Limited Use License No. 1: Thermotolerant polymerases

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AccuPrime™ Pfx SuperMix

Cat. No. 12344-040
Size: 200 Reactions
Store at -20°C in a non-frost-free freezer

Description

AccuPrime™ Pfx SuperMix provides qualified reagents for the high fidelity amplification of DNA templates by polymerase chain reaction (PCR). It includes recombinant DNA polymerase from Thermococcus species KOD, anti-KOD antibodies, thermostable AccuPrime™ proteins, MgSO₄, dNTPs, and stabilizers in a convenient and highly optimized SuperMix formulation for ease of reaction setup (1). It is suitable for targets up to 15 kb in length. AccuPrime™ Pfx DNA polymerase possesses a proofreading 3’ to 5’ exonuclease activity that provides higher fidelity than Pfu DNA polymerase (2). This highly processive enzyme 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 (3).

Thermostable AccuPrime™ proteins enhance specific primer-template hybridization during every cycle of PCR (4). 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.

AccuPrime™ Pfx SuperMix is supplied at 1.1X concentration to allow approximately 10% of the final reaction volume to be used for the addition of primer and template solutions. Reagents sufficient for 200 amplification reactions of 25 µl each are provided.

Component

AccuPrime™ Pfx SuperMix
200-Rxn kit
4 x 1.125 ml

Part. No. 1234400.pps
Rev. Date: 15 Oct 2004

This product is distributed for laboratory research. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Invitrogen Tech-Line™ U.S.A. 800 953 6288

AccuPrime™ Pfx SuperMix

Cat. No. 12344-040
Size: 200 Reactions
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Description

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AccuPrime™ Pfx SuperMix is supplied at 1.1X concentration to allow approximately 10% of the final reaction volume to be used for the addition of primer and template solutions. Reagents sufficient for 200 amplification reactions of 25 µl each are provided.

Component

AccuPrime™ Pfx SuperMix
200-Rxn kit
4 x 1.125 ml

Part. No. 1234400.pps
Rev. Date: 15 Oct 2004

This product is distributed for laboratory research. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
Unit Definition
One unit of AccuPrime™ Pfx DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-insoluble material in 30 min at 74°C.

AccuPrime™ Pfx SuperMix Components
22 U/ml Thermococcus species KOD thermostable polymerase complexed with anti-KOD antibodies, 66 mM Tris-SO4 (pH 8.4), 30.8 mM (NH4)2SO4, 11 mM KCl, 1.1 mM MgSO4, 330 µM dNTPs, AccuPrime™ proteins, stabilizers.

Quality Control
AccuPrime™ Pfx SuperMix 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 with anti-KOD antibodies 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.

Recommendations and Guidelines:
- PCR is a powerful technique capable of amplifying trace amounts of DNA; take all appropriate precautions to avoid cross-contamination.
- PCR Protocol
1. Add the following components in any order to each reaction tube:
   - 22.5 µl AccuPrime™ Pfx SuperMix
   - Forward and reverse primers (200 nM final concentration of each is recommended)*
   - Template DNA solution (10 pg–200 ng)*
   *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 contents of the tubes and overlay with mineral or silicone oil, if necessary.
3. Cap the tubes and load in the thermal cycler.
4. Use the following PCR program as a starting point for your template and primers:
   - 95°C for 5 minutes
   - 35 cycles of:
     - 95°C for 15 seconds
     - 55–65°C for 30 seconds
     - 68°C for 1 minute per kb
5. Maintain reaction at 4°C after cycling. Samples can be stored at -20°C.

References

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

AccuPrime™ Pfx SuperMix Components
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Quality Control
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Recommendations and Guidelines:
- PCR is a powerful technique capable of amplifying trace amounts of DNA; take all appropriate precautions to avoid cross-contamination.
- For multiple reactions, you can prepare a master mix of AccuPrime™ Pfx SuperMix and the component(s) common to all reactions.
- The optimal annealing temperature should be 5-10°C lower than the Tₘ of the primers used; if necessary, 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 to 500 nM, in 100 nM increments.

PCR Protocol
1. Add the following components in any order to each reaction tube:
   - 22.5 µl AccuPrime™ Pfx SuperMix
   - Forward and reverse primers (200 nM final concentration of each is recommended)*
   - Template DNA solution (10 pg–200 ng)*
   *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 contents of the tubes and overlay with mineral or silicone oil, if necessary.
3. Cap the tubes and load in the thermal cycler.
4. Use the following PCR program as a starting point for your template and primers:
   - 95°C for 5 minutes
   - 35 cycles of:
     - 95°C for 15 seconds
     - 55–65°C for 30 seconds
     - 68°C for 1 minute per kb
5. Maintain reaction at 4°C after cycling. Samples can be stored at -20°C.

References
**Limited Use License No. 1: Thermostable polymerases**

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**Limited Use License No. 12: SuperMix Products**

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**Limited Use License No. 15: Pfx DNA Polymerase**

This product is manufactured for Invitrogen by Toyobo Co., Ltd.

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**AccuPrime™ Pfx SuperMix**

**Cat. No. 12344-040**

**Size:** 200 Reactions

**Store at -20°C in a non-frost-free freezer**

**Description**

AccuPrime™ Pfx SuperMix provides qualified reagents for the high fidelity amplification of DNA templates by polymerase chain reaction (PCR). It includes recombinant DNA polymerase from Thermus species KOD, anti-KOD antibodies, thermostable AccuPrime™ proteins, MgSO₄, dNTPs, and stabilizers in a convenient and highly optimized SuperMix formulation for ease of reaction setup (1). It is suitable for targets up to 15 kb in length. AccuPrime™ Pfx DNA polymerase possesses a proofreading 3' to 5' exonuclease activity that provides higher fidelity than Pfu DNA polymerase (2). This highly processive enzyme 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 (3).

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**AccuPrime™ Pfx SuperMix** is supplied at 1.1X concentration to allow approximately 10% of the final reaction volume to be used for the addition of primer and template solutions. Reagents sufficient for 200 amplification reactions of 25 µl each are provided.

**Component**

**AccuPrime™ Pfx SuperMix** [200-Rxn kit]

- **200 x 1.125 ml**

**Part No.** 123440-040.pps

**Rev. Date:** 15 Oct 2004

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**AccuPrime™ Pfx SuperMix**

**Cat. No. 12344-040**

**Size:** 200 Reactions

**Store at -20°C in a non-frost-free freezer**

**Description**

AccuPrime™ Pfx SuperMix provides qualified reagents for the high fidelity amplification of DNA templates by polymerase chain reaction (PCR). It includes recombinant DNA polymerase from Thermus species KOD, anti-KOD antibodies, thermostable AccuPrime™ proteins, MgSO₄, dNTPs, and stabilizers in a convenient and highly optimized SuperMix formulation for ease of reaction setup (1). It is suitable for targets up to 15 kb in length. AccuPrime™ Pfx DNA polymerase possesses a proofreading 3' to 5' exonuclease activity that provides higher fidelity than Pfu DNA polymerase (2). This highly processive enzyme 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 (3).

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**AccuPrime™ Pfx SuperMix** is supplied at 1.1X concentration to allow approximately 10% of the final reaction volume to be used for the addition of primer and template solutions. Reagents sufficient for 200 amplification reactions of 25 µl each are provided.

**Component**

**AccuPrime™ Pfx SuperMix** [200-Rxn kit]

- **200 x 1.125 ml**

**Part No.** 123440-040.pps

**Rev. Date:** 15 Oct 2004