AmpliTaq Gold®

Applied Biosystems

with GeneAmp® 10X PCR Buffer

or GeneAmp 10X PCR Buffer II & $MgCl_2$ Solution

or GeneAmp 10X PCR Gold Buffer & $MgCl_2$ Solution

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES
See notice to purchaser

Enzyme	Enzyme with Buffer		
	GeneAmp 10X PCR Buffer	GeneAmp 10X PCR Buffer II & $MgCl_2$ Solution	GeneAmp 10X PCR Gold Buffer & $MgCl_2$ Solution
AmpliTaq Gold 250 Units (U), 5 U/µL, 200 reactions	N808-0240	N808-0241	4311806
Six Paq, AmpliTaq Gold 6 x 250 U, 5 U/µL, 1200 reactions total	N808-0242	N808-0243	4311814
Twelve Paq, AmpliTaq Gold 12 x 250 U, 5 U/µL, 2400 reactions total	N808-0244	N808-0245	4311820
AmpliTaq Gold 1000 U, 5 U/µL, 800 reactions	N808-0246	N808-0247	4311816
AmpliTaq Gold 5 x 1000 U, 5 U/µL, 4000 reactions total	N808-0248	N808-0249	4311818

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NOTICE TO PURCHASER: LIMITED LICENSE

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STORAGE AND STABILITY

Upon receipt, store the AmpliTaq Gold and reagents at -20°C ± 10°C in a constant temperature freezer. If stored under the recommended conditions, the product will maintain performance through the control date printed on the label.

INTRODUCTION

AmpliTaq Gold is a thermostable DNA polymerase designed to make advanced Polymerase Chain Reaction ("PCR") techniques easy. With AmpliTaq Gold, Hot Start PCR and Time Release PCR (see below) can be introduced into existing amplification systems with modification of cycling parameters or reaction conditions. These techniques improve amplification of most templates by lowering non-specific background and increasing amplification of required specific products.

The PCR process is a simple and powerful method, invented by K. Mullis, for the amplification of DNA sequences *in vitro*.[1,2,3,4,5] PCR is based on a series of incubation steps at different temperatures. One set of these steps is referred to as a PCR cycle. In a typical cycle, the target DNA sequence is melted at a temperature above 90°C. Then the temperature is lowered to 60°-72°C to allow annealing and extension of two primers complementary to the 3' boundaries of the target. The PCR process is a repetition of the cycle. The target is copied with each cycle, resulting in an exponential amplification. With PCR, DNA sequences can be amplified by at least 10^5 fold and potentially as high as 10^9 fold.[1]

High background (non specificity) and low specific product yield can occur in a PCR system when reaction components are mixed at low, or permissive, temperatures (4°-25°C).[6] Reaction setup at room temperature permits non-specific primer annealing, and since active enzyme is present at these temperatures, the mis-primed primers will be extended. These non-specific constructs that begin this way are amplified throughout the remaining PCR cycles, resulting in mis-primed products and primer oligomers. For low copy amplification, excess enzyme in early PCR cycles may give similar results. Mis-primed PCR products can obscure detection of specific target bands in gel analysis and impair quantitative PCR and sequencing of PCR products. Furthermore, amplification of mis-primed products competes for reactants (dNTPs, primers) resulting in poor yields of specific product.

AmpliTaq Gold DNA Polymerase is provided in an inactive state. Heat activates the enzyme. This feature allows flexibility in reaction setup, including pre-mixing of PCR reagents at room temperature. AmpliTaq Gold DNA Polymerase can be completely or partially activated in a pre-PCR heat step, or can be allowed to activate slowly during thermal cycling. Using a pre-PCR heat step provides a PCR Hot Start, since primer extension cannot occur during PCR set-up when AmpliTaq Gold is inactive. Slow activation can provide a Hot Start and a Time Release PCR, where polymerase activity builds as PCR product accumulates.

