invitrogen
by Life technologies

AccuPrime™ SuperMix I

Cat. No:	Size:
12342-010	200 Reactions
12342-028	1,000 Reactions
	Store at -20°C (non-frost-free)

Description

AccuPrime™ SuperMix I provides qualified reagents for the amplification of nucleic acid templates by polymerase chain reaction (PCR). The mixture contains anti-*Taq* DNA polymerase antibodies, thermostable AccuPrime™ protein, Mg^{++}, deoxyribonucleotide triphosphates (dNTPs), and recombinant *Taq* DNA polymerase at concentrations sufficient to allow amplification during PCR.

Anti-*Taq* DNA polymerase antibodies inhibit polymerase activity at room temperature, providing an automatic "hot start" in PCR (Chou *et al.*, 1992; Sharkey *et al.*, 1994). The thermostable AccuPrime™ protein enhances specific primer-template hybridization during every cycle of PCR. Antibody/AccuPrime™ protein-mediated amplification dramatically improves PCR specificity. It also improves the fidelity of *Taq* by 2-fold, and provides the most robust PCR for multiplex PCR and sub-optimal primer sets.

AccuPrime™ SuperMix I may be stored at either -20°C or 4°C. Storage at 4°C avoids the necessity of thawing the mix before assembling the PCR. No detectable reduction of PCR performance or enzyme activity is observed after storage of AccuPrime™ SuperMix I for twelve months at 4°C. Repeated freeze-thaw cycles can reduce performance or activity. Reagents are provided for 200 or 1,000 amplification reactions of 25 µl each.

Component	200 rxns	1,000 rxns
AccuPrime™ SuperMix I	2 × 1.25 ml	12.5 ml

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

SuperMix Components

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

Guidelines for PCR

- AccuPrime™ SuperMix I is designed for the amplification of genomic DNA amplicons (≤200 bp), plasmid DNA, or cDNA templates. AccuPrime™ SuperMix I is *not* recommended for amplification of genomic DNA templates greater than 200 bp.
- 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.
- Optimal reaction conditions (incubation times and temperatures, primers, and template DNA) will vary. Adjust as needed.

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.

Additional Products

Product	Amount	Catalog no.
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

Basic PCR Protocol

The following general procedure is suggested as a guideline and starting point when using AccuPrime™ SuperMix I in any PCR amplification. The reaction size may be scaled as needed.

1. Program the thermal cycler as follows (note that the annealing temperature will vary depending on the T_m of your primers):

   Initial denaturation: 94ºC for 2 minutes

   25–35 cycles of:

   Denaturation: 94ºC for 15–30 seconds
   Annealing: T_m of primers minus 5ºC for 15–30 seconds
   Extension: 68ºC for 1 minute per kb of PCR product

2. Add the following components in any order to each reaction tube/plate well. A final primer concentration of 200 nM each is recommended.

Component	10-µl Rxn	25-µl Rxn	50-µl Rxn
AccuPrime™ SuperMix I	5 µl	12.5 µl	25 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
DNase-free H_2O	Up to 10 µl	Up to 25 µl	Up to 50 µl

3. Cap or seal the tube/plate, 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. After cycling, maintain the reaction at 4ºC. Samples can be stored at –20ºC 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 (see **Additional Products** on page 2).

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