GoTaq® Flexi DNA Polymerase: Robust Performance with Magnesium Optimization

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Abstract

GoTaq® Flexi DNA Polymerase is a new member of the GoTaq® family of products. The two GoTaq® Flexi Buffers offer the flexibility of magnesium optimization in PCR. Green GoTaq® Flexi Buffer allows loading of amplified DNA samples directly onto agarose gels, eliminating the need to add sample loading buffer to PCR samples before electrophoresis. Colorless GoTaq® Flexi Buffer is provided for use when PCR samples are not loaded onto a gel or when the dyes in the Green GoTaq® Flexi Buffer would interfere with downstream applications.

GoTaq® DNA Polymerase gets a partner—GoTaq® Flexi DNA Polymerase—with the same robust, reliable performance plus the ability to optimize magnesium concentration.

Introduction

PCR[a] and RT-PCR[a] are commonly used amplification techniques in biological research, and *Taq* DNA polymerase is the most common thermostable DNA polymerase for these applications. For some amplification reactions, optimization of the magnesium in the reaction is a critical step for ensuring good yield and sensitivity. GoTaq® Flexi DNA Polymerase[b] is a new version of our popular GoTaq® DNA Polymerase[b] and is specially formulated to allow optimization of magnesium concentration in PCR. The 5X Green and 5X Colorless GoTaq® Flexi Buffers provided with GoTaq® Flexi DNA Polymerase have the same formulation as the reaction buffers supplied with the original GoTaq® DNA Polymerase but do not contain magnesium. A separate 25mM $MgCl_2$ solution is provided to allow the flexibility of magnesium optimization in PCR.

After amplification, DNA fragments are frequently analyzed by agarose gel electrophoresis. To streamline this process it is helpful to directly load the PCR sample onto an agarose gel without adding gel loading dye or buffer to each sample. Reactions using GoTaq® Flexi DNA Polymerase and Green GoTaq® Flexi Buffer can be directly loaded onto gels just like reactions using the Green GoTaq® Reaction Buffer with GoTaq® DNA Polymerase (1,2). When the 5X Green GoTaq® Flexi Buffer is diluted to 1X concentration, the buffer has sufficient density to sink into the wells of an agarose gel or nondenaturing TBE polyacrylamide gel. The Green GoTaq® Flexi Buffer also contains two dyes (blue and yellow) that separate during electrophoresis and can be used to monitor the progress of samples on the gel (Figure 1).

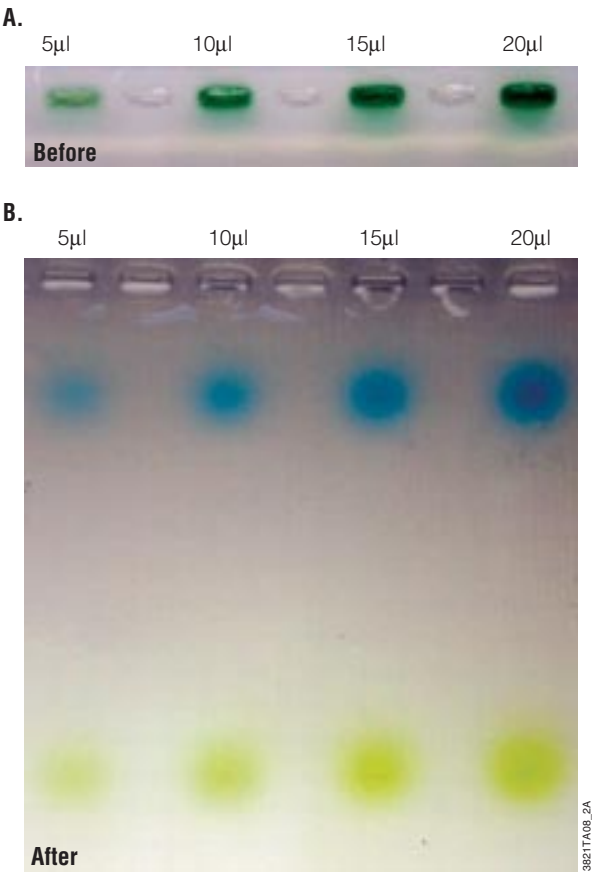

Figure 1. Separation of the blue and yellow dyes used in GoTaq® and GoTaq® Flexi Green Reaction Buffers before and after electrophoresis. Panel A. Loaded wells of an agarose gel. **Panel B.** Blue and yellow dyes after electrophoresis. Volumes of 5, 10, 15 and 20µl of the amplification reactions were loaded onto a 1% agarose gel with TBE buffer and subjected to electrophoresis.