An efficient Hot Start PCR can be added to systems already optimized with AmpliTaq DNA Polymerase by substituting AmpliTaq Gold DNA Polymerase and adding a 5-10 minute 95°C pre-PCR heat step. Little or no change in the optimized system is needed. For Time Release PCR, the pre-PCR heat step can be reduced or eliminated. However, without prior activation it may be necessary to add additional cycles to the GeneAmp PCR Instrument System program. With or without a pre-PCR heat step, more cycles and/or more enzyme can be added to the typical PCR reaction to increase yield of specific product without buildup of mis-primed products.

AmpliTaq Gold is derived from a recombinant, thermostable, 94 kDa DNA polymerase encoded by a modified form of the *Thermus aquaticus* DNA polymerase gene which has been inserted into an *Escherichia coli* host.[7] This ultra-pure, stable polymerase is available in a variety of package sizes with GeneAmp 10X PCR Gold Buffer & $MgCl_2$ Solution, GeneAmp 10X PCR Buffer, and GeneAmp 10X PCR Buffer II & $MgCl_2$ Solution.

AmpliTaq Gold DNA Polymerase gives optimal results under reagent conditions provided by GeneAmp 10X PCR Buffer, GeneAmp 10X PCR Buffer II, and GeneAmp 10X PCR Gold Buffer & $MgCl_2$ Solution. These buffers provide preferred pH and ionic strength for PCR amplification reactions (see below for exact formulations). The magnesium ion concentration required to achieve optimal PCR amplification is dependent on the specific set of primers and template used.[8] The 25 mM $MgCl_2$ Solution supplied with GeneAmp 10X PCR Buffer II and GeneAmp 10X PCR Gold Buffer can be used to adjust magnesium ion concentration for any set of primer-template pairs.

LIST OF COMPONENTS

Reagent	Volume	Description
AmpliTaq Gold	50 µL	1 tube, 250 U of 5 U/µL AmpliTaq Gold.
	or	
	200 µL	1 tube, 1000 U of 5 U/µL AmpliTaq Gold.
with GeneAmp 10X PCR Buffer **(N808-0006)** **(N808-0129)**	1.5 mL	Tube(s) containing 100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01% (w/v) gelatin.
or		
with GeneAmp 10X PCR Buffer II **(N808-0010)** **(N808-0130)**	1.5 mL	Tube(s) containing 100 mM Tris-HCl, pH 8.3, 500 mM KCl.
and	and	and
25 mM $MgCl_2$ Solution	1.5 mL	Tube(s) containing 25 mM $MgCl_2$.
with GeneAmp 10X PCR Gold Buffer **(4306894)** **(4306898)**	1.5 mL	Tube(s) containing 150 mM Tris-HCl, pH 8.0, 500 mM KCl
and	and	and
25 mM $MgCl_2$ Solution	1.5 mL	Tube(s) containing 25 mM $MgCl_2$.
Product Insert	–	Containing AmpliTaq Gold protocols.

ENZYME CHARACTERISTICS

The key component in AmpliTaq Gold is AmpliTaq® DNA Polymerase (Deoxynucleoside triphosphate: DNA Deoxynucleotidyltransferase, E.C.2.7.7.7).

Concentration:	5 Units/µL of AmpliTaq Gold.
Unit Definition:	One unit (U) of AmpliTaq Gold is defined as the amount that will incorporate 10 nmoles of dNTPs into acid insoluble material per 30 minutes in a 10 minute incubation at 74°C under the analysis conditions below. The enzyme is shipped in an inactive form. It is activated by heating for 3 hours at 80°C before the activity is measured.
Analysis conditions for AmpliTaq DNA Polymerase activity:	25 mM TAPS (tris-[hydroxymethyl]-methyl-amino-propane sulfonic acid, sodium salt), pH 9.3 (at room temperature); 50 mM KCl; 2.0 mM $MgCl_2$; 1 mM ß-mercaptoethanol; 200 µM each dATP, dGTP, dTTP; 100 µM [a-^{32}P]-dCTP (0.05 to 0.1 Ci/mmole); salmon sperm DNA activated by a modification of methods in **reference 9**, mixed in a final volume of 50 µL and incubated at 74°C for 10 min.
Storage Buffer:	20 mM Tris-HCl, pH 9.0 (room temperature), 100 mM KCl, 0.1 mM EDTA (ethylenediaminetetraacetic acid), 1.0 mM DTT (dithiothreitol), 0.5% Tween® 20, 50% (v/v) glycerol.
Associated Activities:	Non-specific endonuclease and exonuclease activities were not detectable after one hour incubation of 600 ng of supercoiled pBR322 (dam$^-$, dcm$^-$) or 600 ng of *Msp*I-digested pBR322 DNA, respectively, at 74°C, in the presence of 8 units of AmpliTaq DNA Polymerase. The enzyme has a fork-like-structure dependent, polymerization enhanced 5' to 3' nuclease activity but lacks a 3' to 5' exonuclease activity.[10,11]

AmpliTaq DNA Polymerase is covered by U.S. Patent 4,889,818 owned by Roche Molecular Systems, Inc. and its foreign counterparts owned by F. Hoffmann-La Roche Ltd. Additional patent applications on genes encoding thermostable DNA polymerases and methods and formulations for stable preparations of thermostable DNA polymerase are pending.

MATERIALS REQUIRED BUT NOT SUPPLIED

Unless otherwise noted, the items listed are available from major laboratory suppliers (MLS) such as Baxter Scientific Products (McGaw Park, IL), Fisher Scientific (Pittsburgh, PA) or VWR Scientific (So. Plainfield, NJ).

Reagents	**Source**
Autoclaved deionized, ultrafiltered or glass distilled water, referred to as "water" in this product insert	

Equipment	**Source**
Electrophoresis power supply	MLS
Gel electrophoresis equipment	MLS
GeneAmp PCR Instrument System	**Applied Biosystems** (see **page 16** of this product insert).
Pipettors, adjustable	MLS

Supplies

PCR Reaction Tubes (see **Table 1** for tube/thermal cycler combination)

GeneAmp PCR Reaction Tubes (0.5 mL, polypropylene)	**Applied Biosystems (Part No. N801-0180).**
GeneAmp Thin-Walled Reaction Tubes (0.5 mL, polypropylene)	**Applied Biosystems (Part Nos. N801-0737, N801-0611, N801-0537).**
MicroAmp® Reaction Tubes (0.2 mL, polypropylene)	**Applied Biosystems (Part Nos. N801-0533, N801-0540, N801-0612).**
Pipet tips with hydrophobic filters, used with adjustable pipettors	MLS

PROTOCOLS FOR DNA AMPLIFICATION

1.0 GENERAL ADVICE

1.1 Due to the enormous amplification possible with the PCR process, low levels of DNA contamination, especially from either previous PCR amplification reactions, samples with high DNA levels, or positive control templates, can result in product formation even in the absence of purposefully added template DNA.[12] **Set up all reaction mixes in an area separate from PCR product analysis.** To minimize cross contamination, use dedicated or disposable vessels, solutions and pipettors (preferably pipettors with tips containing hydrophobic filters) for DNA preparation, reaction mixing, and sample analysis.[13,14]

1.2 The recipe for a Master Mix of reagents (**Section 2.2**) is a useful starting place for determining optimal amplification conditions for different DNA targets using primers designed by the user. Prepare a Master Mix of reagents (water, buffer, dNTPs, primers and enzyme) for all samples and aliquot into individual tubes. Then add the template DNA. Using such mixes will minimize reagent pipetting losses, increase accuracy, and reduce the number of reagent transfers. Perform amplifications in the Applied Biosystems PCR reaction tube appropriate for the respective Applied Biosystems GeneAmp PCR Instrument System (**Table 1**). Applied Biosystems PCR reaction tubes provide the best heat transfer because of their uniform fit in the wells of the corresponding instrument.

Table 1. Applied Biosystems GeneAmp PCR Instrument Systems' Cycling Profile Times and Temperatures for AmpliTaq Gold.

Applied Biosystems GeneAmp PCR Instrument System	Tubes and Volumes		Examples of Times and Temperatures for AmpliTaq Gold			
	Tube Type	Volume Range in µL/Tube	Preincubation Step*	Each of 25 or more Cycles		Final Step
				Melt	Anneal/Extend	
DNA Thermal Cycler 480	GeneAmp Thin-walled Reaction	50-100	STEP CYCLE	STEP CYCLE		TIME DELAY
			5-10 min 95°C 1 cycle	45 sec-1 min 94°-96°C	45 sec-1 min 60°-72°C	10 min ≥ 60°-72°C
GeneAmp PCR System 9600 System 2400 System 9700 (9600 emulation mode)	**MicroAmp Reaction**	**50-100**	**HOLD**	**CYCL (CYCLE)**		**HOLD**
			5-10 min 95°C 1 cycle	15-30 sec 94°-96°C	15-30 sec 60°-72°C	10 min** ≥ 60°-72°C

* The pre-PCR heat step can completely activate AmpliTaq Gold. Omit or reduce the duration of this step if using Time Release method (**Sections 2.10 and 3.2**).

** The cycling parameters in **bold type** are recommended for use in protocols outlined in this product insert. After the final step, store amplified samples in refrigerator or freezer until ready to use.

1.3 Use of the GeneAmp PCR Carry-over Prevention Kit (Part No. N808-0068) will prevent the reamplification of PCR products from previous amplifications.

2.0 GENERAL PROTOCOLS FOR PCR AMPLIFICATION

2.1 Thaw and gently mix each of the reagents. Avoid generating bubbles when mixing the enzyme. Spin the enzyme solution down in a microcentrifuge before pipetting. Pipet the enzyme and buffers carefully and slowly; the viscosity of the glycerol in the enzyme storage buffer can lead to pipetting errors.

2.2 Prepare a Master Mix by adding the reagents in the proportions shown below (see **Sections 2.3-2.9** for additional information).

Component	Volume per reaction (µL)	Concentration in Master Mix
Water	*	—
10X PCR Buffer II or 10X PCR Gold Buffer	5	1X
25 mM $MgCl_2$ Solution	2-8	1.0-4.0 mM**
dATP (10 mM each dNTP)	1	200 µM
dCTP (10 mM each dNTP)	1	200 µM
dGTP (10 mM each dNTP)	1	200 µM
dTTP*** (10 mM each dNTP)	1	200 µM
User-provided Primer 1	1-5	0.2-1.0 µM
User-provided Primer 2	1-5	0.2-1.0 µM
User-provided experimental template	*	< 1 µg/reaction****
AmpliTaq Gold	0.25	1.25 Units/reaction
Total volume	50 µL	

* Any combination of water and template can be used as long as the total volume of the Master Mix, sample, and primers equal 50 µL.

** The optimal magnesium chloride concentration may vary, depending on the primer and template used and must be determined empirically. In most cases a final concentration of magnesium chloride in the range of 1.0 to 4.0 mM in the reaction mix will work well. If using the 10X PCR Buffer, which already contains 15 mM $MgCl_2$, the concentration of the $MgCl_2$ in the Master Mix will be 1.5 mM.

*** For dUTP substitution, see **Section 2.9**.

**** Preferably > 10^4 copies of template but < 1 µg DNA/reaction (see **Section 2.5**).

2.3 The range of reaction volumes, listed in Table 1 with the specified Applied Biosystems GeneAmp PCR Instrument System, permits good amplification. If variable volumes of reagents or template are used in the Master Mix, adjust the volume of water in the Master Mix by an equivalent amount to keep the concentrations of other reactants constant.

2.4 Optimization of reactions for each primer-template pair may be necessary and can be achieved by varying magnesium chloride concentration, primer concentration, dNTP concentration, and cycle conditions. The effect of these variations can be monitored by examining the intensity and distribution of bands after electrophoresis on agarose followed by visualization with ethidium bromide staining of the gel.

2.5 The DNA segment to be amplified from the template can be up to 5 kb long, although 100 to 1000 bases are more typical and easier to amplify. Start with enough copies of the template to be sure of obtaining a signal after 25 to 30 cycles: preferably > 10^4 copies but less than 1 µg total sample DNA per 50 µL. Low concentrations of target DNA may require up to 35 or more cycles to produce sufficient product for analysis.

