AmpliTaq® 360 DNA Polymerase

Note: For safety and biohazard guidelines, refer to the *Applied Biosystems AmpliTaq® 360 DNA Polymerase Protocol* (PN 4398942), "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® 360 DNA Polymerase

1 Prepare the reaction mix

IMPORTANT! Prepare the reagents on ice. Avoid generating bubbles when mixing the enzyme.

a. Thaw **AmpliTaq® 360 Buffer, 10×**, 25 mM Magnesium Chloride, dNTP mix, primers, template, and (optional) 360 GC Enhancer on ice, then vortex the reagents before use.

b. Thaw **AmpliTaq® 360 DNA Polymerase** on ice. Mix the enzyme by gently pipetting it up and down, then put the enzyme on ice.

c. Combine the following components on ice 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	—
AmpliTaq® 360 Buffer, 10×	2.5	5	1×
25 mM Magnesium Chloride	1 to 4	2 to 8	1.0 to 4.0 mM§
dNTP mix	2#	4#	200 µM each
(Optional) 360 GC Enhancer	0.5 to 5‡‡	1 to 10‡‡	N/A
AmpliTaq® 360 DNA Polymerase	0.125	0.25	1.25 Units per 50-µL reaction§§

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

§ The optimal magnesium chloride concentration may vary depending on the primer and template that are used and must be determined by experiment. In most cases, a final concentration of magnesium chloride at 1.8 mM in the reaction mix works well.

Contains a 10-mM solution of dNTP (2.5 mM each of dATP, dCTP, dGTP, and dTTP).

‡‡ 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 specificty 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).

§§ For some difficult-to-amplify targets, up to 5.0 U per 50 µL of reaction can be added.

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.

k. Put the plate or tubes on ice.

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-µL reaction (µL)‡	Volume per 50-µL reaction (µL)	Final concentration
Primer 1	0.5 to 2.5	1 to 5	0.2 to 1.0 µM§
Primer 2	0.5 to 2.5	1 to 5	0.2 to 1.0 µM§
DNA	Variable#	Variable#	<1 µg/reaction‡‡
Total PCR volume	25	50	—

‡ If the DNA is difficult to amplify in a 25-µL reaction, performing the PCR in a 50-µL 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 >10^4 copies of template but <1 µg 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.

f. Put the plate or tubes on ice.

3 Set up the run method

Set the:

- Thermal cycling conditions (Table 3):

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

Stage	Step	Temp.	Time
Holding	Initial denaturation	94 °C	3 min‡
Cycling (25 to 40 cycles)	Denature	95 °C	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	∞

‡ For easy-to-amplify targets, the initial denaturation can be reduced to 2 minutes.
§ 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 (µL): **25** or **50**

4 **Load and run the plate or tubes**

a. Remove the plate or PCR tubes from the base.
b. Use a MicroAmp™ Optical Film Compression Pad when you use a MicroAmp™ Clear Adhesive Film.
c. Load the reaction plate or tubes into a PCR instrument.
d. Start the run.
e. Unload the reaction plate or tubes after the run is complete.
f. Store the plate or tubes at 4 °C or at –15 to –25 °C for long-term storage.

Analyze the results

1 **Check the purity of the PCR product**

Analyze the PCR amplification products by agarose gel electrophoresis.

IMPORTANT! To prevent contamination, never bring amplified PCR products into the PCR setup area.

a. 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.
b. 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 10× gel-loading buffer to a 9-µL aliquot of PCR reaction.
c. 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.
d. Dispense the entire volume of the buffer-PCR product aliquot from each well, or tube into a well of the gel.
e. Into one well of the gel, load a DNA-ladder marker appropriate to the PCR product length.
f. 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.
g. Place the gel on a UV transilluminator. Verify that each lane with a PCR-product aliquot contains one distinct band.

2 **For more information**

Refer to the getting started guides for your PCR system for information about analyzing PCR results.

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