The 5X Colorless GoTaq® Flexi Buffer is recommended for use with GoTaq® Flexi DNA Polymerase when performing absorbance or fluorescence measurements without prior purification of the amplimer. The Colorless GoTaq® Flexi Buffer has the same composition as the 5X Green GoTaq® Flexi Buffer but does not contain dye.

In this article, we describe the properties of both the GoTaq® Flexi enzyme and buffers. We examine the characteristics of the dyes in the Green GoTaq® Flexi Buffer and discuss some considerations to assist in choosing the appropriate reaction buffer for your application. We also compare the amplification properties

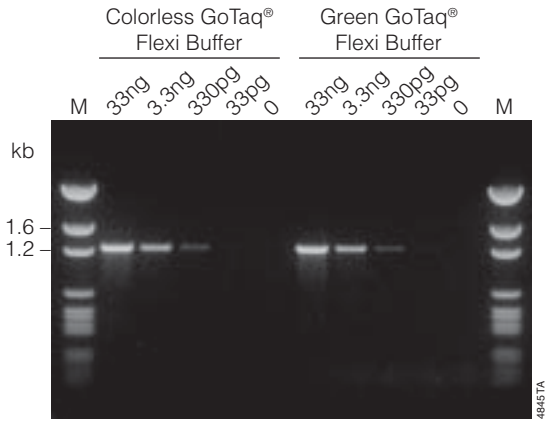

Figure 2. Detection of an α-1-antitrypsin fragment from human genomic DNA using GoTaq® Flexi DNA Polymerase with either Colorless GoTaq® Flexi Buffer or Green GoTaq® Flexi Buffer. A 1.2kb fragment of the α-1-antitrypsin gene was amplified using the indicated amounts of Human Genomic DNA (Cat.# G3041). Lane M, BenchTop pGEM® DNA Markers (Cat.# G7521). All amplifications were performed with PCR Nucleotide Mix (Cat.# C1141) as the dNTP source.

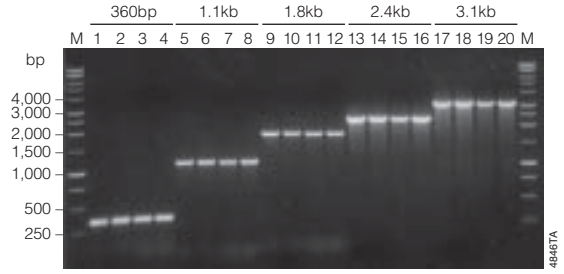

Figure 3. Comparison of amplification reactions using GoTaq® DNA Polymerase and GoTaq® Flexi DNA Polymerase. A 360bp α-1-antitrypsin fragment from 3.3ng Human Genomic DNA (Cat.# G3041), a 1.1kb IL-1β fragment from 10ng Mouse Genomic DNA (Cat. # G3091), a 1.8kb APC fragment from 3.3ng Human Genomic DNA, a 2.4kb APC fragment from 33ng Human Genomic DNA and a 3.1kb APC fragment from 75ng Human Genomic DNA were amplified using the indicated amounts of template DNA. Amplifications were performed using either GoTaq® DNA Polymerase with Colorless GoTaq® Reaction Buffer (lanes 1, 5, 9, 13, 17), GoTaq® Flexi DNA Polymerase with Colorless GoTaq® Flexi Buffer (lanes 2, 6, 10, 14, 18), GoTaq® DNA Polymerase with Green GoTaq® Reaction Buffer (lanes 3, 7, 11, 15, 19), or GoTaq® Flexi DNA Polymerase with Green GoTaq® Flexi Buffer (lanes 4, 8, 12, 16, 20). Lane M, BenchTop 1kb DNA Ladder (Cat.# G7541). All amplifications were performed with PCR Nucleotide Mix (Cat.# C1141) as the dNTP source.

of GoTaq® Flexi DNA Polymerase with those of GoTaq® DNA Polymerase. Finally, we report on the compatibility of GoTaq® Flexi DNA Polymerase with RT-PCR.

