

Overview

With the AmpliTaq® 360 family of products from Applied Biosystems, you can now amplify a broader range of targets with greater specificity from one standardized, highly reproducible reagent. Compared to the original AmpliTaq DNA Polymerases, the most referenced brand of DNA polymerase in the world. AmpliTaq 360 has been ultra-purified to reduce bacterial DNA, and has been optimized with both an improved PCR buffer and a new GC-rich enhancer to bring you greater success with your PCR amplification.

The 360 GC Enhancer, which is supplied in a separate vial, enables you to easily and reproducibly amplify high-GC content targets: either those with an average high GC content or a more localized short-high GC sequence within a larger target. The 360 GC Enhancer increases specificity and yield for these challenging sequences.

This document describes the use of the 360 GC Enhancer with the AmpliTaq 360 family of products: AmpliTaq® 360 DNA Polymerase, AmpliTaq Gold® 360 DNA Polymerase, and AmpliTaq Gold® 360 Master Mix.

Recommended Usage of 360 GC Enhancer

The 360 GC Enhancer is used for difficult-to-amplify templates, especially for templates with high GC content or GC-repeats. The enhancer can also increase specificity in reactions that generate non-specific products for those templates which have a localized GC-rich sequence. You can adjust the amount of the 360 GC Enhancer to optimize your PCR reaction. Reactions that generate nonspecific products may benefit from the addition of small amounts of enhancer to improve specificity. Templates with high-GC content will require more enhancer for best results.

We recommend that customers first try amplification without 360 GC Enhancer. If the amplification fails or results in non-specific products, Table 1 shows the suggested starting volumes of the 360 GC Enhancer to use. The 360 GC Enhancer can reduce nonspecific amplification and improve the yield of specific products. However, for some template sequences, the use of the 360 GC Enhancer can reduce yield, particularly for non GC-rich targets.

360 GC Enhancer—Optimized for Challenging Amplicons

The 360 GC Enhancer has been optimized for robust performance with sequences containing a broad range of GC content. As shown in Figure 1, the 360 GC Enhancer provides significantly increased specificity and yield in the amplification of sequences with up to 80% GC content.

The 360 GC Enhancer increases specificity and yield at slightly different levels for the AmpliTaq® 360 DNA Polymerase and

Purpose	GC Content	Recommended Starting Volume Percentage of 360 GC Enhancer (per 50 µL PCR)
Increased specificity*	Any	2-5% v/v (1-2 µL)
Amplify high GC content	65-75%	10% v/v (5 µL)
Amplify high GC content	>75%	20% v/v (10 µL)

Table 1. Recommended Volumes of 360 GC Enhancer for PCR Optimization. First try amplification without 360 GC Enhancer. If amplification fails or results in nonspecific products, use the recommended starting volumes for PCR optimization. *The 360 GC Enhancer can reduce nonspecific amplification and improve the yield of specific products for those templates which may have a localized high GC sequence. For some template sequences, use of the 360 GC Enhancer can reduce yield, particularly for non GC-rich targets.

