Protocol-at-a-Glance

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

(PT3885-2)

Advantage Genomic LA Polymerase Mix (Cat. No. 639152) is composed of a full-length *Taq* DNA polymerase, a small amount of proofreading enzyme, and a hot-start antibody. This enzyme is suitable for hot-start PCR due to the presence of a neutralizing monoclonal antibody that binds and inhibits the DNA polymerase at ambient temperatures. Nonspecific amplification due to mispriming and/or formation of primer-dimers before thermal cycling is prevented because the antibody inhibits the polymerase activity by binding to the enzyme until the temperature rises.

The key features of this enzyme are synthesis of products up to 30 kb using human genomic DNA template and up to 48 kb using noncomplex templates; 6.5X higher fidelity than wild-type *Taq* DNA polymerase; and less optimization and greater product yields than other "long and accurate polymerases" due to the robust enzyme/buffer system.

The protocol for using the Advantage 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
10X Advantage Genomic LA Buffer (Mg^{2+})[1]	2.5 µl
dNTP Mixture (10 mM each)	1 µl
Primer 1	0.2–1.0 µM
Primer 2	0.2–1.0 µM
Advantage Genomic LA Polymerase Mix (5 units/µl)[2]	0.25 µl
Template DNA	0.1–1 µg

[1] 500 µl of Advantage Genomic LA Buffer is supplied in a vial. This buffer contains 25 mM $MgCl_2$.
[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, using the following parameters (for amplification of a 17.5 kb fragment).
    - 94°C for 1 min
    - 30 cycles
      98°C for 10 sec
      68°C for 15 min
    - 72°C for 10 min

Notes:

- The denaturation conditions vary depending upon the themal cycler and the type of plate used. We recommend 10–30 sec at 94°C or 1–10 sec at 98°C.
- 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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