Advantage® HF 2 PCR Kit Protocol

PT3317-2

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I. Introduction

The **Advantage HF 2 PCR Kit** (Cat. Nos. 639123 & 639124) is a high-performance PCR system designed to amplify cDNA or genomic templates of up to 5 kb with exceptionally high fidelity. This kit contains the Advantage 2 enzyme blend of TITANIUM™ *Taq* DNA Polymerase and a small amount of proofreading enzyme. This blend provides high sensitivity when amplifying a wide range of DNA targets. The kit combines this enzyme blend with a proprietary mix of dNTPs and a specially formulated buffer that work together to achieve 30-fold higher fidelity than that seen with wild-type *Taq* DNA Polymerase. This abbreviated protocol (PT3317-2) is provided for your convenience, but is not intended for first-time users. For additional details, see the Advantage HF 2 PCR Kit User Manual (PT3317-1).

II. General Considerations

A. Using a Master Mix

Using a Master Mix, which contains the appropriate volumes of all reagents required for multiple PCR reactions, saves time and greatly reduces tube-to-tube variation.

1. If multiple templates are being tested with the same primers, include the primers in the Master Mix.
2. If one template is being tested with multiple primer sets, include the template in the Master Mix.
3. If you are setting up several sets of parallel samples, assemble multiple Master Mixes (e.g., each with a different set of primers).
4. For primer design guidelines, see Section IV.A. of the User Manual (PT3317-1).
5. The Master Mix should be **gently but thoroughly mixed before use (e.g., pulse vortex to prevent bubble formation).**

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B. PCR Control Reactions

Always include positive and negative controls (e.g., H_2O instead of DNA template).

III. Protocols

A. PCR Control Reactions Protocol

The following PCR control reactions can be performed in parallel with your experiments to ensure that the Advantage HF 2 Kit is working properly.

1. Allow all components—except the polymerase—to thaw completely at room temperature. Mix each component thoroughly before use.
2. Combine the following reagents in a 0.5 ml PCR tube:

Reagent	Reagent Volume (µl per sample)	
	Positive Control	Negative Control
PCR-Grade H_2O	32	37
5X HF 2 PCR Buffer	5	5
Control DNA template (~0.2 ng/µl)	5	—
Control Primer Mix (10 µM ea.)	2	2
50X HF 2 dNTP Mix	5	5
50X Advantage HF 2 Polymerase Mix	1	1
Total volume per rxn	**50**	**50**

3. Mix well and spin the tubes briefly.
4. If your thermal cycler does not have a "hot lid", add 1–2 drops of mineral oil to each tube to prevent evaporation during cycling. A good "seal" of mineral oil should have a well-defined meniscus between the two phases. Cap the PCR tubes firmly.
5. Begin thermal cycling. Use the cycling parameters described in Section B below. 20–22 cycles with a 4 min annealing/extension time is sufficient for amplification of the positive control template provided in the kit.
6. Transfer 5 µl of your PCR reaction to a fresh tube and add 1 µl of 5X stop/loading buffer. Analyze your sample (s), along with suitable DNA size markers, on a 0.8–1.2 % agarose/EtBr gel.

Expected results: The reaction should produce a major band of 2 kb when using the positive control reagents provided in the kit, and no bands in the negative (i.e., no DNA template) control.

B. Recommended Cycling Parameters

Use the following general guidelines (see next page) when setting up your initial experiments with the Advantage HF 2 Polymerase Mix. The optimal parameters may vary with different thermal cyclers and will depend on your particular primers and templates, and on other experimental variables.

NOTE: When using the Advantage HF 2 Kit with other kits, use the parameters recommended in the protocol for that kit.

Cycle Parameters	
PE 480	**PE 9600**
• 94°C for 1 min • 25–35 cycles[A] 94°C for 30 sec[B] 68°C for 4 min[C] 68°C for 3 min[D]	• 94°C for 15 sec • 25–35 cycles[A] 94°C for 5–15 sec[B] 68°C for 4 min[C] • 68°C for 3 min[D]

[A] Use 25 cycles for multiple-copy genes or medium-to-high abundance cDNA, or 30–35 cycles for single- or low-copy-number genes or rare cDNAs. For most applications, we prefer two-step cycles (denaturation at T_1 followed by annealing and extension at T_2) instead of three-step cycles (denaturation at T_1 followed by annealing at T_2 followed by extension at T_3)—unless the T_m of the primers is <60–65°C and in certain special protocols.

[B] Use the shortest possible denaturation time. Exposure of DNA to high temperatures causes some depurination of single-stranded DNA during denaturation, which eventually leads to truncation. High temperature also leads to gradual loss of enzyme activity.

[C] Use the highest possible annealing/extension temperature. See Note A. Shorter targets may be amplified using shorter extension times. We recommend using 1 min per kb of expected target.

[D] Optional: This final extension may reduce background in some cases.

C. Amplification of Longer Fragments with the Advantage 2 Buffer

The Advantage HF 2 Kit contains two buffers—the HF 2 Buffer and the standard Advantage 2 Buffer. When used with the HF 2 Buffer, this kit delivers the highest possible fidelity. Targets of different sizes can be amplified under the following conditions:

1. Targets of up to ~3.5 kb can be amplified using HF 2 Buffer.
2. To amplify longer targets, some of the increase in fidelity can be sacrificed to improve elongation efficiency by combining the HF 2 and Advantage 2 buffers in varying proportions. We recommend replacing the smallest amount of HF 2 Buffer with Advantage 2 Buffer that allows satisfactory amplification.
3. Optimal conditions for the amplification of other targets should be determined individually. We recommend initially trying HF 2 Buffer concentrations in the 50–100% range for targets up to 10 kb.

D. Analyzing PCR Reactions Using Agarose Gel Electrophoresis

Use the following conditions to analyze a 5 µl sample of your PCR reaction on an agarose gel. The remaining 45 µl of the reaction mixture can be subjected to further cycling if you do not see a product.

Expected Insert Size Range	Recommended % Agarose	Recommended DNA Size Markers
0.3–1.5 kb	1.5	φX174/Hae III
0.5–10 kb	1.2	1 kb DNA ladder
>5 kb	0.8	λ/Hind III

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This document has been reviewed and approved by the Clontech Quality Assurance Department.