

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 × 1.125 ml

Part. No. 12344040.pps

MAN0001080

Rev. Date: 07 Jun 2010

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

The Certificate of Analysis (CofA) provides detailed quality control information for each product. The CofA is available on our website at www.invitrogen.com/cofa, and is searchable by product lot number, which is printed on each box.

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 2–3°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

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

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the U.S.: 5,789,224, 5,618,711, 6,127,155. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Limited Use Label License No. 5: Invitrogen Technology

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. For products that are subject to multiple limited use label licenses, the terms of the most restrictive limited use label license shall control. Life Technologies Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by Life Technologies Corporation which cover 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, Life Technologies is willing to accept return of the product with a full refund. For information about purchasing a license to use this product or the technology embedded in it for any use other than for research use please contact Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008 ; Phone (760) 603-7200 or e-mail: outlicensing@lifetech.com.

Limited Use Label License No. 14 Direct Inhibition by Anti-Polymerase Antibodies

Licensed to Life Technologies 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. 136: Pfx DNA Polymerase

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

©2010 Life Technologies Corporation. All rights reserved. For research use only. Not intended for any animal or human therapeutic or diagnostic use. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.