2.6 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 Gold. This step can be carried out automatically with any of the Applied Biosystems GeneAmp PCR Instrument Systems.

2.7 The single-strand DNA primers should typically be 15 to 30 bases in length. The %G+C of primers should be near 50%, to maximize specificity. Primer sequences should not complement within themselves or to each other, particularly at the 3' ends. To avoid potential problems, primers should be purified by gel electrophoresis or ion-exchange HPLC. The optimal primer concentrations need to be determined empirically, by testing concentrations in the range of 0.2 to 1.0 µM. Primer concentrations that are too low will result in little or no PCR product, while concentrations that are too high may result in amplification of non-target sequences. Primer concentrations in the range of 0.2 to 0.5 µM should work for most PCR amplifications.

2.8 The optimal magnesium chloride concentration needs to be determined empirically, by testing concentrations of 1.0 to 4.0 mM $MgCl_2$ for each primer set. Too little or too much $MgCl_2$ can reduce amplification efficiency or result in non-specific products. If the samples contain EDTA or other chelators, raise the $MgCl_2$ concentration in the reaction mix proportionately. Magnesium chloride concentrations should also be adjusted in parallel with significant changes in the concentrations (higher or lower) of sample DNA and dNTPs.

2.9 Keep concentrations of dNTPs in the reaction mix balanced; if the concentration of any one dNTP is significantly different from the rest, DNA polymerases will tend to misincorporate them, slow down, and terminate prematurely. Substitution of dUTP for dTTP for control of PCR product carryover, however, may require higher concentrations of dUTP (typically twice that of any other dNTP) for optimal amplification.[13,14]

2.10 AmpliTaq Gold DNA Polymerase is provided in an inactive state and is activated by heat. Because of this, the amount of AmpliTaq Gold needed for the typical PCR amplification depends on cycling parameters. Start with a 1.25 U/50 µL reaction volume (**Section 2.2**). Optimal results are obtained with a 5-10 minute pre-PCR heat step at 95°C followed by 25 or more PCR cycles (see **Section 3.0** for further discussion on cycling parameters).

2.11 Because of the enhanced specificity of PCR obtained by using AmpliTaq Gold DNA Polymerase, increasing the AmpliTaq Gold concentration may result in significantly higher yields of specific product without increasing background. Start with the concentration suggested in **Section 2.2** above, then increase the amount of AmpliTaq Gold DNA Polymerase in the reaction mix by 0.5 Unit increments until the desired yield is attained or until non-specific product levels become too high.

2.12 AmpliTaq Gold DNA Polymerase gives optimal results under reagent conditions provided by GeneAmp 10X PCR Gold Buffer, GeneAmp 10X PCR Buffer, and GeneAmp 10X PCR Buffer II. If other PCR buffers are used, or sample preparation reagents are added that change the pH or ionic strength of the PCR, then pH and ionic strength must be optimized.

3.0 CYCLING CONDITIONS

3.1 A typical PCR cycle consists of a melting step (94°-96°C) to separate the complementary strands of DNA followed by a combined primer annealing/extension step (60°-72°C depending on the target). See **Introduction** for complete discussion. **Table 1** shows typical profile times and temperatures.

3.2 As discussed in **Section 2.10**, AmpliTaq Gold DNA Polymerase must be activated by heat, either before or during PCR cycling. For applications already optimized with a conventional thermal stable polymerase such as AmpliTaq DNA polymerase, add a 5-10 minute pre-PCR step at 95°C to the Thermal Cycler program (see the example in Section **4.0**). Because of the enhanced specificity of PCR obtained using AmpliTaq Gold DNA Polymerase, both polymerase concentration and cycle number can be increased if greater product yield is desired. If a pre-PCR incubation is not used, Time Release PCR can be implemented by increasing cycle number. It may take five or more additional PCR cycles to give equivalent product yield. Additionally, a combination of a shorter pre-PCR heat step and extra cycles can improve results.