Green GoTaq® Flexi Buffer

When using the 5X Green GoTaq® Flexi Buffer, a wide range of reaction volumes may be loaded into the wells of a gel. Migration can be monitored by following the progress of the blue and yellow dyes. During electrophoresis the blue dye migrates at the same rate as a 3–5kb DNA fragment in a 1% agarose gel, which is also approximately the same rate as the commonly used loading dye xylene cyanol. The yellow dye migrates at a rate faster than primers (<50bp), making it easy to ensure that the DNA fragments of interest remain in the gel. The yellow dye also runs faster than the orange electrophoresis marker found in the Blue/Orange 6X Loading Dye (Cat.# G1881). Neither dye interferes with the migration of the DNA in agarose gels; fragments from reactions containing these dyes migrate the same distance as corresponding markers. Also, DNA fragments that comigrate with the blue dye are not masked by that dye when ≤20µl of the amplification reaction is loaded onto the gel.

Buffer Choices

The 5X Green GoTaq® Flexi Buffer is not recommended when downstream applications require fluorescence or absorbance measurements. The dyes in the Green Flexi Buffer absorb light between 225 and 300nm, making standard A_{260} determination of DNA concentration unreliable. The dyes also have excitation peaks at 488nm and 600–700nm, which correspond to the excitation wavelengths used in common fluorescence detection instruments. Although the yellow dye has the same excitation wavelength as that used by many fluorescent gel scanners (488nm), it minimally interferes because of its rapid migration in the gel. Gels scanned with a 488nm scanner will have a light-grey dye front (corresponding to the yellow dye) that migrates faster than the primers.

The 5X Colorless GoTaq® Flexi Buffer is recommended for applications requiring absorbance or fluorescence measurements without prior clean-up of the DNA fragments. If the DNA fragments are purified from the amplification reaction, the 5X Green GoTaq® Flexi Buffer may also be used for such applications. Common PCR clean-up systems such as Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281) will easily remove the blue and yellow dyes from amplified DNA. Alternatively, dyes can be removed from the amplified DNA by either ethanol precipitation or agarose gel electrophoresis followed by excision of the DNA fragment from the gel.

PCR Performance

GoTaq® Flexi DNA Polymerase in either 5X Green or 5X Colorless GoTaq® Flexi Buffer amplifies target DNA with approximately equal yield and sensitivity in the amplifications that we have tested. To illustrate this, we amplified a 1.2kb α-1-antitrypsin fragment from Human Genomic DNA (Cat.# G3041). GoTaq® Flexi DNA Polymerase with either 5X Green or 5X Colorless GoTaq® Flexi Buffers amplified this product from different amounts of starting material with similar yield and sensitivity (Figure 2).

GoTaq® Flexi DNA Polymerase performs equivalently to GoTaq® DNA Polymerase in the amplification reactions tested thus far. We compared the ability of the two enzymes to amplify five DNA targets in the buffers provided with each enzyme (GoTaq® Flexi Buffers with GoTaq® Flexi DNA Polymerase and GoTaq® Reaction

