

# Use GoTaq® Hot Start Green and Colorless Master Mixes for Easy, Convenient PCR

## **ABSTRACT**

GoTaq® Hot Start Green Master Mix and GoTaq® Hot Start Colorless Master Mix are two new PCR master mixes offered by Promega. Both mixes contain GoTaq® Hot Start Polymerase, which can be used for hot-start PCR. The master mix format gives you the advantage of easy reaction assembly: just add primers, template and water.

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#### Introduction

Amplification of some PCR targets can be problematic(1) (2) (3) . Without hot-start PCR, nonspecific priming and primer-dimer formation can occur when the reaction is below the optimal primer annealing temperature (e.g., when assembling reactions and during initial ramping of the thermal cycler). These side reactions may lead to secondary amplification products, lower yields and reduced sensitivity.

GoTaq® Hot Start Green and Colorless Master Mixes contain GoTaq® Hot Start Polymerase(4), which reduces the nonspecific priming and primer-dimer formation by minimizing polymerase activity until the reaction is heated to a temperature that supports specific priming. It uses antibody inhibition of *Taq* DNA polymerase as the hot-start method. Activating the GoTaq® Hot Start Polymerase and denaturing the anti-*Taq* DNA polymerase antibodies require a 2-minute initial denaturation at 94–95°C. GoTaq® Hot Start Polymerase has characteristics similar to standard *Taq* DNA polymerase with an extension rate of 1 minute per 1kb of target DNA, a 5′ to 3′ exonuclease activity and lacking a 3′ to 5′ exonuclease activity.

The 2X Master Mixes contain GoTaq® Hot Start Polymerase supplied in 2X GoTaq® Reaction Buffer (either Green or Colorless), 400µM dNTPs, and 4mM MgCl2. The GoTaq® Hot Start Green Master Mix (Cat.# M5122) contains a blue and yellow dye plus a substance that increases density so that reactions can be directly loaded onto gels. The GoTaq® Hot Start Colorless Master Mix (Cat.# M5132) should be used when downstream applications require fluorescence or absorbance readings without prior purification(4) .

#### **Hot-Start PCR Performance**

Amplification of some targets can be improved by using hot-start PCR. To illustrate this, we compared the GoTaq® Hot Start Green and Colorless Master Mixes with a master mix containing standard *Taq* DNA polymerase in amplifications of five targets that typically require hot-start PCR. For this comparison, all reactions were assembled at room temperature and placed in a room-temperature thermal cycler before starting the cycling protocol. The standard *Taq* DNA polymerase amplifications showed secondary products, primer-dimers, low or no yield, or a combination of these. Both GoTaq® Hot Start Master Mixes amplified the target of interest with good yield and minimal secondary products or primer-dimer formation (Figure 1).

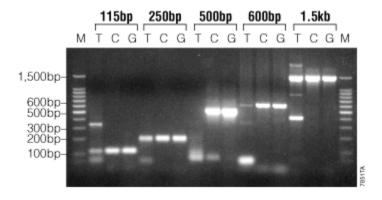


Figure 1. Comparison of amplification reactions using standard *Taq* DNA polymerase and GoTaq® Hot Start Green or Colorless Master Mix. The following fragments were amplified using *Taq* DNA polymerase, GoTaq® Hot Start Colorless or Green Master Mix: 115bp fragment from 5 copies of HIV-1 DNA in 100ng human genomic DNA; 250bp THO1 fragment from 25pg human genomic DNA; 500bp CCR5 fragment from 330pg human genomic DNA; 600bp Lambda fragment from 100 copies of Lambda DNA in 500ng human genomic DNA; and a 1.5kb fragment of Corynephage omega gene from 500pg plasmid DNA template. All reactions were set up at room temperature and placed in a room-temperature thermal cycler before starting the cycling protocol. Lane M, BenchTop 100bp Ladder (Cat.# G8291); lane T, *Taq* DNA polymerase; lane C, GoTaq® Hot Start Colorless Master Mix; lane G, GoTaq® Hot Start Green Master Mix.

