Certificate of Analysis

aTaq DNA Polymerase:

Supplied With:

Cat.#	aTaq DNA Polymerase	Thermophilic DNA Polymerase 10X Reaction Buffer, Mg-Free	Magnesium Chloride Solution, 25mM
M1241	100 units (M124A)	0.75ml (M190A)	0.75ml (A351B)
M1245	500 units (M124B)	2 × 1.2ml (M190G)	2 × 1.2ml (A351H)
M1248	2,500 units (M124F)	10 × 1.2ml (M190G)	10 × 1.2ml (A351H)

Description: aTaq DNA Polymerase contains a nonrecombinant, modified form of *Taq* DNA polymerase that lacks 5 →3 exonuclease activity. The enzyme is supplied in a proprietary formulation containing 50% glycerol. It is supplied with Thermophilic DNA Polymerase 10X Reaction Buffer, Mg-Free, and Magnesium Chloride Solution to allow the optimization of magnesium concentration.

Enzyme Concentration: 5u/µl.

Biological Source: The enzyme is derived from bacteria.

Thermophilic DNA Polymerase 10X Reaction Buffer, Mg-Free (Part# M190A, M190G): When the 10X Buffer supplied with this enzyme is diluted 1:10 it has a composition of 10mM Tris-HCl (pH 9.0 at 25°C), 50mM KCl and 0.1% Triton® X-100. This buffer is optimized for use with 200µM each of dNTPs. Sufficient 25mM $MgCl_2$ is provided separately to allow optimization of enzyme performance under a variety of conditions. The Triton® X-100 in the Buffer is compatible with the detergents in the Storage Buffer of the enzyme for all applications.

Magnesium Chloride Solution, 25mM (Part # A351B, A351H): Provided to allow users to optimize $MgCl_2$ concentration according to their individual requirements. Vortex the $MgCl_2$ thoroughly after thawing and prior to use.

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nanomoles of dNTPs into acid-insoluble material in 30 minutes at 74°C. The reaction conditions are specified below under Standard DNA Polymerase Assay Conditions.

Storage Conditions: Store at –20°C. Avoid exposure to frequent temperature changes. See the expiration date on the Product Information label.

Part# 9PIM124

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Quality Control Assays

Functional Assay: aTaq DNA Polymerase is tested for performance in the polymerase chain reaction (PCR) using 1.25 units of enzyme to amplify a 360bp region of the α-1-antitrypsin gene from 100 molecules (0.35ng) of human genomic DNA. The resulting PCR product is visualized as a single band on an ethidium bromide-stained agarose gel.

Standard DNA Polymerase Assay Conditions (Not PCR Conditions): The polymerase activity is assayed in 50mM Tris-HCl (pH 9.0); 50mM NaCl; 5mM $MgCl_2$; 200µM each of dATP, dGTP, dCTP, dTTP (a mix of unlabeled and [^{3}H]dTTP); 10µg activated calf thymus DNA; 0.1mg/ml BSA in a final volume of 50µl.

Nuclease Assays: No contaminating endonuclease or exonuclease activity detected using λ and/or pGEM® DNA.

PCR Satisfaction Guarantee

Promega's PCR Systems, enzymes and reagents are proven in PCR to ensure reliable, high performance results. Your success is important to us. Our products are backed by a worldwide team of Technical Support scientists. Please contact them for applications or technical assistance. If you are not completely satisfied with any Promega PCR product we will send a replacement or refund your account.

That's Our PCR Guarantee!

Product must be within expiration date and have been stored and used in accordance with product literature. See Promega Product Insert for specific tests performed.