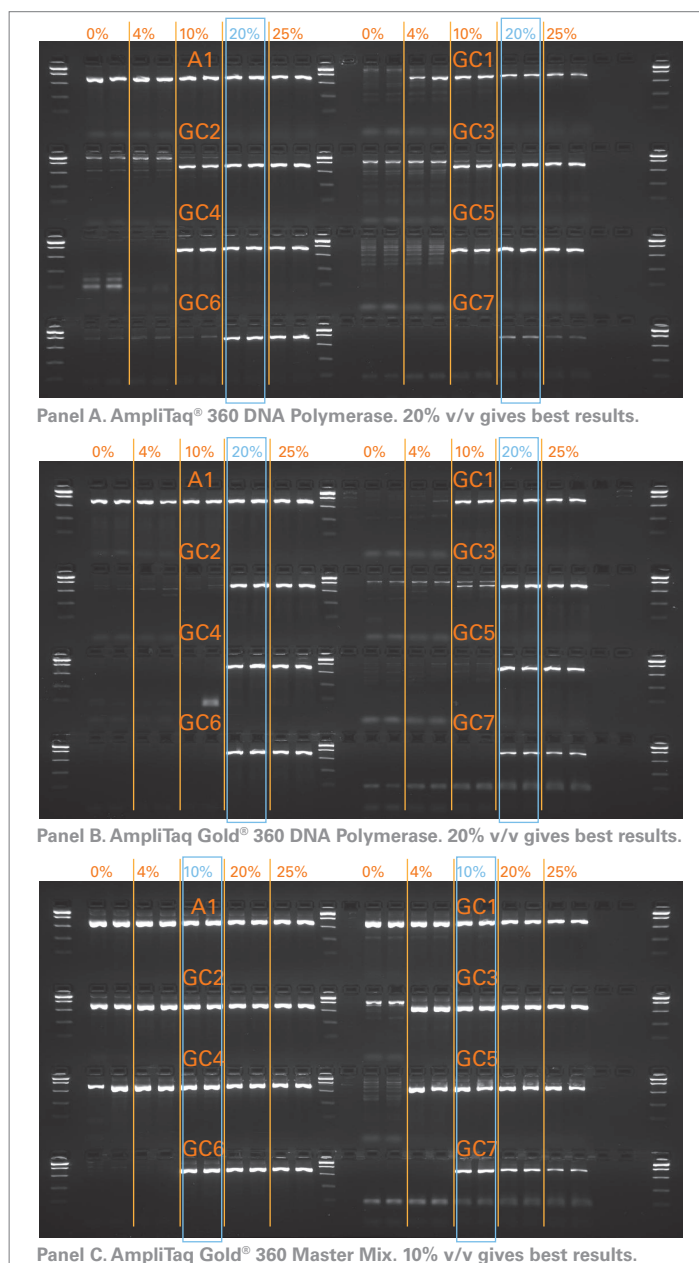


Figure 1. The 360 GC Enhancer Increases Specificity and Yield for Challenging Templates. Five different concentrations of 360 GC Enhancer were used: 0% (v/v), 4% (v/v), 10% (v/v), 20% (v/v) and 25% (v/v). Standard thermal cycling conditions were used, with a 30 sec. extension time. Amplicons are labeled as follows: A1=Easily Amplified (62% GC); GC1=74% GC; GC2=78% GC; GC3=80% GC; GC4=77% GC; GC5=79% GC; GC6=76% GC; GC7=81% GC. Panel A. PCRs were performed in duplicate using AmpliTaq® 360 Polymerase. Panel B. PCRs were performed in duplicate using AmpliTaq Gold® 360 DNA Polymerase. Panel C. PCRs were performed in duplicate using AmpliTaq Gold® 360 Master Mix.

the AmpliTaq Gold® PCR products as described in Table 2. Therefore, both the chemistry and specific GC content may affect the optimum yield and specificity. In some cases, the 360 GC Enhancer may slightly inhibit yield or specificity at higher concentrations, which is observed in Assays A1 and GC1 in Figure 1, Panel A. While Applied Biosystems has performed extensive testing, we encourage customers to perform their own optimization using our recommended starting volumes. Additionally, we recommend using the AmpliTaq Gold® 360 Master Mix for the best overall amplification specificity and yield.

Conclusion

Whether you are using AmpliTaq 360 DNA Polymerase, AmpliTaq Gold 360 DNA Polymerase, or AmpliTaq Gold 360 PCR Master Mix, the 360 GC Enhancer increases the success of PCR

amplification for challenging templates, especially those with high GC content. Overall, the most robust amplification occurs with the use of AmpliTaq Gold 360 Master Mix. Together, this suite of products allows you to amplify a larger range of targets for better success with your PCR experiments.

Product Description	Optimum 360 GC Enhancer Concentration
AmpliTaq® 360 DNA Polymerase	10–20%
AmpliTaq Gold® 360 DNA Polymerase	20%
AmpliTaq Gold® 360 Master Mix	10%

Table 2. Recommended Volumes of 360 GC Enhancer for each AmpliTaq® 360 Product. First try amplification without 360 GC Enhancer. If amplification fails or results in nonspecific products, use the recommended starting volumes for PCR optimization.

ORDERING INFORMATION

Description	Size	P/N
AmpliTaq Gold® 360 DNA Polymerase <i>Includes AmpliTaq® 360 DNA Polymerase, AmpliTaq 360 Buffer, 25mM MgCl₂ and 360 GC Enhancer</i>	100 U	4398813
	250 U	4398823
	1000 U	4398833
	1500 U	4398892
	3000 U	4398894
	5000 U	4398896
	25 x 1000 U	4398898
	25000 U	4398900
AmpliTaq Gold® 360 DNA Polymerase, standalone	25000 U	4398843
AmpliTaq Gold® 360 Master Mix <i>Includes AmpliTaq Gold® 360 Master Mix and 360 GC Enhancer</i>	1 mL	4398876
	5 mL	4398881
	10 x 5 mL	4398901
	50 mL	4398886
AmpliTaq® 360 DNA Polymerase <i>Includes AmpliTaq® 360 DNA Polymerase, AmpliTaq Gold 360 Buffer, 25mM MgCl₂ and 360 GC Enhancer</i>	100 U	4398808
	250 U	4398818
	1000 U	4398828
	1500 U	4398891
	3000 U	4398893
	5000 U	4398895
	25 x 1000 U	4398897
	25000 U	4398899
AmpliTaq® 360 DNA Polymerase, standalone	25000 U	4398838
AmpliTaq® 360 Buffer <i>Includes AmpliTaq® 360 Buffer, 25mM MgCl₂ and 360 GC Enhancer</i>	1.5 mL	4398848
	6 x 1.5 mL	4398858
	150 mL	4398868
AmpliTaq Gold® 360 Buffer <i>Includes Buffer, 25mM MgCl₂ and 360 GC Enhancer</i>	1.5 mL	4398853
	6 x 1.5 mL	4398863
	150 mL	4398872
Protocol: AmpliTaq® 360 DNA Polymerase	1 protocol	4398942
Quick Reference Card: AmpliTaq® 360 DNA Polymerase	1 card	4398952
Protocol: AmpliTaq Gold® 360 DNA Polymerase	1 protocol	4398943
Quick Reference Card: AmpliTaq Gold® 360 DNA Polymerase	1 card	4398953
Protocol: AmpliTaq Gold® 360 Master Mix	1 protocol	4398944
Quick Reference Card: AmpliTaq Gold® 360 Master Mix	1 card	4398954

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