Get the Convenience of Hot-Start PCR with the New GoTaq® Hot Start Polymerase

ABSTRACT GoTaq® Hot Start Polymerase is a new member of the GoTaq® family of products. This enzyme allows hot-start PCR and provides greater specificity, yield and sensitivity. Amplification reactions can be set up at room temperature and put into a room-temperature thermal cycler. In fact, reactions can be assembled and left at room temperature for up to 24 hours before thermal cycling is started. In this article, we describe the properties of GoTaq® Hot Start Polymerase and compare it with standard *Taq* DNA polymerase. We look at sensitivity using GoTaq® Hot Start Polymerase in both the Green and Colorless GoTaq® Flexi Buffers, and we report on the compatibility of GoTaq® Hot Start Polymerase with RT-PCR. Finally, we compare GoTaq® Hot Start Polymerase with two other commercially available hot-start *Taq* DNA polymerases.

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Hot-start PCR can dramatically reduce or eliminate nonspecific products and primer-dimer formation.

INTRODUCTION

Amplification of some targets can be problematic when conventional PCR is used (1,2). Two problems can arise when assembling the reactions at low temperatures and during the initial ramping of the thermal cycler: nonspecific priming and primer-dimer formation. Nonspecific priming occurs at temperatures below the primer-annealing temperature when primers bind to similar but nonhomologous sites in the template DNA and are extended by the polymerase. These nonspecific secondary products contain sequences homologous to the primer and can be amplified with each subsequent round of cycling, resulting in the appearance of secondary products as well as the intended product. Primer-dimer formation also occurs at temperatures below the primer-annealing temperature, but in this case two primers anneal in complementary regions and are extended by the polymerase. The product of primer-dimer formation migrates at a rate slightly slower than the primers. Nonspecific priming and primer-dimer formation result in lower specificity and can affect yield and sensitivity.

Hot-start PCR can dramatically reduce or eliminate these problems. In hot-start PCR the amplification reaction is rendered inactive until the temperature is higher than the primer-annealing temperature. This eliminates or minimizes the extension of misannealed primers. Various hot-start PCR methods have been developed over the years. The most popular include: 1) manual hot-start PCR, where the reactions are assembled without a critical component (i.e., *Taq* DNA polymerase or magnesium), which is added to the reaction once the reaction temperature reaches 80–95 °C (2); 2) reactions assembled on ice prior to being placed in a thermal cycler that has been preheated to 94–95 °C; 3) either *Taq* DNA polymerase or magnesium embedded in a wax bead that melts at high temperatures (1); 4) anti-*Taq* DNA polymerase antibodies that inactivate *Taq* DNA polymerase at low temperatures and then are denatured when the reaction reaches higher temperatures (3,4); 5) chemically modified *Taq* DNA polymerase that is inactive at low temperatures but regains activity when the reactions are heated at high temperatures (5).

GoTaq® Hot Start Polymerase[a,b] combines the familiar GoTaq® DNA Polymerase with proprietary antibodies that bind to the GoTaq® DNA Polymerase to inactivate the polymerase at low temperatures. Polymerase activity is restored by incubating the reactions at 94–95 °C for 2 minutes during the initial denaturation step of the thermal cycling protocol. The polymerase is available with 5X Green and 5X Colorless GoTaq® Flexi Buffers and a 25 mM $MgCl_2$ solution for optimizing the magnesium concentration. Reactions performed with the Green Buffer can be loaded directly onto gels. The Colorless Buffer should be used when downstream applications require fluorescence or absorbance readings without prior purification (6–8).

HOT-START PCR PERFORMANCE

Amplification of some targets can be improved by using GoTaq® Hot Start Polymerase. To illustrate this, we amplified five different targets that typically require hot-start PCR for specific amplification and compared the amplification results of GoTaq® Hot Start Polymerase with both Green and Colorless GoTaq® Flexi Buffers to those of standard *Taq* DNA polymerase. For this comparison, all reactions were assembled at room temperature and placed in

