AmpliTaq Gold® DNA Polymerase, LD

Package Contents

Catalog Number	Size
4338856	250 Units
4338857	1,000 Units

Kit Contents

Storage Conditions

- Store all contents at –20°C.

Required Materials

- Template: cDNA, gDNA, λDNA
- Forward and reverse gene-specific primers
- 10 mM dNTP mix (Cat. no. 18427-088)
- Autoclaved, distilled water
- E-Gel® General Purpose Gels, 1.2% (Cat. no. G5018-01)
- TrackIt™ 1 kb Plus DNA Ladder (Cat. no. 10488-085)
- 0.2 or 0.5-mL nuclease-free microcentrifuge tubes

Timing

Varies depending on amplicon length

Selection Guide

PCR Enzymes and Master Mixes
Go online to view related products.

Product Description

- AmpliTaq Gold® DNA Polymerase, LD (low DNA) is a recombinant, thermostable, 94-kDa modified form of the *Thermus aquaticus* DNA polymerase gene, which is further purified to reduce bacterial DNA introduced from the host.
- This chemically-modified enzyme provides a heat-activated "hot start" and offers increased sensitivity, specificity, and yield, and allows reaction assembly at room temperature.
- This enzyme is recommended for PCR applications that require low background levels of bacterial DNA, and for amplification of low copy number (< 1000) bacterial target sequences.
- This enzyme is QC-tested to verify that ≤ 10 copies of bacterial 16S ribosomal RNA gene sequences are present in a standard 5.0-unit aliquot.

Important Guidelines

- Take precautions to avoid cross-contamination by using aerosol-resistant barrier tips and analyzing PCR products in a separate area from PCR assembly.
- AmpliTaq Gold® DNA Polymerase, LD can be completely or partially activated in a pre-PCR heat step. Slow activation can provide a "hot start" and a "time release" of active enzyme, in which activity builds as product accumulates.

Online Resources

Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.

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Print Options

Enzyme Characteristics

Hot-start:	Chemical
Length:	Up to 5 kb
Fidelity vs. *Taq*:	1X
Format:	Separate components

PCR Reaction Setup

Use the measurements below to prepare your PCR experiment, or enter your own parameters in the column provided.

Component	25-µL rxn	50-µL rxn	Custom	Final Conc.
Autoclaved, distilled water	to 25 µL	to 50 µL	to µL	–
10X PCR Gold Buffer	2.5 µL	5.0 µL	µL	1X
25 mM $MgCl_2$*	1.5 µL	3.0 µL	µL	1.5 mM
10 mM dNTP mix**	0.5 µL	1.0 µL	µL	0.2 mM each
10 µM forward primer	0.5 µL	1.0 µL	µL	0.2 µM
10 µM reverse primer	0.5 µL	1.0 µL	µL	0.2 µM
Template DNA	varies	varies		< 1 µg/rxn
AmpliTaq Gold® DNA Polymerase, LD (5 U/µL)***	0.125 µL	0.25 µL	µL	1.25 U/ 50-µL rxn

* Determine the optimal $MgCl_2$ concentration empirically. Refer to Optimization Guidelines below for additional instructions.

** dUTP substitution for control of PCR product carry-over typically requires a concentration twice that of any other dNTP for optimal amplification.

*** Increasing the enzyme concentration up to 2X may improve the product yield.

PCR Protocol

See page 2 to view a procedure for preparing and running your PCR experiment.

Optimization Strategies

Refer to the pop-up for guidelines to optimize your PCR reactions.

Limited Warranty, Disclaimer, and Licensing Information

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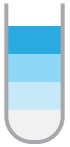
AmpliTaq Gold® DNA Polymerase, LD Protocol

The example PCR procedure below shows appropriate volumes for a single **50-µL** reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, and then dispense appropriate volumes into each 0.2– 0.5 mL PCR reaction tube prior to adding template DNA and primers.

Timeline	Steps	Procedure Details
1 	**Thaw reagents**	Thaw, mix, and briefly centrifuge each component before use. **Note:** Avoid generating bubbles when mixing the enzyme.
2 	**Prepare PCR master mix**	Add the following components to each PCR reaction tube. **Note:** Consider the volumes for all components listed in steps 2 and 3 to determine the correct amount of water required to reach your final reaction volume.
3 	**Add template DNA and primers**	Mix and briefly centrifuge the components. Cap each tube, mix, and then briefly centrifuge the contents.
4 	**Incubate reactions in a thermal cycler**	**Two-Temperature PCR**: Use when primer annealing temperatures are > 60°C. **Three-Temperature PCR**: Use when the templates have high GC content and/or secondary structure, or your desired primer annealing temperatures are < 60°C. **Time-Release PCR**: Skip or perform a 1–2 minute initial denaturation, and then run 35–40 PCR cycles.
5 	**Analyze with gel electrophoresis**	Analyze 10 µL using agarose gel electrophoresis. Use your PCR reaction immediately for down-stream applications, or store it at –20°C.

Component	50-µL rxn	Final Concentration
Autoclaved, distilled water	to 50 µL	
10X PCR Gold Buffer	5.0 µL	1X
25 mM $MgCl_2$	3.0 µL	1.5 mM
10 mM dNTP mix	1.0 µL each	0.2 mM each
AmpliTaq Gold® DNA Polymerase, LD (5 U/µL)	0.25 µL	1.25 Units/rxn

Component	50-µL rxn	Final Concentration
10 µM forward primer	1.0 µL	0.2 µM
10 µM reverse primer	1.0 µL	0.2 µM
Template DNA	varies	< 1.0 µg/rxn*

* Preferably > 10^4 copies of template but < 1 µg DNA/reaction.

Step		Temperature (°C)	Time
Initial Denaturation		95	10 minutes*
25–35 PCR Cycles	Denature	95	15 seconds
	Two-Temp PCR: Anneal/Extend	60–70**	1 minute/kb
	Three-Temp PCR: Anneal	~55**	15 seconds
	Three-Temp PCR: Extend	72	1 minute/kb
Hold		72	7 minutes

* Adjust the time according to the desired initial enzyme activation, from 0–10 minutes.
** Adjust the temperature according to the primer melting temperature.