

AmpliTaQ Gold® 360 Master Mix

Note: For safety and biohazard guidelines, refer to the *Applied Biosystems AmpliTaq Gold® 360 Master Mix Protocol* (PN 4398944), “Safety” section. For all chemicals in **bold red** type, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Perform PCR using AmpliTaq Gold® 360 Master Mix

1 Prepare the reaction mix

IMPORTANT! Avoid generating bubbles when mixing the enzyme.

- a. Thaw the primers, template, and (optional) 360 GC Enhancer, then vortex the reagents before use.
- b. Mix **AmpliTaQ Gold® 360 Master Mix** by gently pipetting it up and down.
- c. Combine the following components in an appropriate tube according to the volumes that are shown in [Table 1](#). Multiply the volume for one reaction component (Table 1) by the total number of reactions, then add that volume to the tube.

Table 1 PCR reaction mix

Component	Volume Per 25-µL reaction (µL)	Volume per 50-µL reaction (µL)	Final concentration
PCR-grade water	Variable	Variable	—
(Optional) 360 GC Enhancer	0.5 to 5 [‡]	1 to 10 [‡]	N/A
AmpliTaQ Gold® 360 Master Mix	12.5	25	1×

[‡] For targets with 65 to 75% GC, start with 2.5 µL in a 25-µL reaction or 5.0 µL in a 50-µL reaction (10% (v/v) of the reaction). For targets with >75% GC, start with 5 µL in a 25-µL reaction or 10 µL in a 50-µL reaction (20% (v/v) of the reaction). In general, if increased specificity is required, add 0.5. to 1 µL 360 GC Enhancer per 25-µL reaction or add 1 to 2 µL 360 GC Enhancer per 50-µL reaction (2 to 5% (v/v) of the reaction).

- d. Cap the tube.
- e. Gently vortex the tube on a low setting for no more than 5 seconds to mix the components.
- f. Centrifuge the tube briefly to spin down the contents and to eliminate air bubbles from the solution.
- g. Dispense equal volumes of the PCR reaction mix to the reaction plate or into PCR tubes (see [Table 1](#)).
- h. Place the plate in a MicroAmp™ Splash-Free 96-Well Base or place the tubes in a MicroAmp™ 96-well Base. Keep the plate or tubes in their respective bases throughout the remainder of the protocol.
- i. Seal the plate with MicroAmp™ Clear Adhesive Film or cap the tubes with MicroAmp™ 8-Cap Strips.
- j. Centrifuge the plate or tubes to collect the liquid at the bottom of the wells.

2 Prepare the reaction plate or tubes

- a. Prepare primers and DNA to their appropriate working dilutions (see [Table 2](#)). For multiple PCR assays, prepare a master mix of components.
- b. With the plate or tubes in the appropriate base, remove the seal from the plate or open the tubes.
- c. Add primers and DNA to the appropriate wells or tubes according to [Table 2](#). Include the no-template controls.

Table 2 Primer and DNA mix for PCR reactions

Component	Volume per 25- μ L reaction (μ L) [‡]	Volume per 50- μ L reaction (μ L)	Final concentration
Primer 1	0.5 to 2.5	1 to 5	0.2 to 1.0 μ M [§]
Primer 2	0.5 to 2.5	1 to 5	0.2 to 1.0 μ M [§]
DNA	Variable [#]	Variable [#]	<1 μ g/reaction ^{††}
Total PCR volume	25	50	—

[‡] If the DNA is difficult to amplify in a 25- μ L reaction, performing the PCR in a 50- μ L reaction may give better results.

[§] Lowering the primer concentration reduces potential secondary products.

[#] For a no-template control, add an equivalent volume of water.

^{††} Preferably >10⁴ copies of template but <1 μ g DNA/reaction

- d. Seal the plate with MicroAmp™ Clear Adhesive Film or cap the tubes with MicroAmp™ 8-Cap Strips.
- e. Centrifuge the plate or tubes to collect the liquid at the bottom of the wells or the tubes. Ensure that the wells are uniformly filled.

3 Set up the run method

Set the:

- Thermal cycling conditions (see [Table 3](#)).

Table 3 Three-temperature thermal cycling on a Veriti™, GeneAmp® PCR System 9700, or 2720 Thermal Cycler

Stage	Step	Temp.	Time
Holding	Activation of AmpliTaq Gold® 360 Master Mix	95 °C	5 to 10 min [‡]
Cycling (25 to 40 cycles)	Denature	95 °C	15 to 30 sec [§]
	Anneal	Primer T _m [#]	30 sec ^{††}
	Extend	72 °C	60 sec/kb
Holding	Final Extension	72 °C	7 min
Holding	Final hold	4 °C	∞

[‡] See “[Adjusting the hold period for activation](#)” in the *AmpliTaq Gold® 360 Master Mix Quick Reference Card*, Appendix B.

[§] For amplicons that are >2 kb, denature for 15 seconds. For amplicons that are ≤ 2 kb, denature for 30 seconds.

[#] Although any primer can be used with this product, Applied Biosystems recommends using primers with T_ms >55 °C. Use the Primer T_m calculator on an Applied Biosystems thermal cycler, or go to www.appliedbiosystems.com/support/techtools/calc.

^{††} Thirty seconds for denaturation and annealing is adequate when you use Veriti™ or GeneAmp® PCR System thermal cyclers that display a calculated sample temperature. Some models of thermal cyclers may require longer times.

- Ramp speed or mode: **Standard**
- Reaction volume (μ L): **25 or 50**

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- 4** Load and run the plate or tubes
- Remove the base from the plate or PCR tubes.
 - Use a MicroAmp™ Optical Film Compression Pad when you use a MicroAmp™ Clear Adhesive Film.
 - Load the reaction plate or tubes into a PCR instrument.
 - Start the run.
 - Unload the reaction plate or tubes after the run is complete.
 - Store the plate at 4 °C or at -15 to -25 °C for longer-term storage until analysis.

Analyze the results

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- 1** Check the purity of the PCR product
- Analyze the PCR amplification products by agarose gel electrophoresis.
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- IMPORTANT!** To prevent contamination, never bring amplified PCR products into the PCR setup area.
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- Obtain a 1% agarose gel with **ethidium bromide** stain. You can use a gel of up to 3% agarose with **ethidium bromide** stain. Set up the electrophoresis apparatus and running buffer according to the manufacturer's instructions.
 - Add an aliquot of the PCR product to a well of a new plate or to an appropriate new tube. Add an appropriate volume of gel-loading buffer to the PCR-product aliquot. For example, add 1 µL of 10X gel-loading buffer to a 9-µL aliquot of PCR reaction.
 - Mix the PCR-product aliquot and buffer in the wells by pipetting up and down, or briefly vortex the samples in the tubes. Spin the plate or pulse-spin the tubes.
 - Dispense the entire volume of the buffer-PCR product aliquot from each well or tube into a well of the gel.
 - Into one well of the gel, load a DNA-ladder marker appropriate to the PCR product length.
 - Run the gel at the voltage or time appropriate to amplicon length and agarose percentage so that the samples run 1/3 to 1/2 the length of the gel. Do not run the dye off the gel.
 - Place the gel on a UV transilluminator. Verify that each lane with a PCR-product aliquot contains one distinct band.
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- 2** For more information
- Refer to the getting started guides for your PCR system for more information about analyzing a PCR results.

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