AccuPrime™ *Taq* DNA Polymerase, High Fidelity

Catalog Numbers 12346-086 and 12346-094

Doc. Part No. 12346.pps **Pub. No.** MAN0001081 **Rev.** A.0

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™ *Taq* DNA Polymerase, High Fidelity, provides qualified reagents for the high-fidelity PCR amplification of nucleic acid templates. It includes an enzyme blend of recombinant *Taq* DNA polymerase, *Pyrococcus* species GB-D polymerase, and Platinum™ *Taq* Antibody. This enzyme blend results in a 9-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 non-template-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. Mixing 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 94°C, providing an automatic "hot start" PCR.

The thermostable AccuPrime™ protein enhances specific primer-template hybridization during every cycle of the PCR. Antibody/AccuPrime™ protein-mediated amplification dramatically improves specificity and provides the most robust PCR for multiplexing and suboptimal primer sets. Two AccuPrime™ PCR Buffers 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.

Contents and storage

Contents	Cat. No.		Storage
	12346-086 (200 rxns)	12346-094 (1,000 rxns)	
AccuPrime™ *Taq* DNA Polymerase, High Fidelity (5 U/µL)	40 µL	200 µL	–30°C to –10°C
$MgSO_4$ (50 mM)	1 mL	1 mL	
10X AccuPrime™ PCR Buffer I[1]	1 mL	4 × 1.25 mL	
10X AccuPrime™ PCR Buffer II[1]	1 mL	4 × 1.25 mL	

[1] PCR 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 each of dGTP, dATP, dTTP, and dCTP, thermostable AccuPrime™ protein, and 10% glycerol.

Note: Unit (U) definition: One unit incorporates 10 nmol of deoxyribonucleotide into DNA in 30 minutes at 74°C.

Storage buffer

- 20 mM Tris–HCl (pH 8.0)
- 0.1 mM EDTA
- 1 mM DTT
- Stabilizers
- 50% (v/v) glycerol

Procedural guidelines

- Assemble PCR reactions in a DNA-free environment. We recommend 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 100–500 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; start at 2 mM, then increase in 0.25 mM increments up to 4 mM (final concentration).
- For longer genomic DNA targets (>15 kb), use 2–2.5 U of the AccuPrime™ polymerase, and increase the extension time as specified (1 minute per kb).
- Do not denature for more than 30 seconds if the target is >12 kb.

Perform the PCR

Use the following protocol as a starting point when preparing your reactions. Use PCR Buffer I for plasmids, cDNA, and λ DNA; use PCR Buffer II for genomic DNA (up to 20 kb).

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.

Component	Amount for one 50-µL reaction[1]	
	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 to 20 ng	10 pg to 500 ng
AccuPrime™ *Taq* DNA Polymerase, High Fidelity [2]	0.2 µL	0.2 µL
Autoclaved, distilled water	to 50 µL	to 50 µL

[1] This table provides amounts for a single 50-µL reaction. Adjust the reaction size as needed.

[2] 0.2 µL = 1.0 unit, which is sufficient for amplifying most targets. In some cases (e.g., longer targets), up to 2.5 units of enzyme may be required.

2. Cap the tube, tap gently to mix, then centrifuge briefly to collect the contents.
3. Place the tube in the thermal cycler, then run the following program:

Step	Temperature	Time
Initial denaturation	94°C	15 seconds to 2 minutes[1]
25–35 cycles of:		
Denature	94°C	15–30 seconds
Anneal	52–64°C	15–30 seconds
Extend	68°C	1 minute per kb

[1] Do not denature for more than 30 seconds if the target is >12 kb.

4. Maintain the reactions at 4°C after cycling. Samples can be stored at –20°C until use.
5. Analyze the amplification products by agarose gel electrophoresis. We recommend using E-Gel™ 1.2% gels and TrackIt™ 100 bp DNA Ladder or 1 kb Plus DNA Ladder. See "Ordering information" on page 4.

Ordering information

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Amount	Source
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™ 1 kb Plus DNA Ladder	100 applications	10488-085

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

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Revision history: Pub. No. MAN0001081

Revision	Date	Description
A.0	5 May 2016	Format, style, and legal updates
—	22 November 2011	Baseline for this revision history

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