

AmpliTaq Gold® Fast PCR Master Mix

	Package Contents	Catalog Number 4390937 4390939 4390941	Size 25 rxns 250 rxns 2,500 rxns	 Kit Contents
	Storage Conditions	<ul style="list-style-type: none"> Store all contents at 4°C. Template: gDNA, cDNA Forward and reverse gene-specific primers MicroAmp® Adhesive Seal Applicator (Cat. no. 4333183) MicroAmp® Clear Adhesive Film (Cat. no. 4306311) MicroAmp® Optical 96-Well Reaction Plate with Barcode (Cat. no. 4306737) ProFlex™ 96-well PCR System (Cat. no. 4484075) Centrifuge with plate holders Autoclaved, distilled water 		
	Required Materials	<ul style="list-style-type: none"> Template: gDNA, cDNA Forward and reverse gene-specific primers MicroAmp® Adhesive Seal Applicator (Cat. no. 4333183) MicroAmp® Clear Adhesive Film (Cat. no. 4306311) MicroAmp® Optical 96-Well Reaction Plate with Barcode (Cat. no. 4306737) ProFlex™ 96-well PCR System (Cat. no. 4484075) Centrifuge with plate holders Autoclaved, distilled water 		
	Timing	Varies depending on amplicon length		
	Selection Guide	PCR Enzymes and Master Mixes Go online to view related products.		
	Product Description	<ul style="list-style-type: none"> The AmpliTaq Gold® Fast PCR Master Mix contains a hot-start polymerase chain reaction, optimized to increase the overall PCR amplification speed. Each 2X Master Mix contains AmpliTaq Gold® DNA Polymerase, UP; PCR Buffer; dNTP; MgCl₂; and stabilizers. Optimized for use in sequencing applications, this kit provides specific, high-yield, and high-quality amplicons that can be easily and robustly sequenced. The mix can be used to amplify either complementary DNA (cDNA) or genomic DNA (gDNA) samples. Assembled reactions are stable up to 18 hours at room temperature. 		
	Important Guidelines	<ul style="list-style-type: none"> Select the correct polymerase, PCR instrument, and cycling conditions for your application. Take precautions to avoid cross-contamination by using aerosol-resistant barrier tips and analyzing PCR products in an area separate from PCR assembly. 		
	Online Resources	Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support .		



Enzyme Characteristics

Hot-start:	Chemical
Length:	Up to 1.5 kb
Fidelity vs. Taq:	1X
Format:	Master mix


PCR Reaction Setup

Use the measurements below to prepare your PCR experiment. The recommended reaction volume is 20 µL for the MicroAmp® Optical 96-well Reaction Plate with Barcode, when using the ProFlex™ 96-well PCR System.

Component	20-µL rxn	Final Conc.
Autoclaved, distilled water	to 20 µL	–
AmpliTaq Gold® Fast Master Mix	10 µL	1X
10 µM forward primer	0.4–1.0 µL	0.2–0.5 µM
10 µM reverse primer	0.4–1.0 µL	0.2–0.5 µM
Template DNA	varies*	< 200 ng/20-µL rxn*

* > 100 copies of template but < 0.2 µg gDNA per reaction. Adjust reaction volumes for your experimental design, keeping the concentrations constant.

PCR Protocol

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





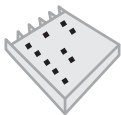
Optimization Strategies

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

Limited Warranty, Disclaimer, and Licensing Information

AmpliQ Gold® Fast PCR Master Mix Protocol

The example PCR procedure below shows appropriate volumes for a single 20- μ L 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 well of an Optical 96-well reaction plate prior to adding template DNA and primers.