Signed by:

R. Wheeler, Quality Assurance

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Usage Information

1. Standard Application

Reagents to Be Supplied by the User:

- PCR Nucleotide Mix (Cat.# C1141, C1145)
- Nuclease-Free Water (Cat.# P1193)
- Upstream primer
- Downstream primer
- Template DNA

1. In a sterile, nuclease-free microcentrifuge tube, combine the following components on ice:

Component	Final Volume	Final Conc.
Thermophilic DNA Polymerase 10X Reaction Buffer[1]	5µl	1X
$MgCl_2$ Solution, 25mM[1]	2–8µl	1.0–4.0mM
PCR Nucleotide Mix, 10mM each	1µl	0.2mM each dNTP
Upstream Primer	Xµl	0.1–1.0µM
Downstream Primer	Yµl	0.1–1.0µM
aTaq DNA Polymerase (5u/µl)	0.25µl	1.25u
Template DNA	Zµl	<0.5µg/50µl
Nuclease-Free Water to	50µl	

[1]Thaw completely and vortex thoroughly prior to use.

2. If using a thermal cycler **without a heated lid**, overlay the reaction mix with 1–2 drops (approximately 50µl) of mineral oil to prevent evaporation during thermal cycling. Centrifuge the reactions in a microcentrifuge for 5 seconds.
3. Perform PCR using your standard parameters. An example profile is given in Table 1. For the cycling protocol, we recommend the following:
   a. Reactions are placed in a thermal cycler that has been preheated to 95°C.
   b. The thermal cycling protocol has an initial denaturation step where samples are heated at 95°C for 2 minutes to ensure that the target DNA is completely denatured. Initial denaturation of longer than 2 minutes at 95°C is unnecessary and may reduce yield.
   c. The extension time should be at least 1 minute/kb target length.

Table 1. Recommended Thermal Cycling Conditions for aTaq DNA Polymerase-Mediated PCR Amplification. These guidelines are optimal for the Perkin Elmer thermal cycler model 480 or comparable thermal cyclers.

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	2 minutes	1 cycle
Denaturation	95°C	0.5–1 minute	
Annealing	42–65°C*	0.5–1 minute	25–35 cycles
Extension	72°C	1 minute/kb	
Final Extension	72°C	5 minutes	1 cycle
Soak	4°C	Indefinite	1 cycle

*Annealing temperature should be optimized for each primer set based on the primer T_m.

4. Separate the PCR products by agarose gel electrophoresis and visualize with ethidium bromide or any other means.

2. General Considerations

2.A. Buffer

aTaq DNA Polymerase in 1X Thermophilic DNA Polymerase Reaction Buffer is compatible with common PCR additives such as DMSO and betaine.

2.B. Enzyme Concentration

Promega has found that 1.25 units of aTaq DNA Polymerase per 50µl amplification reaction is adequate for most amplifications. Adding extra enzyme generally does not produce significant increases in yield. However, in some cases, more enzyme may be beneficial.

2.C. Primer Design

PCR primers generally range in length from 15–30 bases and are designed to flank the region of interest. Primers should contain 40–60% (G + C), and care should be taken to avoid sequences that might produce internal secondary structure. The 3 -ends of the primers should not be complementary to avoid the production of primer-dimers. Primer-dimers unnecessarily deplete primers from the reaction and result in an unwanted polymerase reaction that competes with the desired reaction. Avoid three G or C nucleotides in a row near the 3 -end of the primer, as this may result in nonspecific primer annealing, increasing the synthesis of undesirable reaction products. Ideally, both primers should have nearly identical melting temperatures (T_m); in this manner, the two primers should anneal roughly at the same temperature. The annealing temperature of the reaction is dependent upon the primer with the lowest T_m. For assistance with calculating the T_m of any primer, a T_m Calculator is provided on the BioMath page of the Promega Web site at: **www.promega.com/biomath/**

2.D. More Information on Amplification

More information on amplification is available online at the Promega Web site:
PCR Core Systems Technical Bulletin: **www.promega.com/tbs/tb254/tb254.html**
DNA Analysis Notebook: **www.promega.com/guides/dna_guide/**
PCR Applications: **www.promega.com/paguide/chap1/htm**

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