invitrogen
by life technologies

AccuPrime™ Taq DNA Polymerase High Fidelity

Cat. nos.	Size	Conc. 5 U/µL
12346-086	200 rxns	Store at –30°C to –10°C
12346-094	1000 rxns	

Pub. Part no. 12346.pps MAN0001081 **Rev. Date** 22 November 2011

Description

AccuPrime™ *Taq* DNA Polymerase High Fidelity provides qualified reagents for the high-fidelity amplification of nucleic acid templates by PCR. It includes an enzyme mixture composed of recombinant *Taq* DNA polymerase, *Pyrococcus* species *GB-D* polymerase, and Platinum® *Taq* Antibody.[1,2] This enzyme blend results in a nine-fold increase in fidelity over *Taq* alone and is effective over a wide range of target sizes—up to 20 kb with some optimization. Like regular *Taq*, AccuPrime™ *Taq* DNA Polymerase High Fidelity has a nontemplate-dependent terminal transferase activity that adds a single deoxyadenosine (A) to the 3' ends of PCR products.

Pyrococcus species *GB-D* polymerase is a proofreading enzyme that possesses a 3′ → 5′ exonuclease activity.[3] Mixture of this enzyme with *Taq* DNA polymerase increases fidelity and allows amplification of simple and complex DNA templates over a large range of target sizes. The Platinum® antibody complexes with *Taq* DNA polymerase and inhibits activity at room temperature. Activity is restored after the initial denaturation step at 94ºC, providing an automatic "hot start" PCR.[4,5]

The thermostable AccuPrime™ protein enhances specific primer-template hybridization during every cycle of PCR. Antibody/AccuPrime™ protein-mediated amplification dramatically improves specificity and provides the most robust PCR for multiplexing and suboptimal primer sets. Two AccuPrime™ PCR buffer mixtures are provided for amplifying specific types of templates. Buffer I is optimized for plasmids, cDNA, and λ DNA. Buffer II is optimized for genomic DNA.

Storage Buffer

20 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, 50% (v/v) glycerol, stabilizers.

Unit Definition

One unit incorporates 10 nmol of deoxyribonucleotide into DNA in 30 minutes at 74°C.

Product Use: For research use only.
Not intended for any animal or human therapeutic or diagnostic use.

Contents

Component	Kit Size	
	200 Rxns	1000 Rxns
AccuPrime™ *Taq* DNA Polymerase High Fidelity	40 µL	200 µL
10X AccuPrime™ PCR Buffer I	1 mL	4 × 1.25 mL
10X AccuPrime™ PCR Buffer II	1 mL	4 × 1.25 mL
Mg SO_4 (50 mM)	1 mL	1 mL

10X AccuPrime™ PCR Buffer I and II

Buffer I and II differ in their concentration of thermostable AccuPrime™ protein. Their components are:

600 mM Tris-SO_4 (pH 8.9), 180 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, 2 mM dGTP, 2 mM dATP, 2 mM dTTP, 2 mM dCTP, thermostable AccuPrime™ protein, 10% glycerol.

The supplied PCR Buffer is a 10X concentrate and should be diluted for use.

Protocol Guidelines and Recommendations

- Critical parameters and troubleshooting information are documented in reference 1. Assemble PCR reactions in a DNA-free environment. We recommend use of clean dedicated automatic pipettors and aerosol resistant barrier tips. **Always** keep the control DNA and other templates to be amplified isolated from the other components.
- If PCR efficiency is not optimal, repeat the reaction with different primer concentrations from 100 to 500 nM (final concentration), in 100-nM increments.
- $MgSO_4$ is included in the 10X AccuPrime™ PCR Buffer at a final concentration of 2 mM, which is sufficient for most targets. For some targets, more Mg^{2+} may be required; use the 50-mM $MgSO_4$ provided in the kit to prepare a titration from 2 mM to 4 mM (final concentration) in 0.25-mM increments.
- For longer genomic DNA targets (>15 kb), we recommend using 2–2.5 U of AccuPrime™ *Taq* DNA Polymerase High Fidelity and increasing the extension time as specified (1 min per kb).
- Do not denature for more than 30 seconds if target is larger than 12 kb.

