Certificate of Analysis

GoTaq® Hot Start Polymerase:

Part# 9PIM500

Revised 4/18

Supplied With:

Cat.#	GoTaq® Hot Start Polymerase	5X Green GoTaq® Flexi Buffer	5X Colorless GoTaq® Flexi Buffer	Magnesium Chloride Solution, 25mM
M5001	100 units (M500A)	1ml (M891A)	1ml (M890A)	0.75ml (A351B)
M5005	500 units (M500B)	4 × 1ml (M891A)	4 × 1ml (M890A)	3 × 1.2ml (A351H)
M500B-C	500 units (M500B)			

Description: GoTaq® Hot Start Polymerase[a,b] contains high-performance GoTaq® DNA Polymerase bound to a proprietary antibody that blocks polymerase activity. Polymerase activity is restored during the initial denaturation step, when amplification reactions are heated at 94–95°C for two minutes, allowing hot-start PCR in which polymerase activity is inhibited at temperatures below 70°C for convenient, room-temperature reaction setup. Hot-start PCR is advantageous for some amplification targets because it may eliminate or minimize primer-dimer and nonspecific products. In some cases, hot-start PCR may improve yields. GoTaq® Hot Start Polymerase exhibits 5´→3´ exonuclease activity.

Cat.# M5001 and M5005 are supplied with 5X Green GoTaq® Flexi Buffer, 5X Colorless GoTaq® Flexi Buffer and 25mM $MgCl_2$.

The 5X Green GoTaq® Flexi Buffer contains two dyes (a blue dye and a yellow dye) that separate during electrophoresis to monitor migration progress. The 5X Colorless GoTaq® Flexi Buffer is used when direct fluorescence or absorbance readings are required without prior purification of the amplified DNA. The GoTaq® Flexi Buffers do not contain magnesium, allowing easy optimization of $MgCl_2$ concentration in amplification reactions.

Biological Source: The enzyme is derived from bacteria. The antibody is derived from murine cell culture.

Enzyme Concentration: 5u/µl.

5X Green GoTaq® Flexi Buffer (Part # M891A): Proprietary formulation supplied at pH 8.5 containing blue dye and yellow dye. The blue dye migrates at the same rate as a 3–5kb DNA fragment in a 1% agarose gel. The yellow dye migrates at a rate faster than primers (<50bp) in a 1% agarose gel. Green GoTaq® Flexi Buffer also increases the density of the sample so that it will sink into the well of the agarose gel, allowing reactions to be loaded directly onto gels without loading dye. This buffer does not contain magnesium.

5X Colorless GoTaq® Flexi Buffer (Part # M890A): Proprietary formulation supplied at pH 8.5. This buffer does not contain dyes or magnesium.

Magnesium Chloride Solution, 25mM (Part# A351B, A351H): Provided to allow users to optimize $MgCl_2$ concentration to satisfy individual requirements. Vortex the $MgCl_2$ thoroughly after thawing and prior to use.

Storage Conditions: See the Product Information Label for storage conditions. Avoid exposure to frequent temperature changes. See the expiration date on the Product Information label.

Unit Definition: One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nanomoles of dNTPs into acid-insoluble material in 30 minutes at 74°C.

Quality Control Assays

This lot passes the following Quality Control specifications:

Functional Assay: Performance of GoTaq® Hot Start Polymerase is tested in PCR using 1.25 units of enzyme to amplify a 360bp region of the α-1-antitrypsin gene and a 2.4kb region of the APC gene from 100 molecules (0.35ng) of human genomic DNA in separate reactions. The resulting PCR products are visualized as single bands on an ethidium bromide-stained agarose gel.

Nuclease Assays: No contaminating endonuclease or exonuclease activity detected.

Hot-Start Amplification Assay: GoTaq® Hot Start Polymerase is tested in PCR for its ability to amplify a hot-start model template and produce a single 1.5kb band, eliminating extraneous bands. In a nonhot-start PCR, this template produces an additional band at 410bp.



PCR Satisfaction Guarantee

Promega's PCR Systems, enzymes and reagents are proven in PCR to ensure reliable, high performance results. Your success is important to us. Our products are backed by a worldwide team of Technical Support scientists. Please contact them for applications or technical assistance. If you are not completely satisfied with any Promega PCR product we will send a replacement or refund your account.

That's Our PCR Guarantee!

Product must be within expiration date and have been stored and used in accordance with product literature. See Promega Product Insert for specific tests performed.

[a]Use of this product for basic PCR is outside of any valid US or European patents assigned to Hoffman La-Roche or Applera. This product can be used for basic PCR in research without any license or royalty fees. This product is for research use only.
[b]U.S. Pat. No. 6,242,235, Australian Pat. No. 761757, Canadian Pat. No. 2,335,153, Chinese Pat. No. ZL99808861.7, Hong Kong Pat. No. HK 1040262, Japanese Pat. No. 3673175, European Pat. No. 1088060 and other patents pending.

Signed by:

C. Stollberg, Quality Assurance



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Promega

Usage Information

1. Standard Application

Reagents to Be Supplied by the User

- PCR Nucleotide Mix (Cat.# C1141)
- upstream primer
- template DNA
- Nuclease-Free Water (Cat.# P1193)
- downstream primer
- mineral oil (optional)

1. In a sterile, nuclease-free microcentrifuge tube, combine the following components at room temperature:

Component	Final Volume	Final Concentration
5X Green or Colorless GoTaq® Flexi Buffer[1]	10µl	1X
$MgCl_2$ Solution, 25mM[1]	2–8µl	1.0–4.0mM
PCR Nucleotide Mix, 10mM each	1µl	0.2mM each dNTP
upstream primer	Xµl	0.1–1.0µM
downstream primer	Yµl	0.1–1.0µM
GoTaq® Hot Start Polymerase (5u/µl)	0.25µl	1.25u
template DNA	Zµl	<0.5µg/50µl
Nuclease-Free Water to	50µl	

[1]Thaw completely, and vortex thoroughly prior to use.

