)_2SO_4$, 11 mM KCl, 1.1 mM $MgSO_4$, 330 µM dNTPs, AccuPrime™ proteins, and stabilizers.

**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.

## Procedural guidelines

- Take appropriate precautions to avoid cross-contamination.
- For multiple reactions, prepare a master mix of AccuPrime™ *Pfx* SuperMix and the component(s) common to all reactions.
- 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.
- If the PCR efficiency is not optimal, repeat the reaction with different primer concentrations from 100–500 nM, in 100 nM increments.

## Perform the PCR

1. Add the following components in any order to each reaction tube:

| Component | Amount for one 25-µL reaction |
|---|---|
| AccuPrime™ *Pfx* SuperMix | 22.5 µL |
| Forward and reverse primers | 200 nM final concentration of each is recommended |
| Template DNA solution | 10 pg to 200 ng |

**Note:** A standard 25-µL PCR reaction includes a combined primer and template volume of 2.5 µL. We have observed no decrease in product yield if the amount of primer and template solution is between 0.5 µL and 7.5 µL.

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 load the tubes in a thermal cycler.
4. Use the following PCR program as a starting point for your template and primers:

| Step | Temperature | Time |
|---|---|---|
| Initial denaturation | 95°C | 5 minutes |
| **35 cycles of:** | | |
| Denature | 95°C | 15 seconds |
| Anneal | 55–65°C | 30 seconds |
| Extend | 68°C | 1 minute per kb |

5. Maintain the reactions at 4°C after cycling. Samples can be stored at –20°C until use.

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**Revision history:** Pub. No. MAN0001080

| Revision | Date | Description |
|---|---|---|
| A.0 | 5 May 2016 | Format, style, and legal updates |
| — | 7 June 2010 | Baseline for this revision history |




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