Invitrogen
life technologies

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, $MgSO_4$, 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	200-Rxn kit
AccuPrime™ *Pfx* SuperMix	4 × 1.125 ml

Part. No. 12344040.pps Rev. Date: 15 Oct 2004

This product is distributed for laboratory research only. 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-SO_4 (pH 8.4), 30.8 mM $(NH_4)_2SO_4$, 11 mM KCl, 1.1 mM $MgSO_4$, 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.
- 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 T_m of the primers used; if necessary, gradually increase the annealing temperature by 2–3ºC 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.

©2003–2004 Invitrogen Corporation. All rights reserved. For research use only. Not intended for any animal or human therapeutic or diagnostic use.

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

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. Cline, J., Braman., and Hogrefe, H. H. (1996) *Nucleic Acid Res*., *24*, 3546.
3. Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., Daiss, J.L. (1994) *BioTechnology*, *12*, 506.
4. Rapley, R. (1994) *Mol. Biotechnol., 2,* 295–298.

Limited Use Label License No. 1: Thermostable polymerases

A license under U.S. Patents 4,683,202, 4,683,195, 4,965,188, and 5,075,216 or their foreign counterparts, owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche, Ltd. ("Roche"), has an up-front fee component and a running-royalty component. The purchase price of this product includes limited, nontransferable rights under the running-royalty component to use only this amount of the product to practice the polymerase chain reaction (PCR) and related processes described in said patents solely for the research and development activities of the purchaser when this product is used in conjunction with a thermal cycler whose use is covered by the up-front fee component. The end user must obtain rights to the up-front fee component in order to have a complete license. These rights under the up-front fee component may be purchased from Applied Biosystems or obtained by purchasing an Authorized Thermal Cycler. No right to perform or offer commercial services of any kind using PCR including, without limitation, reporting the results of the purchaser's activities for a fee or other commercial consideration, is hereby granted by implication or estoppel. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

Limited Use Label License No. 12: SuperMix Products

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of patents owned by Invitrogen Corporation and claiming this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500.

Limited Use Label License No. 14: Platinum® Products

Licensed to Invitrogen Corporation, under U.S. Patent Nos. 5,338,671; 5,587,287, and foreign equivalents for use in research only.

Limited Use Label License No. 33: AccuPrime™ Reagent

This product is sold under licensing arrangements with Stratagene. The purchase price of this product includes limited, nontransferable rights under U.S. Patents Nos. 5,449,603; 5,605,824; 5,646,019; and 5,773,257 and foreign equivalents owned by Stratagene to use only this amount of the product to practice the claims in said patents solely for activities of the purchaser within the field of research. Further information on purchasing licenses under the above patents may be obtained by contacting the Director of Business Development, Stratagene, 11011 North Torrey Pines Road, La Jolla, California 92037.

Limited Use Label License No. 134: AccuPrime™ *Taq* and AccuPrime™ *Pfx*

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of patents owned by Invitrogen Corporation and claiming this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500.

Limited Use Label License No. 136: *Pfx* DNA Polymerase

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