2. If using a thermal cycler **without a heated lid**, overlay the reaction mix with 1–2 drops (approximately 50µl) of mineral oil to prevent evaporation during thermal cycling. Centrifuge the reactions in a microcentrifuge for 5 seconds.
3. Reactions can be placed in a room-temperature thermal cycler. **A 2-minute initial denaturation step at 94–95°C is required to inactivate the antibody and initiate hot-start PCR.**

2. General Guidelines for Amplification by PCR

A. Denaturation

- Following the initial 2-minute 94–95°C denaturation step, denaturation steps should be between 30 seconds and 1 minute.

B. Annealing

- Optimize the annealing conditions by performing the reaction with an annealing temperature approximately 5°C below the calculated melting temperature of the primers and increasing the temperature in increments of 1°C.
- The annealing step is typically 30 seconds to 1 minute.

C. Extension

- The extension reaction is typically performed at the optimal temperature for *Taq* DNA polymerase, which is 72–74°C.
- Allow approximately 1 minute for every 1kb of DNA to be amplified.
- A final extension of 5 minutes at 72–74°C is recommended.

D. Soak

- If the thermal cycler has a refrigeration or "soak" cycle, the thermal cycler can be programmed to hold the tubes at 4°C for several hours after amplification.
- This cycle minimizes polymerase activity, which might occur at higher temperatures, although this is not usually a problem.

E. Cycle Number

- Generally, 25–30 cycles result in optimal amplification of desired products.
- Occasionally, up to 40 cycles may be performed, especially to detect low-copy targets.

3. General Considerations

A. Buffer Choice

We recommend using the 5X Green GoTaq® Flexi Buffer in any amplification reaction that will be visualized by agarose gel electrophoresis followed by ethidium-bromide staining. The 5X Green GoTaq® Flexi Buffer is not recommended for any downstream applications using absorbance or fluorescence excitation because the yellow and blue dyes in the reaction buffer may interfere with these applications. The dyes absorb at 225–300nm, making standard A_{260} readings to determine DNA concentration unreliable. Also, the dyes have excitation peaks at 488nm and 600–700nm, which correspond to excitation wavelengths commonly used in fluorescence-detection instrumentation. However, for some instrumentation, such as a fluorescent gel scanner that uses a 488nm excitation wavelength, there will be minimal interference, since it is the yellow dye that absorbs at this wavelength. Gels scanned by this method will have a light gray dye front below the primers that corresponds to the yellow dye front. The Green and Colorless GoTaq® Flexi Buffers give approximately equivalent amplification yields. To obtain equal amplification yields with the two buffers, PCR conditions might require optimization.

For reactions going directly from thermal cycler to an application using absorbance or fluorescence, we recommend the 5X Colorless GoTaq® Flexi Buffer. If both agarose gel analysis and further downstream applications involving absorbance or fluorescence will be used, the two dyes can be removed from the Green GoTaq® Flexi reactions using standard PCR cleanup systems like the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281) or Wizard® SV 96 PCR Clean-Up System (Cat.# A9341).

B. Enzyme Concentration

Promega has found that 1.25 units of GoTaq® Hot Start Polymerase per 50µl amplification reaction is adequate for most amplifications. Adding extra enzyme generally does not produce significant increases in yield. However, in some cases, more enzyme may be beneficial.

C. Primer Design

PCR primers generally range in length from 15 to 30 bases and are designed to flank the region of interest. Primers should contain 40–60% (G + C), and care should be taken to avoid sequences that might produce internal secondary structure. The 3´-ends of the primers should not be complementary to avoid production of primer-dimers. Primer-dimers unnecessarily deplete primers from the reaction and result in an unwanted polymerase reaction that competes with the desired reaction. Avoid three G or C nucleotides in a row near the 3´-end of the primer because this may result in nonspecific primer annealing, increasing synthesis of undesirable reaction products. Ideally, both primers should have nearly identical melting temperatures (T_m) so that the two primers anneal at roughly the same temperature. The annealing temperature of the reaction depends on the T_m of the primer with the lowest T_m. For assistance with calculating the T_m of any primer, a T_m Calculator is provided on the BioMath page of the Promega web site at:
www.promega.com/biomath/

D. Amplification Troubleshooting

To overcome low or no yield in amplifications, we recommend the following suggestions:

- Adjust annealing temperature. The reaction buffer composition affects the melting properties of DNA. See BioMath Calculator to calculate the melting temperature for primers in the GoTaq® reaction (**www.promega.com/biomath/**).
- Minimize the effect of amplification inhibitors. Some DNA isolation procedures, particularly genomic DNA isolation, can result in copurification of amplification inhibitors. Reduce the volume of template DNA in the reaction, or dilute the template DNA prior to addition. Diluting samples up to 1:10,000 can improve results, depending on the initial DNA concentration.
- Increase template DNA purity. Include an ethanol precipitation and wash step prior to amplification to remove inhibitors that copurify with the DNA.
- Add PCR additives. Adding PCR-enhancing agents (e.g., DMSO or betaine) may improve yields. General stabilizing agents such as BSA (Sigma Cat.# A7030; final concentration 0.16mg/ml) also may help to overcome amplification failure.

E. More Information on Amplification

More information on amplification is available online at the Promega web site:
www.promega.com/products/pcr

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