# **Set Up Reactions at Room Temperature**

Room-temperature reaction setup is more convenient than setup on ice for manual hot-start methods. GoTaq® Hot Start Master Mixes allow for room-temperature setup and can even stand at room temperature for 24 hours before starting the cycling procedure. The GoTaq® Hot Start Polymerase in the master mixes remains inactive during this time. To demonstrate this property, we amplified a 1.5kb fragment of the Corynephage omega gene from a plasmid template using the two GoTaq® Hot Start Master Mixes and a master mix with standard *Taq* DNA polymerase. Two sets of reactions were assembled. One set of reactions was amplified immediately, and the second set sat on the lab bench at room temperature for 24 hours before being amplified. As seen in Figure 2, amplifications using the GoTaq® Hot Start Green or Colorless Master Mixes gave good amplification of the 1.5kb product with no secondary products whether the reactions were cycled immediately or left at room temperature for 24 hours. Amplifications using the master mix with standard *Taq* DNA polymerase gave secondary products and less yield of the 1.5kb product regardless of starting conditions.

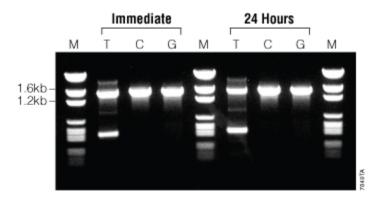


Figure 2. Comparison of PCR products when reactions are cycled immediately after assembly or left at room temperature for 24 hours before cycling. *Taq* DNA polymerase, GoTaq® Hot Start Colorless or Green Master Mix were used to amplify a 1.5kb fragment of the Corynephage omega gene from 500pg plasmid DNA. Reactions were set up at room temperature and placed placed in a room-temperature thermal cycler prior to the cycling protocol, which occurred either immediately or after 24 hours. Lane M, BenchTop pGEM® DNA Markers (Cat.# G7521); lane T, *Taq* DNA polymerase; lane C, GoTaq® Hot Start Colorless Master Mix; lane G, GoTaq® Hot Start Green Master Mix.

### **PCR Performance**

We have amplified fragments from various targets including human genomic DNA, lambda and plasmid DNA (Figure 1) using the GoTaq® Hot Start Master Mixes. We have successfully amplified targets as small as 115bp and as large as 3.1kb as well as a multiplex amplification with 6 products (Table 1, data not shown).

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

Table 1. Compatibility of GoTaq® Hot Start Green and Colorless Master Mixes with Various Applications.

Amplifications with the GoTaq® Hot Start Green and Colorless Master Mixes give similar yield and sensitivity. We amplified a 1.3kb β-globin fragment from human genomic DNA using both GoTaq® Hot Start Master Mixes. Different amounts of template DNA produced approximately equal yield with similar sensitivity (Figure 3).

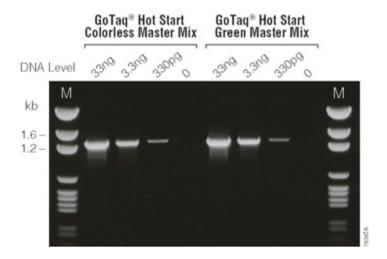


Figure 3. Detection of a beta-globin fragment from human genomic DNA using GoTaq® Hot Start Green Master Mix or GoTaq® Hot Start Colorless Master Mix. A 1.3kb fragment of the β-globin gene was amplified using 0–33ng of human genomic DNA (Cat.# G3041). All reactions were set up at room temperature and placed in a room-temperature thermal cycler before starting the cycling protocol. Lane M, BenchTop pGEM® Markers (Cat.# G7521).

