

Product Contents

GoTaq® Hot Start Polymerase:

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)

Description: GoTaq® Hot Start Polymerase^(a-c) contains GoTaq® Hot Start Polymerase, 5X Green GoTaq® Flexi Buffer, 5X Colorless GoTaq® Flexi Buffer and 25mM MgCl₂. GoTaq® Hot Start Polymerase contains the high-performance GoTaq® DNA polymerase bound to a proprietary antibody that blocks polymerase activity. The polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 94–95°C for two minutes. This allows hot-start PCR in which polymerase activity is inhibited at temperatures below 70°C, allowing convenient, room-temperature reaction setup. Hot-start PCR is advantageous for some amplification targets, because it may eliminate or minimize primer-dimer and secondary products. In some cases, hot-start PCR may improve yields. GoTaq® Hot Start Polymerase exhibits 5'→3' exonuclease activity.

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 colorless buffer is used when direct fluorescence or absorbance readings are required without prior purification of the amplified DNA from the PCR. The Flexi Buffers do not contain magnesium, allowing easy optimization 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 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 magnesium.

Magnesium Chloride Solution, 25mM (Part# A351B, A351H): Provided to allow users to optimize MgCl₂ concentration according to their individual requirements. Vortex the MgCl₂ 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

Functional Assay: GoTaq® Hot Start Polymerase is tested for performance 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 non-hot-start PCR, this template produces an additional band at 410bp.

5X Green GoTaq® Flexi Buffer Migration Pattern: The 5X Green GoTaq® Flexi Buffer does not interfere with the migration of a 1kb DNA ladder when it is used as a loading dye for agarose gel electrophoresis.



PCR Satisfaction Guarantee

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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.

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Promega

Promega Corporation

2800 Woods Hollow Road
Madison, WI 53711-5399 USA
Telephone 608-274-4330
Toll Free 800-356-9526
Fax 608-277-2516
Internet www.promega.com

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I. 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 Conc.
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	

¹Thaw 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.**

II. 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 starting approximately 5°C below the calculated melting temperature of the primers and increasing the temperature in increments of 1°C to the annealing temperature.
- 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. Refrigeration

- If the thermal cycler has a refrigeration or "soak" cycle, the cycling reaction can be programmed to end by holding the tubes at 4°C for several hours.
- This cycle can minimize any polymerase activity that 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 for detection of low-copy targets.

II. 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 between 225–300nm, making standard A₂₆₀ readings to determine DNA concentration unreliable. Also, the dyes have excitation peaks at 488nm and between 600–700nm that correspond to the 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 this wavelength. Gels scanned by this method will have a light gray dye front below the primers corresponding to the yellow dye front. The Green and Colorless GoTaq® Flexi Buffers give approximately equivalent amplification yields. Balanced amplifications between the two buffers may require further optimization.

For reactions going directly from thermal cycler to an application using absorbance or fluorescence, the 5X Colorless GoTaq® Flexi Buffer is recommended. 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 clean-up systems like the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281) or the Wizard® SV 96 PCR Clean-Up System (Cat.# A9341).

B. Enzyme Concentration

Promega has found that 1.25 units of GoTaq® DNA 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 15–30 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 the 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 the synthesis of undesirable reaction products. Ideally, both primers should have nearly identical melting temperatures (T_m); in this manner, the two primers should anneal roughly at the same temperature. The annealing temperature of the reaction depends on 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 yield 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 the copurification of amplification inhibitors. Reduce the volume of template DNA in reaction, or dilute template DNA prior to adding to reaction. Diluting samples up to 1:10,000 has been shown to be effective in improving results, depending on 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:

PCR Applications: www.promega.com/paguide/chap1.html

PCR Core Systems Technical Bulletin: www.promega.com/tbs/tb254/tb254.html

DNA Analysis Notebook: www.promega.com/guides/dna_guide/