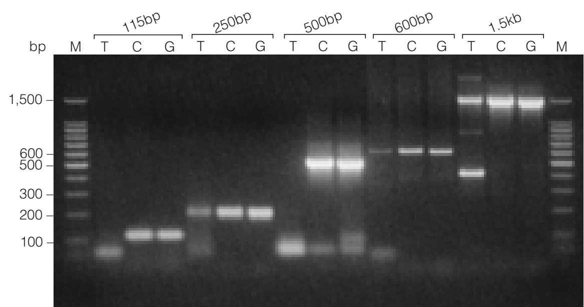

Figure 1. Comparison of amplification reactions using standard *Taq* DNA polymerase and GoTaq® Hot Start Polymerase. The following fragments were amplified using standard *Taq* DNA polymerase (Lanes T), or GoTaq® Hot Start Polymerase with Colorless GoTaq® Flexi Buffer (Lanes C, Cat.# M8901) or Green GoTaq® Flexi Buffer (Lanes G, Cat.# M8911): 115 bp HIV-gag fragment from five copies of HIV-1 DNA; 250 bp THO1 fragment from 250 pg human genomic DNA; 500 bp CCR5 fragment from 330 pg human genomic DNA; 600 bp lambda fragment from 40 copies of lambda DNA in 500 ng human genomic DNA; and a 1.5 kb fragment of Corynephage omega gene from 500 pg plasmid DNA template. Lanes M, BenchTop 100 bp DNA Ladder (Cat.# G8291). All reactions were performed with PCR Nucleotide Mix (Cat.# C1141) and were set up at room temperature and placed in a room-temperature thermal cycler before the cycling protocol was started.

room-temperature thermal cyclers before starting the thermal cycling protocol. The standard *Taq* DNA polymerase amplifications all showed no or low yield, secondary products and/or primer-dimers. The GoTaq® Hot Start Polymerase amplified the desired products with good yield and minimal secondary product or primer-dimer formation (Figure 1).

SET UP REACTIONS AT ROOM TEMPERATURE

Setting up amplification reactions at room temperature is more convenient than setting up reactions on ice. With GoTaq® Hot Start Polymerase, reactions can be kept inactive at room temperature for up to 24 hours before the cycling procedure is started without affecting amplification. To illustrate this, we amplified a 1.5 kb fragment of the Corynephage omega gene from a plasmid DNA template using both GoTaq® Hot Start Polymerase and standard *Taq* DNA polymerase. Two sets of amplification reactions were assembled. One set was amplified immediately after the reactions were assembled, and a second set was allowed to sit at room temperature for 24 hours before thermal cycling was started. As seen in Figure 2, amplifications using GoTaq® Hot Start Polymerase gave good amplification of the 1.5 kb fragment without additional secondary fragments and good yield with both immediate cycling and after 24 hours at room temperature. Amplifications using standard *Taq* DNA polymerase gave poor amplifications, resulting in low yield of the 1.5 kb product, secondary fragments and primer-dimer.

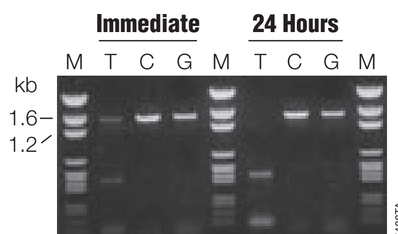

Figure 2. Comparison of amplification when reactions are cycled immediately after assembly or left at room temperature for 24 hours before cycling. Standard *Taq* DNA polymerase (Lanes T), or GoTaq® Hot Start Polymerase with Green GoTaq® Flexi Buffer (Lanes G) or Colorless GoTaq® Flexi Buffer (Lanes C) was used to amplify a 1.5 kb fragment of the Corynephage omega gene from 500 pg plasmid DNA. Reactions with standard *Taq* DNA polymerase and GoTaq® Hot Start Polymerase were set up at room temperature and placed in a room-temperature thermal cycler prior to thermal cycling. Lanes M, BenchTop pGEM® DNA Markers (Cat.# G7521). All amplifications were performed with PCR Nucleotide Mix (Cat.# C1141).

CONSISTENT PCR PERFORMANCE

We have successfully amplified fragments of up to 3.1 kb from human genomic DNA (data not shown) as well as various other targets, including mouse genomic, lambda and plasmid DNA (Figure 1 and data not shown).

GoTaq® Hot Start Polymerase in either Green or Colorless GoTaq® Flexi Buffer results in approximately equal yield and sensitivity. To illustrate this, we amplified a 1.2 kb α-1-antitrypsin fragment from Human Genomic DNA (Cat.# G3041) using GoTaq® Hot Start Polymerase with either Green or Colorless GoTaq® Flexi Buffer. Different amounts of starting material produced similar yield and sensitivity for both GoTaq® Flexi Buffers (Figure 3).

GoTaq® Hot Start Polymerase performs equally well in the Green or Colorless GoTaq® Flexi Buffer.

