TECHNICAL MANUAL

GoTaq® qPCR Master Mix

Instructions for Use of Products
A6001 and A6002

Revised 6/14
TM318

GoTaq® qPCR Master Mix

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

GoTaq® qPCR Master Mix[a,b] is a reagent system for quantitative PCR (qPCR). The system contains a new fluorescent DNA-binding dye that often exhibits greater fluorescence enhancement upon binding to double-stranded DNA (dsDNA) than SYBR® Green I.

GoTaq® qPCR Master Mix is provided as a simple-to-use, stabilized 2X formulation that includes all components for qPCR except sample DNA, primers and water. This formulation, which includes a proprietary dsDNA-binding dye, a low level of carboxy-X-rhodamine (CXR) reference dye (identical to ROX™ dye), GoTaq® Hot Start Polymerase, $MgCl_2$, dNTPs and a proprietary reaction buffer, produces optimal results in qPCR experiments. A separate tube of CXR Reference Dye is included for use with instruments that require a higher level of reference dye than that in the GoTaq® qPCR Master Mix.

Advantages of the GoTaq® qPCR Master Mix

Dye: The proprietary dye provides brighter dsDNA-dependent fluorescence than SYBR® Green I, with less PCR inhibition than SYBR® Green. The dye enables efficient amplification, resulting in earlier quantification cycle (C_q) values and an expanded linear range using the same filters and settings as SYBR® Green I. The CXR reference dye can be detected using the same filters and settings as those used for ROX™ dye.

! Quantification cycle is formerly known as cycle threshold (C_t).

Polymerase/Buffer Formulation: GoTaq® Hot Start Polymerase contains full-length *Taq* DNA polymerase bound to a proprietary antibody that prevents polymerase activity at room temperature. Thermal activation is achieved by incubating the assembled reaction at 95°C for 2 minutes. The proprietary polymerase/buffer formulation accommodates extended cycle numbers (45–50 cycles) and is compatible with thermal cycling programs that require extended activation (95°C for 10 minutes).

Performance: You can expect reliable performance with minimal lot-to-lot variation: efficient, sensitive and linear qPCR amplification over a wide dynamic range.

2. Product Components and Storage Conditions

Product	Size	Cat.#
GoTaq® qPCR Master Mix	**200 reactions**	**A6001**

For Research Use Only. Not for use in diagnostic procedures. Includes:

- 5 × 1ml GoTaq® qPCR Master Mix, 2X
- 100µl CXR Reference Dye
- 2 × 13ml Nuclease-Free Water

Product	Size	Cat.#
GoTaq® qPCR Master Mix	**1,000 reactions**	**A6002**

For Research Use Only. Not for use in diagnostic procedures. Includes:

- 25 × 1ml GoTaq® qPCR Master Mix, 2X
- 5 ×100µl CXR Reference Dye
- 10 × 13ml Nuclease-Free Water

Storage Conditions: GoTaq® qPCR Master Mix is shipped at –20°C. Upon arrival, store all components at –20°C, protected from light. For immediate use, components may be stored at 2–8°C, protected from light, for up to 3 months.

3. General Considerations

3.A. Spectral Properties

The proprietary dye in the GoTaq® qPCR Master Mix has spectral properties similar to those of SYBR® Green I: Excitation at 493nm and emission at 530nm. Instrument optical settings established for SYBR® Green I assays should be used with GoTaq® qPCR Master Mix. The CXR reference dye has the same spectral properties as ROX™: Excitation at 580nm and emission at 602nm. Use the instrument settings for ROX™ dye for reactions containing GoTaq® qPCR Master Mix.

3.B. Magnesium Chloride Concentration

The $MgCl_2$ concentration of the GoTaq® q PCR Master Mix has been determined to be optimal for performance. If desired, the $MgCl_2$ concentration may be adjusted using a PCR-grade stock solution (not provided).

3.C. Instrument Compatibility

GoTaq® qPCR Master Mix can be used with any real-time instrument capable of detecting SYBR® