Certificate of Analysis

GoTaq® Hot Start Colorless Master Mix

Part# 9PIM513

Revised 4/18

Cat.#	Size
M5131	10 reactions
M5132	100 reactions
M5133	1,000 reactions
M513B-C	1 × 1,250µl

Cat.# M5131, M5132 and M5133 include GoTaq® Hot Start Colorless Master Mix, 2X, and Nuclease-Free Water.

Cat.# M513B-C does not include Nuclease-Free Water.

Description: GoTaq® Hot Start Colorless Master Mix[a,b] is a premixed ready-to-use solution containing GoTaq® Hot Start Polymerase, dNTPs, $MgCl_2$ and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR. GoTaq® Hot Start Polymerase contains the high-performance GoTaq® DNA Polymerase bound to a proprietary antibody that blocks polymerase activity. The polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 94–95°C for two minutes. This allows hot-start PCR in which polymerase activity is inhibited at temperatures below 70°C, allowing convenient room-temperature reaction setup. Hot-start PCR is advantageous for some amplification targets, because it may eliminate or minimize primer-dimer and secondary products. In some cases, hot-start PCR may improve yields. GoTaq® Hot Start Polymerase exhibits 5´→3´ exonuclease activity.

GoTaq® Hot Start Colorless Master Mix, 2X: GoTaq® Hot Start DNA Polymerase is supplied in 2X Colorless GoTaq® Reaction Buffer (pH 8.5), 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP and 4mM $MgCl_2$.

Biological Source for GoTaq® Hot Start Polymerase: The enzyme is derived from bacteria. The antibody is derived from murine cell culture.

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

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Promega

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Quality Control Assays

Functional Assay: GoTaq® Hot Start 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 and a 2.4kb region of the APC gene from 100 molecules of human genomic DNA. The resulting PCR products are visualized on an ethidium bromide-stained agarose gel.

Hot-Start Amplification Assay: GoTaq® Hot Start Colorless Master Mix is tested in PCR for its ability to amplify a hot-start model template to produce a single 1.5kb band, eliminating extraneous bands. In a nonhot-start PCR, this template produces an additional band at 410bp.

Nuclease Assays: No contaminating endonuclease or exonuclease activity detected.

SATISFACTION PCR GUARANTEE

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 without any license or royalty fees. This product is for research use only. [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

template DNA	downstream primer
upstream primer	mineral oil (optional)

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

For a 25µl reaction volume:

Component	Volume	Final Conc.
GoTaq® Hot Start 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® Hot Start 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® Hot Start 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.

Note: Although typically not necessary, magnesium optimization may be required to improve yield for some targets.

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 room-temperature thermal cycler. Perform PCR using your standard parameters. **A 2-minute initial denaturation step at 94–95°C is required to inactivate the antibody and initiate hot-start PCR.**

2. General Guidelines for Amplification by PCR

A. Denaturation

- All denaturation steps after the 2-minute initial denaturation step should 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. 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 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/**

B. 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 the copurification of amplification inhibitors. Reduce the volume of template DNA in reaction, or dilute template DNA prior to adding to reaction. Diluting samples up to 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.

C. 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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