Protocol

Use the following protocol as a starting point and guideline when preparing your reactions. Adjust the reaction size as needed. Use Buffer I for plasmids, cDNA, and λ DNA and Buffer II for genomic DNA (up to 20 kb) as indicated.

1. Add the following components to a DNase/RNase-free, thin-walled PCR tube. For multiple reactions, prepare a master mix of common components to minimize reagent loss and enable accurate pipeting.

	Template Type	
Component	**Plasmids/cDNA/λ DNA**	**Genomic DNA**
10X AccuPrime™ PCR Buffer I	5 µl	—
10X AccuPrime™ PCR Buffer II	—	5 µl
Sense primer (10 µM)	1 µl	1 µl
Anti-sense primer (10 µM)	1 µl	1 µl
Template DNA	0.1 pg–20 ng	10 pg–500 ng
AccuPrime™ *Taq* High Fidelity	0.2 µl*	0.2 µl*
Autoclaved, distilled water	to 50 µl	to 50 µl

*0.2 µl = 1.0 unit, which is sufficient for amplifying most targets. In some cases (e.g., longer targets), more enzyme may be required, up to 2.5 units.

2. Cap the tube, tap gently to mix, and centrifuge briefly to collect the contents.

3. Place the tube in the thermal cycler and run the following program.

   Initial denaturation: 94ºC for 15 seconds to 2 minutes (do not denature for more than 30 seconds if target is larger than 12 kb)

   25–35 cycles of:
   Denature: 94ºC for 15–30 seconds
   Anneal: 52–64°C for 15–30 seconds
   Extend: 68ºC for 1 minute per kb of PCR product

   After cycling, maintain the reaction at 4ºC. Samples can be stored at -20ºC until use.

4. Analyze the amplification products by agarose gel electrophoresis .We recommend using E-Gel® 1.2% gels and TrackIt™ 100 bp or 1kb Plus DNA ladders (see **Additional Products** on page 4).

References

1. Innis, M.A., Myambo, K.B., Gelfand, D.H. and Brow, M.A.D. (1988) Proc. Natl. Acad. Sci. USA 85, 9436.
2. Barnes, W.M. (1994) *Proc. Natl. Acad. Sci. USA* 91, 2216.
3. Tindall, K.R. and Kunkel, T.A. (1988) *Biochemistry 27*, 6008.
4. Chou, Q., Russel, M., Birch, D., Raymond, J., Bloch, W. (1992) *Nucl. Acids Res., 20*, 1717.
5. Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., Daiss, J.L. (1994) *BioTechnology, 12*, 506.
6. Westfall, B., Sitaraman, K., Solus, J., Hughes, J., Rashtchian, A. (1997) *Focus® 19*, 46.

Additional Products

Product	Amount	Catalog no.
10 mM dNTP Mix, PCR Grade	100 µL	18427-013
10 mM dNTP Mix, PCR Grade	1 mL	18427-088
E-Gel® 1.2% Starter Pak	6 gels plus PowerBase™	G6000-01
E-Gel® 1.2% 18-Pak	18 gels	G5018-01
TrackIt™ 100 bp DNA Ladder	100 applications	10488-058
TrackIt™ 1kb Plus DNA Ladder	100 applications	10488-085

Product Qualification and SDS

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/sds**.

Limited Use Label License No. 358: Research Use Only

The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008

Limited Use Label License

This product is sold under licensing arrangements with Stratagene. The purchase price of this product includes limited, nontransferable rights 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.

©2011 Life Technologies Corporation. All rights reserved. The trademarks mentioned herein are the property of Life Technologies Corporation or their respective owners.

For support visit www.lifetechnologies.com/support or email techsupport@lifetech.com
www.lifetechnologies.com

life technologies™