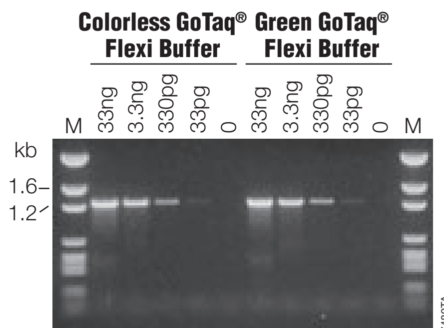

Figure 3. Detection of a 1.2 kb α-1-antitrypsin fragment from human genomic DNA using GoTaq® Hot Start Polymerase with either Colorless GoTaq® Flexi Buffer or Green GoTaq® Flexi Buffer. A 1.2 kb fragment of the α-1-antitrypsin gene was amplified using the indicated amounts of Human Genomic DNA (Cat.# G3041). Lanes M, BenchTop pGEM® DNA Markers (Cat.# G7521). All amplifications were performed with PCR Nucleotide Mix (Cat.# C1141).

USE IN RT-PCR

GoTaq® Hot Start Polymerase with either GoTaq® Flexi Buffer can be used for PCR in uncoupled RT-PCR. We amplified a 540 bp β-actin target from a cDNA template generated using the ImProm-II™ Reverse Transcription System (Cat.# A3800). GoTaq® Hot Start Polymerase successfully amplified the target, and the results were similar for both GoTaq® Flexi Buffers (Figure 4). GoTaq® Hot Start Polymerase also can be used for PCR after generation of cDNA using the AMV RT-based Reverse Transcription System (Cat.# A3500; Table 1; data not shown).

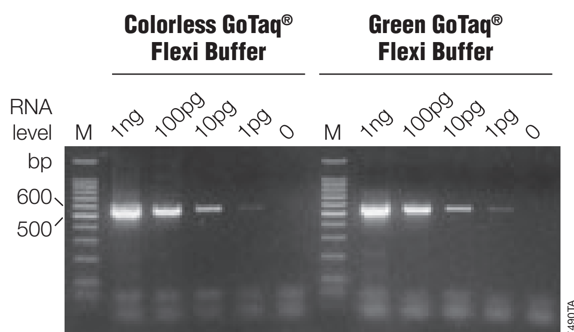

Figure 4. RT-PCR detection of a β-actin fragment from total mouse liver RNA. Total RNA from mouse liver was isolated using RNAgents® Total RNA Isolation System (Cat.# Z5110), and the indicated amounts were used as template in 20 µl cDNA synthesis reactions. cDNA was generated as directed in the *ImProm-II™ Reverse Transcription System Technical Manual* (9) using Oligo(dT)$_{15}$ Primer (Cat.# C1101). A 540 bp β-actin fragment was amplified using 20 µl of the cDNA synthesis reactions in 100 µl PCR amplifications as directed in the *ImProm-II™ Reverse Transcription System Technical Manual* (9), except the amplification reactions were set up at room temperature and placed in a room-temperature thermal cycler. GoTaq® Hot Start Polymerase with either Colorless or Green GoTaq® Flexi Buffer was used for the amplification reactions. Lanes M, BenchTop 100 bp DNA Ladder (Cat.# G8291). All amplifications were preformed with PCR Nucleotide Mix (Cat.# C1141) as the dNTP source.

Table 1. Compatibility of GoTaq® Flexi Buffers with Various Applications.

Application	GoTaq® Flexi Buffer	
	Green	Colorless
PCR		
Amplify 115 bp to 3.1 kb fragments	Yes	Yes
Uncoupled RT-PCR		
Amplify cDNA generated by the ImProm-II™ Reverse Transcription System	Yes	Yes
Amplify cDNA generated by the Reverse Transcription System (AMV RT)	Yes	Yes
Downstream Applications		
T-vector cloning	Yes	Yes
Direct loading onto agarose or nondenaturing TBE polyacrylamide gel	Yes	No
Applications involving absorbance or fluorescence	No	Yes

CHARACTERISTICS AND APPLICATIONS

GoTaq® Hot Start Polymerase has characteristics similar to standard *Taq* DNA polymerase. It has an extension rate of 1 minute per 1 kb of target DNA, has the 5´→3´ exonuclease activity and no 3´→5´ exonuclease activity and gives template-independent addition of single deoxyadenosine to 3´ ends of DNA. Adding a single deoxyadenosine to the 3´ end allows simple T-vector cloning of the PCR fragment following purification (Table 1).

