AmpliTaq® DNA Polymerase

applied biosystems by life technologies™

Print Options

Package Contents

Catalog Number	Size
N808-0160 AmpliTaq® with Buffer I	250 Units
N808-0161 AmpliTaq® with Buffer II	250 Units

Kit Contents

Storage Conditions

- Store all contents at –20°C.

Required Materials

- 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

- AmpliTaq® DNA Polymerase is an ultra-pure, recombinant, thermostable, 94kDa DNA polymerase encoded by a modified form of the *Thermus aquaticus* 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

- 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 AmpliTaq® 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. *Taq*: 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
AmpliTaq® DNA Polymerase (5 U/µL)	0.125 µL	0.25 µL	µL	1.25 U/ 50-µL rxn

* Use $MgCl_2$ 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

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AmpliTaq® DNA Polymerase Protocol

The example PCR procedure below shows appropriate volumes for a single **50-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 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**	Add the following components to appropriate wells or tubes. **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.
3 	**Add template DNA and primers**	Mix and briefly centrifuge the components.
4 	**Incubate reactions in a thermal cycler**	Cap each tube, mix, and then briefly centrifuge the contents.
5 	**Analyze with gel electrophoresis**	Analyze 10 µL using agarose gel electrophoresis. Use your PCR reaction immediately for down-stream applications, or store it at –20°C.

Component	50-µL rxn	Final Concentration
Autoclaved, distilled water	to 50 µL	–
10X PCR Buffer I or II	5.0 µL	1X
10 mM dNTP mix	1.0 µL each	0.2 mM each
25 mM $MgCl_2$ (with Buffer II only)	3 µL	1.5 mM
AmpliTaq® DNA Polymerase (5 U/µL)	0.25 µL	1.25 U/ 50-µL rxn*

* The amount of AmpliTaq® DNA Polymerase needed for the typical PCR amplification depends on cycling parameters. Start with 1.25 U/reaction.

Component	50-µL rxn	Final Concentration
10 µM forward primer	1.0 µL	0.2 µM
10 µM reverse primer	1.0 µL	0.2 µM
Template DNA	varies	< 500 ng/rxn*

* Preferably > 10^4 copies of template but < 500 ng DNA/reaction.

Note: You can use two-step cycling (skipping the anneal step) when the annealing temperature is > 50°C.

Step		Temperature (°C)	Time
Initial Denaturation		95	2 minutes
25–35 PCR Cycles	Denature	95	15 seconds
	Anneal	~55 (depends on primer T_m)	30 seconds
	Extend	72	1 minute/kb
Final Extension		72	5 minutes
Hold		4	indefinitely