

Certificate of Analysis

GoTaq® G2 Flexi DNA Polymerase:

Supplied With:

Cat.#	GoTaq® G2 Flexi DNA Polymerase	5X Green GoTaq® Flexi Buffer	5X Colorless GoTaq® Flexi Buffer	Magnesium Chloride Solution, 25mM
M7801	100 units (M780A)	1ml (M891A)	1ml (M890A)	0.75ml (A351B)
M7802	100 units (M780A)			
M7805	500 units (M780B)	4 × 1ml (M891A)	4 × 1ml (M890A)	3 × 1.2ml (A351H)
M7806	5 × 500 units (M780B)	20 × 1ml (M891A)	20 × 1ml (M890A)	15 × 1.2ml (A351H)
M7808	20 × 500 units (M780B)	80 × 1ml (M891A)	80 × 1ml (M890A)	60 × 1.2ml (A351H)

Description: GoTaq® G2 Flexi DNA polymerase^(a,b) contains GoTaq® G2 Flexi DNA Polymerase, 5X Green GoTaq® Flexi Buffer, 5X Colorless GoTaq® Flexi Buffer, and 25mM MgCl₂. The enzyme is supplied in a proprietary formulation containing 50% glycerol with buffers designed for enhanced amplification. The enzyme is a full-length form of *Taq* DNA polymerase that 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 indicate 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 of magnesium concentration in amplification reactions.

Biological Source: The enzyme is derived from bacteria.

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. The reaction conditions are specified below under Standard DNA Polymerase Assay Conditions.

Quality Control Assays

Functional Assay: GoTaq® G2 Flexi DNA Polymerase is tested for performance in the polymerase chain reaction (PCR) 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.

Standard DNA Polymerase Assay Conditions (Not PCR Conditions): The polymerase activity is assayed in 50mM Tris-HCl (pH 9.0); 50mM NaCl; 5mM MgCl₂; 200μM each of dATP, dGTP, dCTP, dTTP (a mix of unlabeled and [³H]dTTP); 10μg activated calf thymus DNA; 0.1mg/ml BSA in a final volume of 50μl.

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

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, commercial or diagnostic applications without any license or royalty fees.

^(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:

R. Wheeler, Quality Assurance

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Part# 9PIM780

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1. Standard Application

Reagents to Be Supplied by the User

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

1. In a sterile, nuclease-free microcentrifuge tube, combine the following components on ice:

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® G2 Flexi DNA 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. Place reactions into a thermal cycler that has been heated to 94–95°C and begin PCR.

2. General Guidelines for Amplification by PCR

2.A. Denaturation

- Following an initial 2-minute 94–95°C denaturation, denaturation steps should be between 15 seconds and 1 minute per cycle.

2.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 15 seconds to 1 minute.

2.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.

2.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.

2.E. Cycle Number

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

3. General Considerations

3.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 A₂₆₀ 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 clean-up systems like the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281) or Wizard® SV 96 PCR Clean-Up System (Cat.# A9341).

3.B. Enzyme Concentration

Promega has found that 1.25 units of GoTaq® G2 Flexi 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 or less enzyme may be beneficial.

3.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/

3.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 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 also may help to overcome amplification failure.

3.E. More Information on Amplification

More information on amplification is available online at the Promega web site:

www.promega.com/products/pcr/