

AmpliQ[®] DNA Polymerase

	Package Contents	Catalog Number N808-0160 AmpliQ [®] with Buffer I N808-0161 AmpliQ [®] with Buffer II  Kit Contents	Size 250 Units 250 Units
	Storage Conditions	<ul style="list-style-type: none"> Store all contents at -20°C. 	
	Required Materials	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> AmpliQ[®] DNA Polymerase is an ultra-pure, recombinant, thermostable, 94kDa DNA polymerase encoded by a modified form of the <i>Thermus aquaticus</i> DNA polymerase gene, which provides optimal results under reagent conditions supplied by 10X PCR Buffer I or II. The enzyme has a fork-like-structure dependent, polymerization-enhanced, 5' to 3' nuclease activity, but lacks a 3' to 5' exonuclease activity. 	
	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 a separate area from PCR assembly. 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 AmpliQ[®] DNA Polymerase. GC-rich DNA needs very high annealing (> 65°C) and melting temperatures, or the use of 7-deaza-2'-deoxy-GTP mixed with dGTP, to overcome secondary structures. 	

 **Online Resources** Visit our [product page](#) for additional information and protocols. For support, visit www.lifetechnologies.com/support.



Enzyme Characteristics

Hot-start:	None
Length:	Up to 5 kb
Fidelity vs. <i>Taq</i>:	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 or II	2.5 μL	5.0 μL	μL	1X
10 mM dNTP Mix	0.5 μL	1.0 μL	μL	0.2 mM each
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		< 500 ng/ rxn
AmpliQ [®] DNA Polymerase (5 U/μL)	0.125 μL	0.25 μL	μL	1.25 U/ 50-μL rxn

* Use MgCl₂ with Buffer II only. Buffer I already contains Mg.

PCR Protocol

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

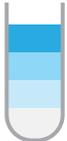
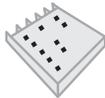
Optimization Strategies

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

Limited Warranty, Disclaimer, and Licensing Information

AmpliTaq® DNA Polymerase Protocol

The example PCR procedure below shows appropriate volumes for a single 50- 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 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. Keep components on ice.																														
2		Prepare PCR master mix	<p>Add the following components to appropriate wells or tubes.</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>50-μL rxn</th> <th>Final Concentration</th> </tr> </thead> <tbody> <tr> <td>Autoclaved, distilled water</td> <td>to 50 μL</td> <td>–</td> </tr> <tr> <td>10X PCR Buffer I or II</td> <td>5.0 μL</td> <td>1X</td> </tr> <tr> <td>10 mM dNTP mix</td> <td>1.0 μL each</td> <td>0.2 mM each</td> </tr> <tr> <td>25 mM MgCl₂ (with Buffer II only)</td> <td>3 μL</td> <td>1.5 mM</td> </tr> <tr> <td>AmpliTaq® DNA Polymerase (5 U/μL)</td> <td>0.25 μL</td> <td>1.25 U/ 50-μL rxn*</td> </tr> </tbody> </table> <p>* The amount of AmpliTaq® DNA Polymerase needed for the typical PCR amplification depends on cycling parameters. Start with 1.25 U/reaction. Mix and briefly centrifuge the components.</p> <table border="1"> <thead> <tr> <th>Component</th> <th>50-μL rxn</th> <th>Final Concentration</th> </tr> </thead> <tbody> <tr> <td>10 μM forward primer</td> <td>1.0 μL</td> <td>0.2 μM</td> </tr> <tr> <td>10 μM reverse primer</td> <td>1.0 μL</td> <td>0.2 μM</td> </tr> <tr> <td>Template DNA</td> <td>varies</td> <td>< 500 ng/rxn*</td> </tr> </tbody> </table> <p>* Preferably > 10⁴ copies of template but < 500 ng DNA/reaction. Cap each tube, mix, and then briefly centrifuge the contents.</p>	Component	50- μ L rxn	Final Concentration	Autoclaved, distilled water	to 50 μ L	–	10X PCR Buffer I or II	5.0 μ L	1X	10 mM dNTP mix	1.0 μ L each	0.2 mM each	25 mM MgCl ₂ (with Buffer II only)	3 μ L	1.5 mM	AmpliTaq® DNA Polymerase (5 U/ μ L)	0.25 μ L	1.25 U/ 50- μ L rxn*	Component	50- μ L rxn	Final Concentration	10 μ M forward primer	1.0 μ L	0.2 μ M	10 μ M reverse primer	1.0 μ L	0.2 μ M	Template DNA	varies	< 500 ng/rxn*
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3		Add template DNA and primers	<p>Note: You can use two-step cycling (skipping the anneal step) when the annealing temperature is > 50°C.</p> <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</td> <td>2 minutes</td> </tr> <tr> <td rowspan="3">25–35 PCR Cycles</td> <td>Denature</td> <td>95</td> </tr> <tr> <td>Anneal</td> <td>~55 (depends on primer T_m)</td> </tr> <tr> <td>Extend</td> <td>72</td> </tr> <tr> <td>Final Extension</td> <td>72</td> <td>5 minutes</td> </tr> <tr> <td>Hold</td> <td>4</td> <td>indefinitely</td> </tr> </tbody> </table>	Step	Temperature (°C)	Time	Initial Denaturation	95	2 minutes	25–35 PCR Cycles	Denature	95	Anneal	~55 (depends on primer T _m)	Extend	72	Final Extension	72	5 minutes	Hold	4	indefinitely											
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4		Incubate reactions in a thermal cycler																															
5		Analyze with gel electrophoresis	<p>Analyze 10 μL using agarose gel electrophoresis. Use your PCR reaction immediately for down-stream applications, or store it at –20°C.</p>																														