Advantage® GC 2 PCR Kit Protocol

PT3316-2

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I. Introduction

Advantage GC 2 Polymerase Mix (Cat. No. 639114) is optimized for amplifying complex, GC-rich templates. The **Advantage GC 2 PCR Kit** (Cat. Nos. 639119 & 639120) includes Advantage GC 2 Polymerase Mix, our proprietary GC-Melt Reagent, and a reaction buffer containing DMSO. This kit allows the amplification of virtually all GC-rich sequences that are resistant to PCR amplification by standard techniques, including sequences that are up to 90% GC, with minimal buffer optimization. This abbreviated protocol (PT3316-2) is provided for your convenience, but is not intended for first-time users. For additional details, see the Advantage GC 2 PCR Kit User Manual (PT3316-1).

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 (PT3316-1).
5. The Master Mix should be **gently but thoroughly mixed before use (e.g., pulse vortex to prevent bubble formation).**

B. PCR Control Reactions

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

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III. Protocols

A. Protocol for Determining the Optimal Concentration of GC-Melt

The PCR reaction mixture composition for Advantage GC 2 Polymerase Mix will vary depending on your specific application. Use the following example, with a 50 µl reaction volume, as a guide to determine the optimal concentration of GC-Melt for amplifying your specific sequence. The PCR parameters provided here (i.e., amount of template and primer, cycling recommendations) are intended as guidelines only. Refer to Section V. of the User Manual (PT3316-1) for suggestions on improving your results.

NOTES:

- The concentration of GC-Melt that yields maximal amplification of the desired product with a given sample may be used in all subsequent reactions involving that sample.
- On many templates, we have found a GC-Melt concentration of **0.5 M** to be optimal.

1. Place all components—except the polymerase—at room temperature and allow them to thaw completely. Mix each component thoroughly before use.
2. Combine the following reagents in a 0.5 ml PCR tube (all component amounts are in µl; 50 µl final reaction volume). See Section II of the User Manual (PT3316-1) for final concentrations of enzyme and buffer components.

	Final GC-Melt conc. (M)			
Reagent	**0**	**0.5**	**1.0**	**1.5**
PCR-Grade H_2O	35	30	25	20
5X GC 2 PCR Buffer	10	10	10	10
GC Melt (5 M)	**0**	**5**	**10**	**15**
DNA template (~100 ng/µl)	1	1	1	1
Primer mix (10 µM each)	2	2	2	2
50X dNTP Mix (10 mM each)	1	1	1	1
50X Advantage GC 2 Polymerase Mix	1	1	1	1
Total volume per rxn	50	50	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. For a guide to cycling parameters, refer to Section III.C.

B. PCR Control Reactions Protocol

The following PCR control reactions can be performed in parallel with your experiments to ensure that the Advantage GC 2 Polymerase Mix 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 (50 µl reaction volume). See Section II of the User Manual (PT3316-1) for final concentrations of enzyme and buffer components.

Reagent	Reagent Volume (µl per sample)		
	Positive Control	Negative Control	[Optional] Negative Control*
PCR-Grade H_2O	30	31	35
5X GC 2 PCR Buffer	10	10	10
GC-Melt (5 M)	5	5	—
Control DNA template	1	—	1
Control primer mix	2	2	2
50X dNTP mix	1	1	1
50X Advantage GC 2 Polymerase Mix	1	1	1
Total volume per rxn	50	50	50

* This negative control reaction, **without GC-Melt**, may be used to demonstrate GC-Melt benefits.

NOTE: Since this kit is intended for amplifying GC-rich sequences that fail to amplify with other PCR kits or enzyme mixes/PCR reaction buffers, another negative control reaction may be performed with your DNA template and a conventional enzyme mix and PCR reaction buffer.

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 using the following parameters:
   - 94°C for 3 min
   - 25–30 cycles*
     94°C for 30 sec
     68°C for 1.5 min
   - 68°C for 3 min

   * 25–30 cycles with a 1.5 min annealing/extension time is sufficient for amplifying the positive control template provided in the kit. Other templates may require more cycles and different annealing/extension times (see Section III.C).
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 1.5% agarose/EtBr gel.

Expected results: The reaction should produce a major band of 510 bp when using the positive control reagents provided in the kit and no bands in the negative (e.g., no DNA template) control.

C. Recommended Cycling Parameters

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

Target Size	Cycle Parameters
< 5 kb:	• 94°C for 3 min • 25–35 cycles[A] 94°C for 30 sec[B] 68°C for 3 min[C] • 68°C for 3 min[D]
5–9 kb	• 94°C for 3 min • 25–35 cycles[A] 94°C for 30 sec[B] 68°C for 6 min[C] • 68°C for 6 min[D]
10–20 kb	• 94°C for 3 min • 25–35 cycles[A] 94°C for 30 sec[B] 68°C for 12 min[C] • 68°C for 12 min[D] • Hold at 15°C

[A] Initially, run 25 amplification cycles. If the expected product is not observed upon analysis, run an additional 5–10 cycles. 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 minimal possible denaturation time, especially with very large templates. In some cases, better results may be obtained by using a 15 sec denaturation at 94°C. With fast cyclers (e.g., the PE 9600), denaturation times as short as 10 sec may be used.

[C] Use the maximum possible annealing/extension temperature for 1 min per kb of expected target.

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

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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