

Certificate of Analysis

GoTaq® Flexi DNA Polymerase:

Supplied With:

Cat.#	GoTaq® DNA Polymerase	5X Green GoTaq® Flexi Buffer	5X Colorless GoTaq® Flexi Buffer	Magnesium Chloride Solution, 25mM
M8291	100 units (M829A)	1ml (M891A)	1ml (M890A)	0.75ml (A351B)
M8295	500 units (M829B)	4 × 1ml (M891A)	4 × 1ml (M890A)	3 × 1.2ml (A351H)
M829B-C	500 units (M829B)			

Description: GoTaq® Flexi DNA Polymerase^(a,b) is supplied in a proprietary formulation containing 50% glycerol with buffers designed for enhanced amplification. Cat.# M8291 and M8295 are provided with 5X Green and 5X Colorless GoTaq® Flexi Buffers and Magnesium Chloride Solution. The 5X Green GoTaq® Flexi Buffer, contains two dyes (blue and yellow) 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.

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 recommendations. 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.

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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

Quality Control Assays

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.

Enzyme Physical Purity: GoTaq® DNA Polymerase is >90% pure as judged by SDS-polyacrylamide gel electrophoresis followed by Coomassie® Blue staining.

Functional Assay: GoTaq® DNA Polymerase is tested for performance in PCR using 1.25 units of enzyme to amplify a 360bp region of the α -1-antitrypsin gene from 100 molecules (0.35ng) of human genomic DNA. The resulting PCR product is visualized as a single band 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.



PCR Satisfaction Guarantee

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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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^(a)Use of this product in the US for basic PCR is outside of any valid US patents assigned to Hoffman La-Roche or Applera. This product can be used in the US 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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1. Standard Application

Reagents to Be Supplied by the User

- PCR Nucleotide Mix (Cat.# C1141, C1145)
- Nuclease-Free Water (Cat.# P1193)
- upstream primer
- downstream primer
- template DNA

1. In a sterile, nuclease-free microcentrifuge tube, combine the following 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® 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. Perform PCR using your standard parameters. An example profile is given in Table 1. For the cycling protocol, we recommend the following:
 - a. Reactions are placed in a thermal cycler that has been preheated to 95°C.
 - b. The thermal cycling protocol has an initial denaturation step where samples are heated at 95°C for 2 minutes to ensure that the target DNA is completely denatured. Initial denaturation of longer than 2 minutes at 95°C is unnecessary and may reduce yield.
 - c. The extension time should be at least 1min/kb target length.

Table 1. Recommended Thermal Cycling Conditions for GoTaq® DNA Polymerase-Mediated PCR Amplification. These guidelines are optimal for the Perkin Elmer thermal cycler model 480 or comparable thermal cyclers.

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	2 minutes	1 cycle
Denaturation	95°C	0.5–1 minute	
Annealing	42–65°C*	0.5–1 minute	25–35 cycles
Extension	72°C	1min/kb	
Final Extension	72°C	5 minutes	1 cycle
Soak	4°C	Indefinite	1 cycle

*Annealing temperature should be optimized for each primer set based on the primer T_m.

4. Separate the PCR products by agarose gel electrophoresis and visualize with ethidium bromide or any other means. For reactions containing the 5X Green GoTaq® Flexi Buffer, load the amplification reaction onto the gel directly after amplification. Reactions containing the 5X Colorless GoTaq® Flexi Buffer also can be loaded directly into the wells of an agarose gel, but a tracking dye will need to be added to monitor the progress of electrophoresis.

2. General Considerations

A. Enzyme Concentration

We have 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. Please be aware that excessive amounts of enzyme and excessively long extension times increase the likelihood of generating artifacts due to the intrinsic 5' → 3' exonuclease activity of Taq DNA polymerase.

B. 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, as 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 at 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 because 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 Wizard® SV 96 PCR Clean-Up System (Cat.# A9341).

Both reaction buffers are compatible with common PCR additives such as DMSO and betaine. These additives do not change the color of the Green GoTaq® Flexi Buffer or affect dye migration.

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 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, as 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 (e.g., mouse tail genotyping applications), 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 template DNA prior to adding to reaction. Diluting samples 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.