Certificate of Analysis



Advantage® GC Genomic LA Polymerase Mix

Catalog No. Amount Lot Number

639153 200 rxns Specified on product label.

Description

The 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 that inhibits polymerase activity at ambient temperature. The 2X Advantage GC-Melt Buffer is specifically designed to amplify DNA templates with high GC content or a significant amount of secondary structure. This combination allows synthesis of products up to 20 kb for complex templates, such as genomic DNA containing GC-rich regions, and up to 48 kb for less complex templates, such as λ DNA. The polymerase mix has 6.5X higher fidelity than wild-type Taq DNA polymerase due to the presence of the proofreading 3'-to-5' exonuclease. The robust enzyme/buffer system requires less optimization and produces higher yields than other "long and accurate polymerases." Sufficient polymerase mix and buffer are provided for 200 reactions (25 μ l each).

Package Contents

- 50 μl Advantage GC Genomic LA Polymerase (5 units/μl)
- 2 x 1.25 ml 2X Advantage GC-Melt Buffer (containing 5 mM MgCl₂)

Storage Conditions

• Store at -20° C.

Expiration Date

• Specified on product label.

Shipping Conditions

• Dry ice

Product Documents

Documents for our products are available for download at <u>takarabio.com/manuals</u> The following documents apply to this product:

Advantage GC Genomic LA Polymerase Mix Protocol-At-A-Glance

Quality Control Data

Raw Material Quality Control

Purity

No nicking activity, endonuclease activity, or exonuclease activity was detected after incubation of 0.6 mg of supercoiled pBR322 DNA, 0.65 mg of λ DNA, or 0.6 mg of λ -Hind III digest with 10 units of this enzyme for 1 hr at 74°C.

Advantage GC Genomic LA Polymerase Mix

Unit definition

One unit is the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble products in 30 min at 74°C using activated salmon sperm DNA as template.

Reaction mixture for unit definition

 $\begin{array}{cccc} 25 \text{ mM} & TAPS \text{ (pH 9.3 at 25°C)} \\ 50 \text{ mM} & KCl \\ & 2 \text{ mM} & MgCl_2 \\ & 1 \text{ mM} & 2\text{-mercaptoethanol} \\ 200 \text{ mM} & Each dATP, dGTP, dTTP} \\ 100 \text{ mM} & [\alpha^{-32}P]\text{-dCTP} \\ 0.25 \text{ mg/ml} & Activated salmon sperm DNA \\ \end{array}$

Functional Quality Control

PCR performance

The Advantage GC Genomic LA Polymerase Mix was tested for PCR performance using a λ DNA control template and primers. Reactions (50 μ l) were assembled and performed using a standard PCR protocol. When the PCR products were examined by electrophoresis on a 1.0% agarose/EtBr gel, a major band of 35 kb was observed.

To ensure optimum PCR performance, the Advantage GC Genomic LA Polymerase Mix was also tested using human genomic DNA as template. Reactions were assembled and performed using a standard PCR protocol. When the PCR products were examined by electrophoresis on a 1.0% agarose/EtBr gel, a major band of 17.5 kb was observed.

To ensure optimal performance of the GC-Melt Buffer, a GC-rich fragment (65% GC content) from the *c-jun* gene was amplified using human genomic DNA as template. Reactions were assembled using either the 2X Advantage GC-Melt Buffer or a standard buffer, and performed using a standard PCR protocol. When the PCR products were examined by electrophoresis on a 1.0% agarose/EtBr gel, a major 1.2 kb band was observed in reactions containing the GC-Melt Buffer. The improvement (no non-specific band) was observed in GC-Melt Buffer by comparison with LA Buffer.

Hot-start antibody

Hot-start antibody inhibition of the Advantage GC Genomic LA enzyme was confirmed to be greater than 80% following a 10 min incubation of the reaction at 55°C.

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

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NOTICE TO PURCHASER:

Our products are to be used for **Research Use Only**. They may not be used for any other purpose, including, but not limited to, use in humans, therapeutic or diagnostic use, or commercial use of any kind. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without our prior written approval.

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