ADVANTAGES OF GOTAQ® HOT START POLYMERASE

Using anti-*Taq* DNA polymerase antibody to inactivate *Taq* DNA polymerase for hot-start PCR has several advantages over other hot-start methods. Unlike chemically modified *Taq* DNA polymerases that require a long (5- to 15-minute) initial denaturation step at 95 °C to restore polymerase activity, GoTaq® Hot Start Polymerase activity is restored with a shorter, two-minute initial denaturation at 94–95 °C. With hot-start methods that use either magnesium or *Taq* DNA polymerase embedded in wax, amplified DNA can be difficult to remove because of the wax barrier, and the component sequestered in the bead cannot be titrated or adjusted. Manual hot-start methods easily can lead to contamination and are inconvenient to assemble. Methods using reaction setup on ice and a preheated thermal cycler can be inconvenient and may not work well.

To compare competitor hot-start products, we amplified a 2.4 kb APC target from human genomic DNA using the manufacturer's instructions. Competitor A used a chemically modified *Taq* DNA polymerase, and competitor I used the antibody method to inactivate *Taq* DNA polymerase. These two products were compared with GoTaq® Hot Start Polymerase using Green and Colorless GoTaq® Flexi Buffers. The GoTaq® Hot Start Polymerase performed well, providing good yield, sensitivity and specificity (Figure 5). Competitor I's product gave less yield and lower sensitivity, and competitor A's product gave less specificity than the GoTaq® Hot Start Polymerase with the same template.

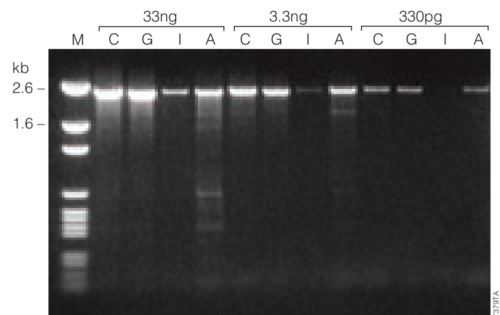

Figure 5. Detection of an APC fragment from human genomic DNA using GoTaq® Hot Start Polymerase and two competitors' hot-start *Taq* DNA polymerases. A 2.4 kb fragment of the APC gene was amplified using the indicated amounts of Human Genomic DNA (Cat.# G3041). Lanes C, GoTaq® Hot Start Polymerase with Colorless GoTaq® Flexi Buffer; Lanes G, GoTaq® Hot Start Polymerase with Green GoTaq® Flexi Buffer; Lanes I, Competitor I; Lanes A, Competitor A; Lanes M, BenchTop pGEM® DNA Markers (Cat.# G7521).

CONCLUSION

GoTaq® Hot Start Polymerase provides an easy method to amplify targets and obtain good specificity, yield and sensitivity. It provides the convenience of room-temperature assembly of reactions and requires a short incubation to restore polymerase activity. Assembled reactions can sit at room temperature for up to 24 hours before starting the thermal cycling procedure, which could be important for scientists using robotics platforms. As always with the GoTaq® products, you can choose the convenience of loading gels directly using the 5X Green GoTaq® Flexi Buffer, or use the Colorless GoTaq® Flexi Buffer if the dyes will interfere with your downstream applications.

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ORDERING INFORMATION

Product	Size	Cat.#
GoTaq® Hot Start Polymerase	100 u	M5001
	500 u	M5005
	2,500 u	M5006
	10,000 u	M5008

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[a]U.S. Pat. No. 6,242,235, Australian Pat. No. 761757, Canadian Pat. No. 2,335,153 and other patents and patents pending.

[b]Licensed under U.S. Pat. Nos. 5,338,671 and 5,587,287 and corresponding patents in other countries.

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GoTaq® DNA Polymerase Family

	Superior Amplification	High Specificity	Direct-to-Gel Loading	Easy Fluorescence and Absorbance Applications	Master Mix Formulation	dNTPs Included	Colorless Reaction Buffer	Green Reaction Buffer	Magnesium Supplied Separately	Easy and Convenient Room-Temperature Setup	Controls Supplied Separately
GoTaq® Hot Start Polymerase	✓	✓	✓	✓			✓	✓	✓	✓	
GoTaq® DNA Polymerase	✓		✓	✓			✓	✓			
GoTaq® Flexi DNA Polymerase	✓		✓	✓			✓	✓	✓		
GoTaq® Green Master Mix	✓		✓		✓	✓		✓			
GoTaq® Colorless Master Mix	✓			✓	✓	✓	✓				
GoTaq® PCR Core System I	✓		✓	✓		✓*	✓	✓	✓		
GoTaq® PCR Core System II	✓		✓	✓		✓*	✓	✓	✓		✓

* dNTPs supplied as a separate kit component.

GoTaq® DNA
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