Buffers with GoTaq® DNA Polymerase). For this comparison, $MgCl_2$ solution was added to the GoTaq® Flexi DNA Polymerase amplifications to give the appropriate final concentration. Since $MgCl_2$ is present at 1.5mM in the GoTaq® Reaction Buffers, no magnesium was added to the GoTaq® DNA Polymerase amplifications unless a higher concentration of magnesium was necessary for the specific target. In these cases, a 25mM $MgCl_2$ stock (Cat.# A3511) was used for this adjustment. The final magnesium concentrations for the amplification reactions for both enzymes were equivalent. The GoTaq® Flexi DNA Polymerase in either Green or Colorless GoTaq® Flexi Buffer amplified as well as GoTaq® DNA Polymerase in either Green or Colorless GoTaq® Reaction Buffer (Figure 3). Both products successfully amplified fragments of a wide size range. In addition to those targets tested in Figure 3, we have successfully amplified fragments up to 3.9kb from a genomic DNA template using GoTaq® Flexi DNA Polymerase (data not shown). Previous work (2) demonstrated that GoTaq® DNA Polymerase performed as well as, and in some cases better than, standard *Taq* DNA Polymerase (Cat.# M1661, M1861) in a similar experiment.

Some targets may prove difficult to amplify due to DNA secondary structure or high GC content. Frequently the addition of PCR-enhancing agents, such as dimethyl sulfoxide (DMSO) or betaine, to the reaction enable successful amplification (3–6). We tested the performance of GoTaq® Flexi DNA Polymerase in Green and Colorless Reaction Buffers in amplifications containing DMSO or betaine. Both DMSO and betaine were compatible with GoTaq® Flexi DNA Polymerase in these amplifications (Table 1, specific data not shown). In addition, neither enhancing agent has an adverse effect on the blue or yellow dyes. Both dyes retain their color and migrate as expected in agarose gels during electrophoresis.

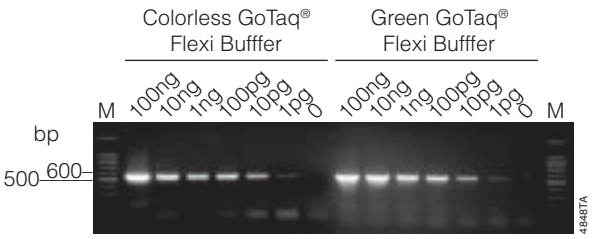

Figure 4. Detection of a β-actin fragment from total mouse liver RNA. Indicated amounts of total RNA from mouse liver isolated using RNAgents® Total RNA Isolation System (Cat.# Z5110) served as the template in the 20µl cDNA synthesis reactions. cDNA was generated as directed in the *ImProm-II™ Reverse Transcription System Technical Manual* (7) using the Oligo$(dT)_{15}$ Primer (Cat.# C1101). A 540bp β-actin fragment was amplified using 20µl of the cDNA synthesis reactions in 100µl PCR amplifications as directed in the *ImProm-II™ Reverse Transcription System Technical Manual* (7). GoTaq® Flexi DNA Polymerase with either Colorless or Green GoTaq® Flexi Buffer was used for the amplification reactions. Lane M, 100bp DNA Ladder (Cat.# G2101). All amplifications were performed with PCR Nucleotide Mix (Cat.# C1141) as the dNTP source.

Table 1. Compatibility of GoTaq® Flexi DNA Polymerase with Various Applications.

Application	Green GoTaq® Flexi Buffer	Colorless GoTaq® Flexi Buffer
PCR		
Amplification of targets from 360bp to 3.9kb in length	Yes	Yes
Addition of PCR-enhancing agents DMSO (5%) and betaine (1M)	Yes	Yes
Uncoupled RT-PCR		
Amplifying fragment from cDNA generated by the ImProm-II™ Reverse Transcription System	Yes	Yes
Amplifying fragment from cDNA generated by the Reverse Transcription System (AMV RT)	Yes	Yes
Direct Gel Loading		
Direct loading onto agarose or nondenaturing TBE polyacrylamide gel	Yes	No

Use in RT-PCR

When performing uncoupled RT-PCR, cDNA is generated by the reverse transcriptase (RT), then all or part of this reaction is used in PCR. When setting up the amplification reactions carryover of magnesium, dNTPs and buffer from the RT reaction must be considered. Since the Green and Colorless GoTaq® Flexi Buffers do not contain $MgCl_2$ it is easier to optimize the magnesium concentration of the amplification reaction. The range of magnesium concentration that can be achieved in the amplification reaction with GoTaq® Flexi Buffer is much greater than that achieved if the DNA polymerase reaction buffer contains magnesium. We found GoTaq® Flexi DNA Polymerase to be particularly useful when large volumes of the RT reaction were used as template in the subsequent amplification, since higher magnesium concentrations are generally required for RT reactions than amplification reactions.

