# AmpliTaq Gold® DNA Polymerase

## Package Contents

| Catalog Number | Size |
|---|---|
| N808-0240 AmpliTaq Gold® with Buffer I | 250 Units |
| N808-0241 AmpliTaq Gold® with Buffer II | 250 Units |
| 4311806 AmpliTaq Gold® with Gold Buffer | 250 Units |

Kit Contents

## Storage Conditions

- Store all contents at –20°C.

## Required Materials

- Template: cDNA, gDNA, λDNA
- 10 mM dNTP mix (Cat. no. 18427-088)
- Forward and reverse gene-specific primers
- 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® is derived from recombinant, thermostable, 94 kDa DNA polymerase, encoded by a modified form of the *Thermus aquaticus* DNA polymerase gene. It is available with 3 different buffers, which provide preferred pH and ionic strength for PCR amplification reactions.
- With AmpliTaq Gold® DNA Polymerase, Hot Start and Time Release PCR can be introduced into existing amplification systems by modifying cycling parameters or reaction conditions for increased specificity, sensitivity, and yield.
- AmpliTaq Gold® is provided in an inactive state and can be completely or partially activated in a pre-PCR heat step, allowing flexibility and assembly of reactions at room temperature.

## 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.
- If the samples contain EDTA or other chelators, raise the $MgCl_2$ concentration in the reaction mix proportionately.
- If proteases are present in the sample DNA (e.g. impure genomic DNA), inactivate the proteases by heating samples to 95°C for 5 minutes before adding AmpliTaq Gold®.

## 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 Buffer I, II, or Gold Buffer | 2.5 µL | 5.0 µL | µL | 1X |
| 10 mM dNTP Mix* | 0.5–5.0 µL | 1.0 µL | µL | 0.2 mM |
| 25 mM $MgCl_2$** | 1.5 µL | 3.0 µL | µL | 1.5 mM |
| 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 (5 U/µL) | 0.125 µL | 0.25 µL | µL | 1.25 U/ 50-µL rxn |

* Substituting dUTP for dTTP to control PCR product carryover may require higher concentrations of dUTP (typically twice that of any other dNTP) for optimal amplification.

** Use $MgCl_2$ with Buffer II or Gold only. Buffer I already contains Mg.

## 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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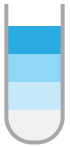
# AmpliTaq Gold® DNA Polymerase Protocol

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.<br>Keep components on ice. |
| 2  | **Prepare PCR master mix** | Add the following components into appropriate wells or tubes.<br>**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.<br>Cap each tube, mix, and then briefly centrifuge the contents. |
| 4  | **Incubate reactions in a thermal cycler** | **Note:** The pre-PCR heat step can completely activate AmpliTaq Gold®. If using the Time Release method, skip or reduce the duration of the initial denaturation step. |
| 5  | **Analyze with gel electrophoresis** | Analyze 10 µL using agarose gel electrophoresis.<br>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 Buffer I, II, or Gold Buffer | 5.0 µL | 1X |
| 10 mM dNTP mix | 1.0 µL | 0.2 mM each |
| 25 mM $MgCl_2$ (with Buffer II or Gold only) | 3.0 µL | 1.5 mM |
| AmpliTaq Gold® DNA Polymerase (5 U/µL) | 0.25 µL | 1.25 U/rxn* |

* The amount of AmpliTaq Gold® needed for the typical PCR amplification depends on cycling parameters. Start with 1.25 U/reaction.

| 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 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 |
| | Anneal | ~55 (depending on primer $T_m$) | 30 seconds |
| | Extend | 72 | 1 minute/kb |
| Final Extension | | 72 | 5 minutes |
| Hold | | 4 | indefinitely |