Certificate of Analysis

GoTaq® MDx Hot Start Polymerase:

For Laboratory Use. *Supplied with:*

Cat.#	GoTaq® MDx Hot Start Polymerase	5X Green GoTaq® Flexi Buffer	5X Colorless GoTaq® Flexi Buffer	Magnesium Chloride Solution, 25mM
D6001	100 units (D600A)	1ml (M891A)	1ml (M890A)	0.75ml (A351B)
D6005	500 units (D600B)	4 × 1ml (M891A)	4 × 1ml (M890A)	3 × 0.75ml (A351B)
D6006	5 × 500 units (D600B)	20 × 1ml (M891A)	20 × 1ml (M890A)	15 × 0.75ml (A351B)
D6008	20 × 500 units (D600B)	80 × 1ml (M891A)	80 × 1ml (M890A)	60 × 0.75ml (A351B)

Description: GoTaq® MDx Hot Start Polymerase[a] contains GoTaq® MDx 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® MDx DNA polymerase is manufactured under cGMP.

Biological Source: The enzyme is derived from bacteria. The antibody is derived from murine cell culture.

Concentration: See product label for measured unit activity.

Storage Conditions: Store at –30°C to –10°C.

Expiration Date: See product label for expiration date.

5X Green GoTaq® Flexi Buffer (Part# M891A): Proprietary formulation supplied at pH 8.5 containing blue dye and yellow dye. The blue dye migrates at the same rate as a 3–5kb DNA fragment in a 1% agarose gel. The yellow dye migrates at a rate faster than primers (<50bp) in a 1% agarose gel. Green GoTaq® Flexi 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 does not contain magnesium.

5X Colorless GoTaq® Flexi Buffer (Part# M890A): Proprietary formulation supplied at pH 8.5. This buffer does not contain magnesium.

Magnesium Chloride Solution, 25mM (Part# A351B): Provided to allow you to optimize $MgCl_2$ concentration according to your individual requirements. Vortex the $MgCl_2$ thoroughly after thawing and prior to use.

Quality Control Assays

Test			Specification	Result
Concentration			6.5–9.5u/µl One unit is defined as the amount of enzyme required to catalyze the incorporation of 2 nanomoles of dNTPs in 30 minutes at 55°C in a fluorescent extension assay.	Pass See attached label.
Purity	DNA Contamination	Bacterial DNA	One unit of enzyme contains less than 10 copies of bacterial genomic DNA determined by quantitative amplification of a 16S rRNA gene.	Pass
		Fungal DNA	One unit of enzyme contains less than 1 genome equivalent of fungal genomic DNA by quantitative amplification of a 18S rRNA gene.	Pass
		Mammalian DNA	One unit of enzyme contains less than 1 genome equivalent of mammalian gDNA by quantitative amplification of mitochondrial genomic DNA.	Pass
	Nuclease Contamination	Endonuclease/ Nicking	No observable nicking of 0.5µg of supercoiled DNA after incubation for 8 hours at 22°C, followed by 8 hours at 45°C in the presence of 15 units of enzyme.	Pass
		Exonuclease	No observable degradation of 1.0µg of Lambda DNA/HindIII markers after incubation for 8 hours at 22°C, followed by 8 hours at 45°C in the presence of 15 units of enzyme.	Pass
		Ribonuclease	No observable degradation of RNA target after incubation for 1 hour at 37°C in the presence of 10 units of enzyme.	Pass
Function			1 unit of enzyme quantitatively amplifies mitochondrial genomic DNA.	Pass
			Amplify a hot-start model template to produce a single 1.5kb product, eliminating extraneous amplification products. In a non-hot start PCR, this template produces an additional 410bp amplification product.	Pass

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:

R. Wheeler, Quality Assurance

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[a]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.

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Promega

1. Standard Application

Reagents to Be Supplied by the User

- dNTP Mix (Cat.# U1511)
- 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 at room temperature:

Component	Final Volume	Final Conc.
5X GoTaq® Flexi Buffer[1] (Green or Colorless)	10µl	1X
$MgCl_2$ Solution, 25mM[1]	2–8µl	1.0–4.0mM
dNTP Mix, 10mM each	1µl	0.2mM each dNTP
upstream primer	Xµl	0.1–1.0µM
downstream primer	Yµl	0.1–1.0µM
GoTaq® MDx Hot Start Polymerase	Zµl	1.25u[2]
template DNA	Aµl	<0.5µg/50µl
Nuclease-Free Water to	50µl	

[1]Thaw completely and vortex thoroughly prior to use.
[2]Recommended optimization of enzyme quantity specific to assay.

2. If using a thermal cycler **without a heated lid**, overlay the reaction with 1–2 drops (approximately 50µl) of mineral oil to prevent evaporation during thermal cycling.
3. Place reactions in a room-temperature thermal cycler. **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

- Following the initial 2-minute 94–95°C denaturation step, denaturation steps 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.

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. Buffer Choice

We recommend using the 5X Green GoTaq® Flexi Buffer in any amplification reaction that will be visualized by agarose gel electrophoresis followed by ethidium-bromide staining. The 5X Green GoTaq® Flexi 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 between 225–300nm, making standard A_{260} readings to determine DNA concentration unreliable. Also, 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 this wavelength. Gels scanned by this method will have a light gray dye front below the primers corresponding to the yellow dye front. The Green and Colorless GoTaq® Flexi 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® Flexi Buffer is recommended.

B. Enzyme Concentration

We have found that 1.25 units of GoTaq® MDx Hot Start Polymerase per 50µl amplification reaction is adequate for most amplifications. However, optimization of enzyme concentration specific to the amplification reaction may be required to achieve optimal assay performance.

C. 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 because 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/**

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 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.

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