3.3 Except for amplifications in the Applied Biosystems GeneAmp PCR System 2400, System 9600, and System 9700, control evaporation and refluxing by overlaying the mix with 50 to 100 µL of mineral oil (**Part No. 0186-2302**). The oil should not interfere when withdrawing samples. If the entire volume is to be recovered, 100 µL of high purity chloroform should be added after amplification. The aqueous phase containing the DNA will then float on the chloroform-oil mixture, allowing easy collection.

3.4 Higher annealing temperatures generally result in better specificity and higher product yield.[1,15] The optimum annealing temperature can be determined empirically by testing at 5°C or smaller increments until the maximum in specificity is reached. At annealing temperatures, activated AmpliTaq Gold has significant activity so extension of primed templates occurs.

4.0 PROTOCOL FOR QUALITY CONTROL ASSAY

The following protocol, used in quality control testing of the enzyme, specifically demonstrates the amplification activities of AmpliTaq Gold DNA Polymerase.

4.1 Materials Required But Not Supplied.

In addition to the materials required for general PCR, the following materials are required for this protocol.

Reagents	Source
HIV-1 Positive Control DNA	**Applied Biosystems (Part No. N808-0016)**
HIV-1 Negative Control DNA (human placental DNA)	**Applied Biosystems (Part No. N808-0131)**
Primer SK145 5' AGTGGGGGGACATCA-AGCAGCCATGCAAAT 3' (forward)	Commercial oligo synthesis service laboratories
Primer SK431 5' TGCTATGTCAGTTCC-CCTTGGTTCTCT 3' (reverse)	Commercial oligo synthesis service laboratories
AmpErase® UNG	**Applied Biosystems (Part No. N808-0096)**

4.2 Master Mix Preparation.

To perform this assay, use the following concentrations of components in a 100 µL PCR: 1X PCR Buffer II; 2.5 mM $MgCl_2$; 200 µM each of dATP, dGTP, dCTP, and 400 µM of dUTP; 50 pmol of each primer SK145 and SK431; 1.0 Unit of AmpErase UNG; 10 ng HIV-1 Negative Control DNA; 10 copies HIV-1 Positive Control DNA; and 2.5 Units of AmpliTaq Gold. Refer to **Section 2.2** on preparation of the Master Mix.

4.3 PCR Cycling Parameters.

Optimal PCR amplification is achieved using the thermal profiles outlined below.

4.3.1 For the GeneAmp PCR System 9600 and the GeneAmp PCR System 2400 and using MicroAmp Reaction Tubes with PCR volumes of 100 µL (no oil overlay):

HOLD:	**95°C - 9 min**
AUTO (CYCLE with AutoX):	**94°C - 30 sec, 60°C - 1 min; 43 cycles**
HOLD:	**60°C - 10 min**

Store at -20°C until post-PCR analysis.

4.3.2 For the DNA Thermal Cycler 480 and using GeneAmp PCR Reaction Tubes with PCR volumes of 50 µL (add an oil overlay):

STEP-CYCLE:	**95°C - 9 min; 1 cycle**
STEP-CYCLE:	**94°C - 1 min, 60°C - 1 min; 43 cycles**
TIME-DELAY:	**60°C - 10 min**

Store at -20°C until post-PCR analysis.

