Certificate of Analysis

GoTaq® G2 DNA Polymerase:

Supplied With:

Cat.#	GoTaq® G2 DNA Polymerase	5X Green GoTaq® Reaction Buffer	5X Colorless GoTaq® Reaction Buffer
M7841	100 units (M784A)	1ml (M791A)	1ml (M792A)
M7842	100 units (M784A)		
M7845	500 units (M784B)	4 × 1ml (M791A)	4 × 1ml (M792A)
M7848	2,500 units (M784B)	20 × 1ml (M791A)	20 × 1ml (M792A)

Description: GoTaq® G2 DNA Polymerase[a,b] contains GoTaq® G2 DNA Polymerase, 5X Green GoTaq® Reaction Buffer and 5X Colorless GoTaq® Reaction Buffer. 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® Reaction Buffer, contains two dyes (blue and yellow) 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. 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.

Part# 9PIM784

Revised 03/18

AF9PIM784 0318M784

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

Functional Assay: GoTaq® G2 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 $MgCl_2$; 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.

5X Green GoTaq® Reaction Buffer Migration Pattern: The 5X Green GoTaq® Reaction 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

PRODUCT USE LIMITATIONS, WARRANTY DISCLAIMER

Promega manufactures products for a number of intended uses. Please refer to the product label for the intended use statements for specific products. Promega products contain chemicals which may be harmful if misused. Due care should be exercised with all Promega products to prevent direct human contact.

Each Promega product is shipped with documentation stating specifications and other technical information. Promega products are warranted to meet or exceed the stated specifications. Promega's sole obligation and the customer's sole remedy is limited to replacement of products free of charge in the event products fail to perform as warranted. Promega makes no other warranty of any kind whatsoever, and SPECIFICALLY DISCLAIMS AND EXCLUDES ALL OTHER WARRANTIES OF ANY KIND OR NATURE WHATSOEVER, DIRECTLY OR INDIRECTLY, EXPRESS OR IMPLIED, INCLUDING, WITHOUT LIMITATION, AS TO THE SUITABILITY, PRODUCTIVITY, DURABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, MERCHANTABILITY, CONDITION, OR ANY OTHER MATTER WITH RESPECT TO PROMEGA PRODUCTS. In no event shall Promega be liable for claims for any other damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Promega products to perform in accordance with the stated specifications.

Part# 9PIM784

Printed in USA. Revised 03/18.

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 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® G2 DNA 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]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. 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® 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 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® Reaction Buffers give approximately equivalent amplification yields. To obtain equal amplification yields with the two buffers, PCR conditions will likely require optimization.

For reactions going directly from thermal cycler to an application using absorbance or fluorescence, we recommend the 5X Colorless GoTaq® Reaction 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® Reaction 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 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:
PCR Amplification: **www.promega.com/resources/product-guides-and-selectors/protocols-and-applications-paguide/**
PCR Core Systems Technical Bulletin: **www.promega.com/resources/protocols/**
DNA Analysis Notebook: **www.promega.com/resources/product-guides-and-selectors/dna-analysis-notebook/**

Part# 9PIM784
Printed in USA. Revised 03/18.