# **Characteristics and Applications**

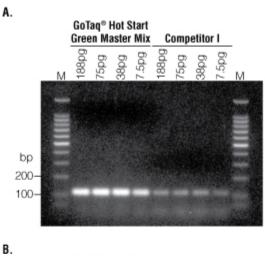
GoTaq® Hot Start Polymerase adds a single deoxyadenosine to the 3′ ends of DNA in a template-independent fashion. This allows for simple T-vector cloning of the PCR fragment following purification (Table 1). In addition, the GoTaq® Hot Start Master Mixes can be used for the PCR step in uncoupled RT-PCR after synthesizing cDNA using either the ImProm-II™ Reverse Transcription System (Cat.# A3800) or the Reverse Transcription System (Cat.# A3500; data not shown and Table 1).

# The Advantages of GoTaq® Hot Start Master Mixes

The use of the antibody inactivation of *Taq* DNA polymerase in the GoTaq® Hot Start Master Mixes has advantages over other hot-start methods. Chemical modification of *Taq* DNA polymerase requires a long initial denaturation (5–15 minutes) to restore polymerase activity unlike the shorter, 2-minute initial denaturation time for the GoTaq® Hot Start Polymerase. Manual hot-start methods can easily lead to contamination, are inconvenient to set up and may not work as well as other hot-start methods.

To compare competitor *Taq* DNA polymerase hot-start products, we amplified a 115bp HIV-1 gag fragment (4) in a human genomic DNA background using the manufacturer's instructions. Amplification of this target requires hot-start PCR. GoTaq® Hot Start Green Master Mix was compared to the direct-to-gel loading hot-start master mix from Competitor I. This competitor's product also uses the antibody method of *Taq* DNA polymerase inhibition. The GoTaq® Hot Start Green Master Mix performed well with good yield, specificity and sensitivity, while the competitor's product gave lower yield (Figure 4, Panel A). GoTaq® Hot Start Colorless Master Mix was compared with two competitor products. The product from Competitor I used the antibody inhibition hot-start method while the product from Competitor A used the chemical modification hot-start method. The GoTaq®

Hot Start Colorless Master Mix performed well giving good yield, specificity, and sensitivity while the competitor's products gave lower yields (Figure 4, Panel B).



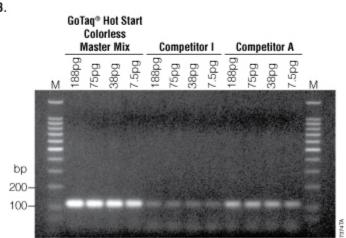


Figure 4. Detection of an HIV-gag fragment from HIV-1 DNA template using GoTaq® Hot Start Green or Colorless Master Mix and two competitors' hot-start *Taq* DNA polymerase master mixes. A 115bp fragment was amplified using the indicated amounts of HIV-1 template in a background of 100ng Human Genomic DNA (Cat.# G3041). Panel A. Direct load hot-start master mixes: GoTaq® Hot Start Green Master Mix and the master mix from Competitor I were used side-by-side. Panel B. Colorless hot-start master mixes: GoTaq® Hot Start Colorless Master Mix, the master mix from Competitor I, and the master mix from Competitor A were tested together. Amplifications were set up as directed by the protocols given for each vendor's product. Lane M, BenchTop 100bp DNA Ladder (Cat.# G8291).

# Conclusion

GoTaq® Hot Start Master Mixes provide a convenient method to amplify targets and improve specificity, sensitivity and yield for some amplifications. These two products provide the convenience of master mix format, room-temperature setup and a short initial denaturation 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 high-throughput or robotic platforms. The cycling and reaction components are the same as the familiar GoTaq® Master Mixes. To assemble reactions, you only need to add template, primer and water. As for all the GoTaq® Amplification family products, you can choose the

convenience of loading directly onto gels using the GoTaq® Hot Start Green Master Mix, or choose the GoTaq® Hot Start Colorless Master Mix if the dyes may interfere with your downstream applications.

## **REFERENCES**

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## **HOW TO CITE THIS ARTICLE**

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American Medical Association, Manual of Style, 10th edition, 2007

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