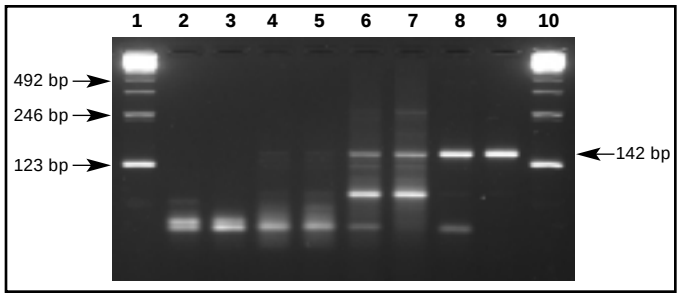

Figure 1. 100 µL PCR amplifications of a 142 bp product from 10 input copies of HIV-1 Positive Control DNA using Manual or No Manual Hot Start methods. This procedure uses 2.5 Units of AmpliTaq DNA Polymerase or AmpliTaq Gold DNA Polymerase with 1X PCR Buffer II, 2.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, 400 µM dUTP, and 50 pmol of each primer in MicroAmp Reaction Tubes and a GeneAmp® PCR System 9600. Following 43 cycles of amplification (cycled as described in Section 4.3), the samples were analyzed as described in Section 4.4. Lanes 2 to 3: 0 input copies, AmpliTaq DNA Polymerase, no preincubation, No Manual Hot Start; Lanes 4 to 5: 10 input copies, AmpliTaq DNA Polymerase, no preincubation, No Manual Hot Start; Lanes 6 to 7: 10 input copies, AmpliTaq DNA Polymerase, no preincubation, Manual Hot Start; Lanes 8 to 9: 10 input copies, AmpliTaq Gold DNA Polymerase, 9 minute preincubation, No Manual Hot Start; Lanes 1 and 10: 123 bp DNA ladder, 1 µg.

4.4 Analysis of amplified sample.

4.4.1 Subject 10 µL of each amplification product to agarose gel electrophoresis using a 3% NuSieve® GTG® and 1% SeaKem® GTG agarose gel. Run in 1X TBE (89 mM Tris-borate, 2 mM EDTA) stained with 0.5 µg/mL ethidium bromide. Electrophorese at 150-200 V/25 cm for 1.5-2.0 hours.

4.4.2 Analyze agarose gels, referring to Figure 1.

PERFORMANCE CHARACTERISTICS

Each lot of AmpliTaq Gold DNA Polymerase has been shown under the conditions described in **Sections 4.2 and 4.3** (bold typeface) to yield a visible band which corresponds to approximately 142 bp product when starting with 10 copies of HIV-1 Positive Control DNA per reaction.

TROUBLESHOOTING

Observation	Possible Cause	Recommended Action
Reduced or no product band is observed.	Experimental sample concentration too low.	Increase experimental sample concentration.
	Experimental sample DNA damaged or degraded.	Use experimental sample that has been processed to minimize nicking and shearing.
	Enzyme concentration too low.	Increase enzyme concentration in increments of 0.25 Unit per 50 µL reaction.
	Magnesium concentration too low.	Increase magnesium concentration in increments of 0.2 mM.
	Denaturation time too long or too short.	Adjust denaturation time in increments of 5 seconds.
	Denaturation temperature too high or too low.	Adjust denaturation temperature in increments of 1 degree.
	Anneal/extend temperature too high.	Lower temperature in increments of 2°C.
	Anneal/extend time too short.	Lengthen time in increments of 30 seconds.
	Cycle number too low.	Increase cycle number in increments of 3.
	pH or ionic strength not optimal.	See **Section 2.12**.
	Primer design not optimal.	Review primer design and composition.
	Preincubation time not optimized.	Increase pre-PCR heat step time in increments of 1 minute or use time release protocol.

Observation	Possible Cause	Recommended Action
Product is multi-banded.	Anneal/extend temperature too low.	Raise temperature in increments of 2 degrees.
	Primer design not optimal.	Review primer design and composition.
	Excess initial active enzyme.	Decrease pre-PCR heat step time in increments of 1 minute or use time release protocol.
Product band is smeared.	Carry-over contamination.	Set up PCR reactions in a separate area.
	Enzyme concentration too high.	Decrease enzyme concentration in increments of 0.25 Unit per 50 µL reaction or use time release protocol.
	Magnesium concentration too high.	Decrease magnesium concentration in increments of 0.1 mM.
	Denaturation time too short.	Lengthen denaturation time in increments of 5 seconds.
	Denaturation temperature too low.	Raise denaturation temperature in increments of 1 degree.
	Anneal/extend time too long.	Shorten time in increments of 1 minute.
	Cycle number too high.	Reduce cycle number in increments of 3 cycles.
Partial or complete loss of product band.	Carry-over contamination.	Set up PCR reactions in a separate area.
	Experimental sample DNA degraded.	Test a new aliquot of sample.

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