2X GeneAmp® Fast PCR Master Mix

applied biosystems by life technologies™

Print Options

Package Contents

Catalog Number	Size
4359187	250 rxns

Kit Contents

Storage Conditions

- Store all contents at 4°C.

Required Materials

- Template: cDNA, gDNA, λDNA
- Forward and reverse gene-specific primers
- MicroAmp® Adhesive Seal Applicator (Cat. no. 4333183)
- MicroAmp® Clear Adhesive Film (Cat. no. 4306311)
- Applied Biosystems 9800 Fast Thermal Cycler With 96-Well Aluminum Sample Block Module (Cat. no. 4352604)
- Optical 96-Well Fast Thermal Cycling Plates with Barcode (code 128), 20 plates (Cat. no. 4346906)
- Centrifuge with plate holders
- Autoclaved, distilled water

Timing

Varies depending on amplicon length

Selection Guide

PCR Enzymes and Master Mixes
Go online to view related products.

Product Description

- The 2X GeneAmp® Fast PCR Master Mix contains a hot-start polymerase system that has been optimized to decrease the overall PCR amplification time and eliminate an activation step.
- The 2X Master Mix contains AmpliTaq® DNA Polymerase, GeneAmp PCR Buffer, dNTPs, $MgCl_2$, and stabilizers.
- Performance is similar to that of AmpliTaq Gold® DNA Polymerase. The reaction mix can be prepared up to 2 hours before starting the run.

Important Guidelines

- 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.
- Empirically determine the optimal annealing/extension time and temperature.

Online Resources

Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.

Enzyme Characteristics

Hot-start:	Chemical
Length:	Up to 2 kb
Fidelity vs. *Taq*:	1X
Format:	Master mix

PCR Reaction Setup

Use the measurements below to prepare your PCR experiment.

Component	20-µL rxn	Final Conc.
Autoclaved, distilled water	to 20 µL	–
2X GeneAmp® Fast PCR Master Mix	10 µL	1X
10 µM forward primer	0.4 µL	0.2 µM
10 µM reverse primer	0.4 µL	0.2 µM
Template DNA	varies*	< 200 ng/rxn*

* > 10^2 copies of template but < 0.2 µg gDNA per reaction. Adjust reaction volumes for your experimental design, keeping the concentrations constant.

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

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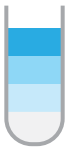
2X GeneAmp® Fast PCR Master Mix Protocol

The example PCR procedure below shows appropriate volumes for a single **20-µL** 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 PCR reaction tube prior to adding template DNA and primers.

Timeline	Steps	Procedure Details
1 	**Thaw reagents**	Thaw the reagents on ice. Mix and briefly centrifuge each component before use. **Note:** Prepare the reaction plates for Fast DNA amplification using 2X GeneAmp® Fast PCR Master Mix no more than 2 hours before starting the instrument run.
2 	**Prepare PCR master mix**	Add the following components into your desired number of wells in an Optical 96-Well Fast Thermal Cycling Plate. **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. Adjust the reaction volumes as needed for different sizes or master mixes.

Component	20-µL rxn	Final Concentration
Autoclaved, distilled water	to 20 µL	-
2X GeneAmp® Fast PCR Master Mix	10 µL	1X

Mix the contents by swirling gently and centrifuging briefly. Do not introduce bubbles into the master mix solution.

3 	**Add template DNA and primers**	

Component	20-µL rxn	Final Concentration
10 µM forward primer	0.4 µL	0.2 µM
10 µM reverse primer	0.4 µL	0.2 µM
Template DNA	varies	< 200 ng/rxn

a. Add your template DNA and primers to each well for a final reaction volume of 20 µL.
b. Seal the plate with MicroAmp® Clear Adhesive Film, and briefly centrifuge the contents.

Assembled reactions can be stable at room temperature for up to 18 hours.

4 	**Incubate reactions in a thermal cycler**	**Note:** You can use two-step cycling by combining the annealing and extension steps.

Step		Temperature (°C)	Time
Initial Denaturation		95	10 seconds
35 PCR Cycles	Denature	94	0 seconds
	Anneal/ Extend	62–72 (depending on primer T_m)	~25 seconds/kb
Final Extension		72	10 seconds
Hold		4	indefinitely

5 	**Analyze with gel electrophoresis**	Analyze 5 µL using agarose gel electrophoresis. Use your PCR reaction immediately for down-stream applications, or store it at -20°C.