Timeline		Steps	Procedure Details																						
1		Thaw reagents	<p>Thaw the reagents on ice. Mix and briefly centrifuge each component before use.</p>																						
2		Prepare PCR master mix	<p>Add the following components into your desired number of wells in an Optical 96-Well Reaction Plate.</p> <p>Adjust the reaction volumes as needed for different sizes or master mixes, keeping concentrations constant.</p> <p>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.</p> <table border="1"> <thead> <tr> <th>Component</th> <th>20-μL rxn</th> <th>Final Concentration</th> </tr> </thead> <tbody> <tr> <td>Autoclaved, distilled water</td> <td>to 20 μL</td> <td>–</td> </tr> <tr> <td>AmpliQ Gold® Fast Master Mix</td> <td>10 μL</td> <td>1X</td> </tr> </tbody> </table>	Component	20- μ L rxn	Final Concentration	Autoclaved, distilled water	to 20 μ L	–	AmpliQ Gold® Fast Master Mix	10 μ L	1X													
Component	20- μ L rxn	Final Concentration																							
Autoclaved, distilled water	to 20 μ L	–																							
AmpliQ Gold® Fast Master Mix	10 μ L	1X																							
3		Add template DNA and primers	<p>Mix the contents by swirling gently. Do not introduce bubbles into the master mix solution.</p> <table border="1"> <thead> <tr> <th>Component</th> <th>20-μL rxn</th> <th>Final Concentration</th> </tr> </thead> <tbody> <tr> <td>10 μM forward primers</td> <td>0.4 μL</td> <td>0.2 μM</td> </tr> <tr> <td>10 μM reverse primers</td> <td>0.4 μL</td> <td>0.2 μM</td> </tr> <tr> <td>Template DNA</td> <td>varies</td> <td>< 200 ng/ 20-μL rxn</td> </tr> </tbody> </table> <p>a. Add your template DNA and primers to each tube for a final reaction volume of 20 μL. b. Seal the plate with MicroAmp® Clear Adhesive Film, and briefly centrifuge the contents.</p> <p>Assembled reactions can be stable at room temperature for up to 18 hours.</p>	Component	20- μ L rxn	Final Concentration	10 μ M forward primers	0.4 μ L	0.2 μ M	10 μ M reverse primers	0.4 μ L	0.2 μ M	Template DNA	varies	< 200 ng/ 20- μ L rxn										
Component	20- μ L rxn	Final Concentration																							
10 μ M forward primers	0.4 μ L	0.2 μ M																							
10 μ M reverse primers	0.4 μ L	0.2 μ M																							
Template DNA	varies	< 200 ng/ 20- μ L rxn																							
4		Incubate reactions in a thermal cycler	<table border="1"> <thead> <tr> <th>Step</th> <th>Temperature (°C)</th> <th>Time</th> </tr> </thead> <tbody> <tr> <td>Initial Denaturation</td> <td>95°C</td> <td>10 minutes</td> </tr> <tr> <td rowspan="3">35 PCR Cycles</td> <td>Denature</td> <td>96°C</td> <td>3 seconds</td> </tr> <tr> <td>Anneal</td> <td>primer T_m</td> <td>3 seconds</td> </tr> <tr> <td>Extend</td> <td>68°C</td> <td>≤ 5 kb—5 seconds 0.5–1.0 kb—15 seconds 1–1.5 kb—30 seconds</td> </tr> <tr> <td>Final Extension</td> <td>72°C</td> <td>10 seconds</td> </tr> <tr> <td>Hold</td> <td>4°C</td> <td>indefinitely</td> </tr> </tbody> </table>	Step	Temperature (°C)	Time	Initial Denaturation	95°C	10 minutes	35 PCR Cycles	Denature	96°C	3 seconds	Anneal	primer T _m	3 seconds	Extend	68°C	≤ 5 kb—5 seconds 0.5–1.0 kb—15 seconds 1–1.5 kb—30 seconds	Final Extension	72°C	10 seconds	Hold	4°C	indefinitely
Step	Temperature (°C)	Time																							
Initial Denaturation	95°C	10 minutes																							
35 PCR Cycles	Denature	96°C	3 seconds																						
	Anneal	primer T _m	3 seconds																						
	Extend	68°C	≤ 5 kb—5 seconds 0.5–1.0 kb—15 seconds 1–1.5 kb—30 seconds																						
Final Extension	72°C	10 seconds																							
Hold	4°C	indefinitely																							
5		Analyze with gel electrophoresis	<p>Analyze 5 μL using agarose gel electrophoresis. Use your PCR reaction immediately for down-stream applications, or store it at –20°C.</p>																						