Certificate of Analysis

GoTaq® Colorless Master Mix

Part# 9PIM713

Revised 8/13

Cat.#	Size
M7131	10 reactions
M7132	100 reactions
M7133	1,000 reactions

Includes GoTaq® Colorless Master Mix, 2X, and Nuclease-Free Water.

Description: GoTaq® Colorless 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® Colorless Master Mix, 2X: GoTaq® DNA Polymerase is supplied in 2X Colorless GoTaq® Reaction Buffer (pH 8.5), 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP and 3mM $MgCl_2$.

Storage Conditions: See the Product Information Label for storage recommendations. Minimize the number of freeze-thaw cycles by storing in working aliquots. Product may be stored at 4°C for up to 6 weeks. Mix well prior to use.

Quality Control Assays

Functional Assay: GoTaq® Colorless Master Mix is tested for performance in the polymerase chain reaction (PCR). GoTaq® Colorless 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.

Signed by: J. Stevens

J. Stevens, Quality Assurance

[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 and European Pat. No. 1088060 and other patents pending.

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

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Usage Information

1. Standard Application

Reagents to be Supplied by the User

template DNA	downstream primer
upstream primer	mineral oil (optional)

1. Thaw the GoTaq® Colorless 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® Colorless 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® Colorless 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® Colorless 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.

2. 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.

3. General Considerations

A. GoTaq® Colorless Master Mix Compatibility

GoTaq® Colorless Master Mix is compatible with common PCR additives such as DMSO and betaine.

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:
PCR Applications: **www.promega.com/paguide/chap1.htm**
PCR Protocols and Reference: **www.promega.com/guides/pcr_guide/default.htm**

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