Protocol-at-a-Glance

Advantage® HD Polymerase Mix Protocol-at-a-Glance

(PT3921-2)

Advantage HD Polymerase (Cat. No. 639241) is a novel DNA polymerase that offers both high accuracy and high efficiency for PCR amplification of DNA. Its unmatched performance is achieved by its superior proofreading function, due to a robust 3'→5' exonuclease activity. Additionally, high specificity is achieved by inclusion of a Hot Start antibody that inhibits polymerase activity at ambient temperatures, preventing false initiation events during reaction assembly due to mispriming or primer digestion. When used with Clontech's optimized reaction buffer, Advantage HD Polymerase achieves the high fidelity, high sensitivity, and high specificity required for critical applications such as gene cloning and amplification of cDNAs for library construction.

The key features of this enzyme mix are high accuracy, with only 12 errors per 250,000 bp, rapid annealing, and improved specificity due to increased priming efficiency. It also exhibits higher amplification efficiency than *Taq* Polymerase and excellent performance even on GC-rich templates. This allows the use of a single PCR cycling protocol for products of varying sizes, including up to 8.5 kb on human genomic DNA, 10 kb on *E. coli* genomic DNA, and 22 kb on λ DNA.

The protocol for using Advantage HD Polymerase Mix is outlined below.

PCR Protocol

1. Thaw all reagents on ice.
2. Assemble the PCR reactions at room temperature, by adding each of the components listed in Table I to tubes or plates.

TABLE I. COMPONENTS OF PCR REACTIONS

Reagents	Volume/Final Concentration per Reaction
Sterile deionized H_2O	up to 25 µl
5X Advantage HD Buffer (Mg^{2+})[a]	5 µl
dNTP Mixture (2.5 mM each)	2 µl
Primer 1	0.2–0.3 µM
Primer 2	0.2–0.3 µM
Advantage HD Polymerase (2.5 units/µl)[b]	0.25 µl
Template	200 ng

[a] Each vial contains 1 ml of 5X Advantage HD Buffer (5 mM $MgCl_2$).
[b] The enzyme is supplied in a storage buffer containing 50 mM Tris-HCl (pH 8.2 at 4°C), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Tween®20, 0.1% Nonidet P-40, and 50% glycerol.

3. Mix well and spin down briefly to collect all the liquid at the bottom of the wells.

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4. Use one of the following PCR Protocols.

Note: Clontech recommends first trying the Three-Step PCR Method with Advantage HD Polymerase.

Three-Step PCR Method		Two-Step PCR Method	
30 cycles:		30 cycles:	
98°C	10 sec[a]	98°C	10 sec
55°C[b]	5 sec or 15 sec[c]	68°C	1 min/kb
72°C	1 min/kb		

[a] Denaturation Conditions: Denature at 98°C for 5–10 sec. If a lower denaturation temperature of 94°C is used, increase time to 10–15 sec.

[b] Annealing Temperature: Anneal at 55°C initially. Annealing temperature optimization may be required.

[c] Annealing Time: Annealing time is dependent on primer length and on T_m values. For primers <25mers, anneal for 5 sec. For primers ≥25mers, calculate T_m values using the following formula, and choose the appropriate annealing time:

T_m value (°C) = 2(NA+NT) + 4(NG+NC) – 5

When T_m ≥55°C, anneal for 5 sec.

When T_m <55°C, anneal for 15 sec.

Important Note: Advantage HD Polymerase has an extremely high priming efficiency. Annealing times longer than 5–15 sec may cause smearing of PCR products. Please try the Two-Step PCR Method when smeared DNA products are observed with the Three-Step PCR Method, or when using primers with T_m values ≥ 70°C.

PCR products obtained using Advantage HD Polymerase cannot be used directly for TA cloning. The termini of PCR products obtained with Advantage HD Polymerase are blunt-ended due to this enzyme's 3'-5' exonuclease activity. Advantage HD Polymerase products should be blunt-end cloned into appropriate vectors. When performing blunt-end cloning using a dephosphorylated vector, PCR products should also be phosphorylated, or PCR primers having phosphoric acid residues at their 5' termini must be used.