






AmpliTaq Gold® 360 Master Mix

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|  Package Contents | Catalog Number 4398876 4398881 4398886 4398901 | Size 40 rxns 200 rxns 2,000 rxns 2,000 rxns |  Kit Contents |
|  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 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"> AmpliTaq Gold® 360 Master Mix contains AmpliTaq Gold® 360 DNA Polymerase and optional 360 GC Enhancer. Activity is restored after the denaturation step in PCR cycling at 94°C, providing an automatic “hot start” and offering increased sensitivity, specificity, and yield, while allowing assembly of reactions at room temperature. AmpliTaq Gold® 360 Master Mix comes in a 2X format and was designed for 360° coverage of a full range of targets. | | |
|  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. Avoid creating bubbles when mixing the enzyme. If your application requires increased specificity, add 1–2 µL of 360 GC Enhancer per 50-µL reaction—2 to 5% (v/v) of the reaction. | | |

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|  Online Resources | Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support . |
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Enzyme Characteristics

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|---------------------------------|------------|
| Chemical | Chemical |
| Length: | Up to 5 kb |
| Fidelity vs. <i>Taq</i>: | 1X |
| Format: | Master mix |

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 | – |
| AmpliTaq Gold® 360 Master Mix | 12.5 µL | 25 µL | µL | 1X |
| 360 GC Enhancer (optional)* | 0.5–5.0 µL | 1.0–10 µL | µL | 0–20% |
| 10 µM forward primer | 0.5 µL | 1 µL | µL | 0.2 µM |
| 10 µM reverse primer | 0.5 µL | 1 µL | µL | 0.2 µM |
| Template DNA | varies | varies | varies | < 1 µg |

* For targets with 65–75% GC, start with 5 µL in a 50-µL reaction (10% (v/v) of the reaction). For targets with > 75% GC, start with 10 µL in a 50-µL reaction (20% (v/v) of the reaction). For increased specificity, add 1–2 µL of 360 GC Enhancer per 50-µL reaction (2 to 5% (v/v) of the reaction).

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

AmpliAq Gold® 360 Master Mix Protocol

The example PCR procedure below shows appropriate volumes for a single, **70% GC-rich, 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. Avoid generating bubbles when mixing the MasterMix. | | | |
| 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. | | | |
| | | | Component | 50-μL rxn | Final Concentration | |
| | | | Autoclaved, distilled water | to 50 μ L | – | |
| | | | AmpliAq Gold® 360 Master Mix | 25 μ L | 1X | |
| | | | 360 GC Enhancer (optional) | 5.0 μ L* | 10% | |
| | | | * Targets 65–75% GC, start with 5.0 μ L/rxn. Targets > 75% GC, start with 10 μ L/rxn. For increased specificity, add 1–2 μ L. | | | |
| | | | Cap each tube, mix, and then briefly centrifuge the contents. | | | |
| 3 |  | Add template DNA and primers | Add your template DNA and primers to each tube for a final reaction volume of 50 μ L. | | | |
| | | | Component | 50-μL 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 | < 1 μ g/reaction | |
| | | | Cap each tube, mix, and then briefly centrifuge the contents. | | | |
| 4 |  | Incubate reactions in a thermal cycler | Step | Temperature (°C) | Time | |
| | | | Initial Denaturation | 95°C | 10 minutes  | |
| | | | 25–40 PCR Cycles | Denature | 95°C | Amplicons > 2 kb: 15 seconds Amplicons \leq 2 kb: 30 seconds |
| | | | | Anneal | ~55°C (depending on primer T _m) | 30 seconds |
| | | | | Extend | 72°C | 1 minute/kb |
| | | | Final Extension | 72°C | 7 minutes | |
| | | | Hold | 4°C | indefinitely | |
| 5 |  | Analyze with gel electrophoresis | Analyze 10 μ L using agarose gel electrophoresis. Use your PCR reaction immediately for down-stream applications, or store it at –20°C. | | | |