Certificate of Analysis

GoTaq® Green Master Mix

Cat.#	Size
M7121	10 reactions
M7122	100 reactions
M7123	1,000 reactions
M712B-C	1 × 1,250µl

Cat.# M7121, M7122 and M7123 include GoTaq® Green Master Mix, 2X, and Nuclease-Free Water.

Cat.# M712B-C does not include Nuclease-Free Water.

Description: GoTaq® Green Master Mix[a,b] is a premixed ready-to-use solution containing bacterially derived *Taq* DNA polymerase, dNTPs, $MgCl_2$ and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR. GoTaq® Green Master Mix contains two dyes (blue and yellow) that allow monitoring of progress during electrophoresis. Reactions assembled with GoTaq® Green Master Mix have sufficient density for direct loading onto agarose gels.

GoTaq® Green Master Mix is recommended for any amplification reaction that will be visualized by agarose gel electrophoresis and ethidium bromide staining. The master mix is not recommended if any downstream applications use absorbance or fluorescence excitation, as the yellow and blue dyes in the reaction buffer may interfere with these applications. The dyes absorb between 225–300nm, making standard A_{260} readings to determine DNA concentration unreliable. 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 at this wavelength. Gels scanned by this method will have a light grey dye front (corresponding to the yellow dye front) below the primers.

GoTaq® Green Master Mix, 2X: GoTaq® DNA Polymerase is supplied in 2X Green GoTaq® Reaction Buffer (pH 8.5), 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP and 3mM $MgCl_2$. Green GoTaq® Reaction Buffer is a proprietary buffer containing a compound that increases sample density, and yellow and blue dyes, which function as loading dyes when reaction products are analyzed by agarose gel electrophoresis. 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), in a 1% agarose gel.

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

Quality Control Assays

Functional Assay: GoTaq® Green Master Mix is tested for performance in the polymerase chain reaction (PCR). GoTaq® Green Master Mix, 1X, is used to amplify a 360bp region of the α-1-antitrypsin gene from 100 molecules of human genomic DNA. The resulting PCR product is visualized on an ethidium bromide-stained agarose gel.

Nuclease Assays: No contaminating endonuclease or exonuclease activity detected.



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 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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Promega

Usage Information

I. Standard Application

Reagents to be Supplied by the User

template DNA	downstream primer
upstream primer	mineral oil (optional)

1. Thaw the GoTaq® Green Master Mix at room temperature. Vortex the Master Mix, then spin it briefly in a microcentrifuge to collect the material at the bottom of the tube.
2. Prepare one of the following reaction mixes on ice:

For a 25µl reaction volume:

Component	Volume	Final Conc.
GoTaq® Green Master Mix, 2X	12.5µl	1X
upstream primer, 10µM	0.25–2.5µl	0.1–1.0µM
downstream primer, 10µM	0.25–2.5µl	0.1–1.0µM
DNA template	1–5µl	<250ng
Nuclease-Free Water to	25µl	N.A.

For a 50µl reaction volume:

Component	Volume	Final Conc.
GoTaq® Green Master Mix, 2X	25µl	1X
upstream primer, 10µM	0.5–5.0µl	0.1–1.0µM
downstream primer, 10µM	0.5–5.0µl	0.1–1.0µM
DNA template	1–5µl	<250ng
Nuclease-Free Water to	50µl	N.A.

For a 100µl reaction volume:

Component	Volume	Final Conc.
GoTaq® Green Master Mix, 2X	50µl	1X
upstream primer, 10µM	1.0–10.0µl	0.1–1.0µM
downstream primer, 10µM	1.0–10.0µl	0.1–1.0µM
DNA template	1–5µl	<250ng
Nuclease-Free Water to	100µl	N.A.

3. 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.
4. Place the reactions in a thermal cycler that has been preheated to 95°C. Perform PCR using your standard parameters.

II. General Guidelines for Amplification by PCR

A. Denaturation

- Generally, a 2-minute initial denaturation step at 95°C is sufficient.
- Subsequent denaturation steps will 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.

III. General Considerations

A. GoTaq® Green Master Mix Compatibility

GoTaq® Green Master Mix is compatible with common PCR additives such as DMSO and betaine. These additives neither change the color of GoTaq® Green Master Mix nor affect dye migration.

If both agarose gel analysis and further downstream applications involving absorbance or fluorescence will be used, the two dyes can be removed from reactions using standard PCR clean-up systems such as the Wizard® SV Gel and PCR Clean-Up System (Cat.# A9281).

B. 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, 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 is dependent upon 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/**

C. 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 the copurification of amplification inhibitors. Reduce the volume of template DNA in reaction or dilute template DNA prior to adding to reaction. Diluting samples even 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.

D. More Information on Amplification

More information on amplification is available online at the Promega web site: **www.promega.com/products/pcr/**

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