Protocol-at-a-Glance

# Advantage® GC Genomic LA Polymerase Mix Protocol-at-a-Glance

**(PT3938-2)**

Advantage GC Genomic LA Polymerase Mix is composed of a full-length *Taq* DNA polymerase, a small amount of proofreading enzyme, and a hot-start antibody. The neutralizing hot-start antibody binds and inhibits the *Taq* DNA polymerase at ambient temperatures, enabling reactions to be assembled at room temperature and preventing non-specific amplification due to mispriming and/or formation of primer-dimers before thermal cycling begins. The 2X Advantage GC-Melt Buffer is specifically designed to amplify DNA templates having high GC content or a significant level of secondary structure

The protocol for using the Advantage GC Genomic LA Polymerase Mix is outlined below.

## PCR Protocol

1. Thaw all reagents on ice.
2. Assemble the PCR reactions by adding each of the components listed in Table I. This can be done at room temperature.

**TABLE I. COMPONENTS OF PCR REACTIONS**

| Reagents | Volume/Final Concentration per Reaction |
|---|---|
| Sterile deionized $H_2O$ | up to 25 µl |
| 2X Advantage GC-Melt LA Buffer[1] | 12.5 µl |
| dNTP Mixture (10 mM each) | 1 µl |
| Primer 1 | 0.1–0.5 µM |
| Primer 2 | 0.1–0.5 µM |
| Advantage GC Genomic LA Polymerase Mix (5 units/µl)[2] | 0.25 µl |
| Template DNA | 10–100 ng |

[1] 2 vials (1.25 ml each) of Advantage GC-Melt Buffer are supplied with the kit. This buffer contains 5 mM $Mg^{2+}$.
[2] The enzyme is supplied in a storage buffer containing 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween®20, 0.5% Nonidet P-40, and 50% glycerol.

3. Mix well and spin down briefly to collect all the liquid at the bottom of the wells.
4. Place the plates in a thermal cycler and begin thermal cycling immediately. The following parameters are useful starting points for amplifying a 2 kb fragment, but annealing temperatures will depend on the primers used.
    - 94°C for 1 min
    - 30 cycles
        94°C for 30 sec
        60°C for 30 sec
        72°C for 2 min*
    - 72°C for 5 min

***Note:** The extension time should be determined by the size of the amplicon. As a guideline, use 1 min per 1 kb of amplicon.

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