Certificate of Analysis

GoTaq® DNA Polymerase:

Supplied With:

Cat.#	Size	5X Green GoTaq® Buffer	5X Colorless GoTaq® Buffer
M3001	100 units	1ml (M791A)	1ml (M792A)
M3005	500 units	4 × 1ml (M791A)	4 × 1ml (M792A)
M3008	2,500 units	4 × 5ml (M7911)	4 × 5ml (M7921)
M300B–C	500 units		

Description: GoTaq® DNA Polymerase[a,b] is a *Taq* DNA polymerase supplied in a proprietary formulation containing 50% glycerol with buffers designed for enhanced amplification. Cat.# M3001, M3005 and M3008 are supplied with 5X Green and 5X Colorless GoTaq® Reaction Buffers. The 5X Green GoTaq® Reaction 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. Both buffers contain $MgCl_2$ at a concentration of 7.5mM for a final concentration of 1.5mM in the 1X reaction.

Biological Source: The enzyme is derived from bacteria.

Enzyme Concentration: 5u/µl.

5X Green GoTaq® Reaction Buffer (Part# M791A, M791B): Proprietary formulation supplied at pH 8.5 containing blue and yellow dyes. In a 1% agarose gel, the blue dye migrates at the same rate as 3–5kb DNA fragments, and the yellow dye migrates at a rate faster than primers (<50bp). Green GoTaq® Reaction 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 contains 7.5mM magnesium. Vortex thoroughly after thawing and prior to use.

5X Colorless GoTaq® Reaction Buffer (Part# M792A, M792B): Proprietary formulation supplied at pH 8.5. This buffer contains 7.5mM magnesium. Vortex thoroughly after thawing and prior to use.

Storage Conditions: See the Product Information Label for storage recommendations. 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

Activity Assays

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

Standard DNA Polymerase Assay Conditions (Not PCR Conditions): The polymerase activity is assayed in 50mM Tris-HCl (pH 9.0); 50mM NaCl; 5mM $MgCl_2$; 200µM each of dATP, dGTP, dCTP, dTTP (a mix of unlabeled and [^{3}H]dTTP); 10µg activated calf thymus DNA; 0.1mg/ml BSA in a final volume of 50µl.

Contaminant Assays

5X Green GoTaq® Reaction Buffer Migration Pattern: The 5X Green GoTaq® Reaction Buffer is tested to ensure that it does not interfere with DNA migration when it is used as a loading dye for agarose gel electrophoresis. 1kb DNA Ladder (Cat.# G5711) is mixed with either 1X Green GoTaq® Reaction Buffer or the Blue/Orange Loading Dye supplied with the markers and loaded on a 1% agarose gel. Following electrophoresis and ethidium bromide staining, the migration distance and pattern of the bands in both lanes are identical.

Enzyme Physical Purity: GoTaq® DNA Polymerase is determined to be >90% pure as judged by SDS-polyacrylamide gel electrophoresis with Coomassie® blue staining.

Nuclease Assays: No contaminating endonuclease or exonuclease activity detected.

PCR Satisfaction Guarantee

Promega 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 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 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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Usage Information

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® Reaction Buffer[1]	10µl	1X (1.5mM $MgCl_2$)[2]
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	

[1]Completely thaw and thoroughly vortex the buffer prior to use.
[2]More $MgCl_2$ can be added to the reaction using 25mM $MgCl_2$ Solution (Cat.# A3511).

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	25–35 cycles
Annealing	42–65°C*	0.5–1 minute	
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® Reaction Buffer, load the amplification reaction onto the gel directly after amplification. Reactions containing the 5X Colorless GoTaq® Reaction 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® Reaction Buffer in any amplification reaction that will be visualized by agarose gel electrophoresis followed by ethidium bromide staining. The 5X Green GoTaq® Reaction 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 A_{260} 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 grey dye front below the primers corresponding to the yellow dye front. The Green and Colorless GoTaq® Reaction 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® Reaction 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® reactions using standard PCR clean-up systems like the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281) or Wizard® PCR Preps DNA Purification System (Cat.# A2180).

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® Reaction 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 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, 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 at roughly 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.

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