

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.



For Research Use Only. Not for use in diagnostic procedures.

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

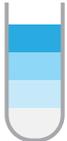
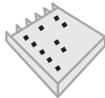
Optimization Strategies

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* |
| 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* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 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 | | | | | | | | | | | |
| 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 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 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> | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |