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by Life technologies

AccuPrime™ SuperMix II

Cat. no: 12341-012 **Kit Size: 200 reactions**
12341-020 **1,000 reactions**

Store at 4°C or –20°C (non-frost-free)

Description

AccuPrime™ SuperMix II is designed for amplification of genomic DNA templates 200 bp–4 kb in size. It is **not recommended** for amplification of small genomic templates (≤200 bp), plasmid DNA, or cDNA templates.

AccuPrime™ SuperMix II is a specially formulated master mix for the amplification of nucleic acid templates by PCR. The mixture contains recombinant *Taq* DNA polymerase, anti-*Taq* antibodies, thermostable AccuPrime™ protein, Mg^{++}, and dNTPs at optimized concentrations. The SuperMix is supplied at 2X concentration to allow for the addition of primers and template.

Anti-*Taq* DNA polymerase antibodies inhibit polymerase activity at ambient temperatures, allowing room-temperature reaction setup and providing an automatic "hot start" in PCR (Chou *et al.*, 1992; Sharkey *et al.*, 1994). Thermostable AccuPrime™ protein enhances specific primer-template hybridization during every cycle of PCR. Antibody- and AccuPrime™ protein-mediated amplification dramatically improves PCR specificity and fidelity, providing the most robust PCR for multiplex PCR and sub-optimal primer sets.

Reagents are provided for 200 or 1,000 amplification reactions of 25 µl each.

Storage

Store at 4°C or –20°C. No reduction of PCR performance or enzyme activity has been observed after storage for 12 months at 4°C. Note that repeated freeze-thaw cycles may reduce enzyme performance.

	200-rxn kit	1,000-rxn kit
AccuPrime™ SuperMix II	2 × 1.25 ml	12.5 ml

For technical support, email tech_support@invitrogen.com.
For country-specific contact information, visit **www.invitrogen.com**.

Components

40 mM Tris-HCl (pH 8.4); 100 mM KCl; 3 mM $MgCl_2$, 400 µM each dGTP, dATP, dTTP, dCTP; AccuPrime™ *Taq* DNA Polymerase; thermostable AccuPrime™ protein; stabilizers

Terminal Transferase Activity

Like regular *Taq* DNA polymerase, AccuPrime™ *Taq* DNA Polymerase has a nontemplate-dependent terminal transferase activity that adds a single deoxyadenosine (A) to the 3' ends of PCR products.

Guidelines for PCR

General PCR parameters and troubleshooting information are documented in Innis, et al (Innis et al., 1990). PCR reactions should be assembled in a DNA-free environment using 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.

Recommendations for PCR

The protocol on the following page is designed as a starting point for PCR amplification. Optimal reaction conditions—incubation times and temperatures, primers, and template DNA—may vary.

- **Starting material:** 10 pg to 200 ng of genomic DNA (200 bp–4 kb in size).
- **Annealing temperature:** In general, the optimal annealing temperature should be 5–10° lower than the T_m of the primers used.

References

Chou, Q., Russell, M., Birch, D., Raymond, J., and Bloch, W. (1992) Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. *Nucl. Acids Res., 20*, 1717-1723

Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. S. (eds) (1990) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, San Diego, CA

Sharkey, D. J., Scalice, E. R., Christy, K. G., Atwood, S. M., and Daiss, J. L. (1994) Antibodies as thermolabile switches: high temperature triggering for the polymerase chain reaction. *Biotechnology, 12*, 506-509

PCR Protocol

The following general protocol is suggested as a starting point when using AccuPrime™ SuperMix II in PCR.

Due to the "hot-start" capability of AccuPrime™ SuperMix II, the reaction can be set up at room temperature.

1. Program the thermal cycler as follows:

   Initial denaturation: 94ºC for 2 minutes

   25–35 cycles of:
   - Denaturation: 94ºC for 15–30 seconds
   - Annealing: 55–60ºC for 15–30 seconds
   - Extension: 68ºC for 1 minute per kb of PCR product

2. Add the following components to a DNase/RNase-free microcentrifuge tube. The 25-µl reaction size may be scaled up or down as needed.

Component	Volume	Final Concentration
AccuPrime™ SuperMix II	12.5 µl	1X
Primer mix (10 µM each)	0.5 µl	0.2 µM each
Template DNA (10 pg–200 ng)	≥ 1 µl	as required
Autoclaved distilled water	to 25 µl	n/a

3. Cap the tube, tap gently to mix, and centrifuge briefly to collect the contents.
4. Place the tube in the thermal cycler and run the program from Step 1.
5. After cycling, maintain the reaction at 4°C. Samples can be stored at –20°C until use.

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).

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.

Additional Products

Product	Amount	Catalog no.
E-Gel® 1.2% Starter Pak	6 gels plus PowerBase™	G6000-01
TrackIt™ 100 bp DNA Ladder	100 applications	10488-058
TrackIt™ 1kb Plus DNA Ladder	100 applications	10488-085

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