AccuPrime™ *Taq* DNA Polymerase System

Cat. No. 12339-016 **Size: 200 Reactions**
Cat. No. 12339-024 **Size: 1,000 Reactions**

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

Description

The AccuPrime™ *Taq* DNA Polymerase System provides qualified reagents for the amplification of nucleic acid templates by polymerase chain reaction (PCR). AccuPrime™ *Taq* DNA polymerase contains anti-*Taq* DNA polymerase antibodies. The 10X AccuPrime™ buffers contain thermostable AccuPrime™ protein, Mg^{++}, and deoxyribonucleotide triphosphates at concentrations sufficient to allow amplification during PCR. Two individual buffer systems (10X AccuPrime™ PCR Buffer I and II) are provided for amplification of specific types of templates.* Reagents sufficient for 200 or 1,000 amplification reactions of 25 µl each are provided.

Anti-*Taq* DNA polymerase antibodies inhibit polymerase activity, providing an automatic "hot start" (1,2) and permitting room temperature set-up. The thermostable AccuPrime™ protein enhances specific primer-template hybridization during every cycle of PCR. Antibody/AccuPrime™ protein-mediated amplification dramatically improves PCR specificity, improves the fidelity of *Taq* by two fold, and provides the most robust PCR for multiplex PCR and suboptimal primer sets.

	Amount	
Component	**200 rxn kit**	**1,000 rxn kit**
AccuPrime™ *Taq* DNA Polymerase	100 µl	500 µl
10X AccuPrime™ PCR Buffer I*	500 µl	2 × 1.25 ml
10X AccuPrime™ PCR Buffer II*	500 µl	2 × 1.25 ml
50 mM Magnesium Chloride	500 µl	500 µl

*10X AccuPrime™ PCR Buffer I is designed for small genomic DNA amplicons (≤200 bp), cDNA, or plasmids.
10X AccuPrime™ PCR Buffer II is designed for genomic DNA (200 bp–4 kb).

Storage Buffer

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

10X AccuPrime™ PCR Buffer I and II

200 mM Tris-HCl (pH 8.4), 500 mM KCl, 15 mM $MgCl_2$, 2 mM dGTP, 2 mM dATP, 2 mM dTTP, 2 mM dCTP, thermostable AccuPrime™ protein, 10% glycerol

Product Qualification

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.

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.

PCR Precautions

Since PCR is a powerful technique capable of amplifying trace amounts of DNA, all appropriate precautions should be taken to avoid cross-contamination. Ideally, amplification reactions should be assembled in a DNA-free environment. Use of aerosol-resistant barrier tips is recommended. Take care to avoid contamination with the primers or template DNA used in individual reactions. PCR products should be analyzed in an area separate from the reaction assembly area.

General Protocol

The following procedure is suggested as a general guideline when using AccuPrime™ *Taq* DNA Polymerase in any PCR amplification. Optimal reaction conditions (incubation times and temperatures; the amounts of AccuPrime™ *Taq* DNA Polymerase, primers, $MgCl_2$, and template DNA) will vary and may need to be optimized. Reaction sizes may be altered to suit user preference, as shown in the tables below.

1. Add the following components to a sterile thin walled 0.25-ml or 0.5-ml PCR tube either at room temperature or on ice:

For Small Genomic DNA (≤200 bp), Plasmids, or cDNA:

Component	10-µl Reaction	25-µl Reaction	50-µl Reaction
10X AccuPrime™ PCR Buffer I	1 µl	2.5 µl	5 µl
Primer Mix (10 µM each)	0.2 µl	0.5 µl	1 µl
Template DNA	10 pg–200 ng	10 pg–200 ng	10 pg–200 ng
AccuPrime™ *Taq* DNA Polymerase	0.25 µl	0.5 µl	1 µl
Autoclaved distilled water	To 10 µl	To 25 µl	To 50 µl

For Genomic DNA (200 bp-4 kb):

Component	10-µl Reaction	25-µl Reaction	50-µl Reaction
10X AccuPrime™ PCR Buffer II	1 µl	2.5 µl	5 µl
Primer Mix (10 µM each)	0.2 µl	0.5 µl	1 µl
Template DNA	1–200 ng	1–200 ng	1–200 ng
AccuPrime™ *Taq* DNA Polymerase	0.25 µl	0.5 µl	1 µl
Autoclaved distilled water	To 10 µl	To 25 µl	To 50 µl

Note: Prepare a master mix for multiple reactions to minimize reagent loss and variation due to pipetting.

2. Mix contents of the tubes and overlay with 50 µl of mineral or silicone oil, if necessary.
3. Cap the tubes and centrifuge briefly to collect the contents.
4. Incubate tubes in a thermal cycler at 94°C for 2 min to completely denature the template and activate the enzyme.
5. Perform 25-35 cycles of PCR amplification as follows:

   Denature: 94°C for 15-30 s
   Anneal: 55°C-60°C for 15-30 s
   Extend: 68°C for 1 min per kb
6. Maintain the reaction at 4°C after cycling. The samples can be stored at -20°C until use.
7. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.

Multiplex PCR Protocol

The following specialized procedure is suggested as a guideline and as a starting point when using AccuPrime™ *Taq* DNA Polymerase in multiplex PCR amplification. Optimal reaction conditions (incubation times and temperatures; the amounts of AccuPrime™ *Taq* DNA Polymerase, primers, $MgCl_2$, and template DNA) will vary and may need to be optimized. Reaction sizes may be altered to suit user preference, as shown in the tables below.

1. Add the following components to a sterile, thin-walled 0.25-ml or 0.5-ml PCR tube either at room temperature or on ice:

Component	Amount
10X AccuPrime™ PCR Buffer I (for genomic DNA ≤200 bp, cDNA, or plasmids) ***or*** 10X AccuPrime™ PCR Buffer II (for genomic DNA 200 bp–4 kb)	5 µl
Primer mix (10 µM each)	1 µl each (0.2 µM each)
Template DNA	100-200 ng
AccuPrime™ *Taq* DNA Polymerase*	1-2.5 µl
Autoclaved, distilled water	to 50 µl

*For primer mixes up to 5 sets, 1 µl of enzyme is sufficient.

If desired, a master mix can be prepared for multiple reactions, to minimize reagent loss and to enable accurate pipetting.

2. Continue with steps 2-7 of the General Protocol.

References

1. Chou, Q., Russel, M., Birch, D., Raymond, J., Bloch, W. (1992) *Nucl. Acids Res. 20*, 1717.
2. Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., Daiss, J.L. (1994) *BioTechnology 12*, 506.
3. Westfall, B., Sitaraman, K., Solus, J., Hughes, J., Rashtchian, A. (1997) *Focus® 19*, 46.

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