GoTaq® Flexi DNA Polymerase with either Green or Colorless Flexi Buffer can be used for PCR after generation of cDNA in uncoupled RT-PCR. We tested this by generating cDNA from total mouse liver RNA using the ImProm-II™ Reverse Transcription System (Cat.# A3800) and then amplifying a 540bp β-actin target using GoTaq® Flexi DNA Polymerase with either the Green or Colorless GoTaq® Flexi Buffer. GoTaq® Flexi DNA Polymerase was able to amplify this target, and the yield and sensitivity were similar using either buffer (Figure 4). GoTaq® Flexi DNA Polymerase with either the Green or Colorless Flexi Buffer can also be used for PCR after generation of cDNA using the AMV RT-based Reverse Transcription System (Cat.# A3500; Table 1, specific data not shown).

Conclusion

GoTaq® Flexi DNA Polymerase with 5X Green and 5X Colorless GoTaq® Flexi Buffers provides a flexible choice for amplifications that require magnesium optimization. As with the familiar 5X GoTaq® Green Reaction Buffer, amplifications performed using the 5X Green GoTaq® Flexi Buffer provide the convenience of direct loading of samples onto gels without the need to add loading dye. GoTaq® Flexi DNA Polymerase can be used to amplify a wide range of target sizes. Yield and sensitivity are similar for a variety of targets using either the Green or Colorless GoTaq® Flexi Buffer. GoTaq® Flexi DNA Polymerase can also be used in the PCR step of uncoupled RT-PCR when either the ImProm-II™ Reverse Transcription System or the AMV RT-based Reverse Transcription System are used to generate the cDNA.

Acknowledgments

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References

1. Knoche, K. (2002) *Neural Notes* **21**, 2–5.
2. Glebs, A. *et al.* (2003) *Promega Notes* **83**, 21–4.
3. Frackman, S. *et al.* (1998) *Promega Notes* **65**, 27–9.
4. Baskaran, N. *et al.* (1996) *Genome Res.* **6**, 633–8.
5. Weissensteiner, T. and Lanchbury, J.S. (1996) *Biotechniques* **21**, 1102–8.
6. Hengen, P.N. (1997) *Trends Biochem. Sci.* **22**, 225–6.
7. *ImProm-II™ Reverse Transcription System Technical Manual* #TM236, Promega Corporation.

Protocol

◆ *GoTaq® Flexi DNA Polymerase Product Information* #9PIM829, Promega Corporation.
www.promega.com/tbs/9pim829/9pim829.html

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Ordering Information

Product	Size	Cat.#
GoTaq® Flexi DNA Polymerase	100u	M8291
	500u	M8295
	2,500u (5 × 500u)	M8296
	5,000u (10 × 500u)	M8297
	10,000u (20 × 500u)	M8298

For Laboratory Use.

Related Products

Product	Size	Cat.#
GoTaq® DNA Polymerase	100u	M3001
	500u	M3005
	2,500u	M3008
ImProm-II™ Reverse Transcription System	100 reactions	A3800
Reverse Transcription System	100 reactions	A3500

For Laboratory Use.

[a] The PCR process is covered by patents issued and applicable in certain countries*. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.

*In the U.S., effective March 29, 2005, U.S. Pat. Nos. 4,683,195, 4,965,188 and 4,683,202 will expire. In Europe, effective March 28, 2006, European Pat. Nos. 201,184 and 200,362 will expire.

[b] Certain applications of this product are covered by patents issued and applicable in certain countries. Because purchase of this product does not include a license to perform any patented application, users of this product may be required to obtain a patent license depending upon the particular application and country in which the product is used.

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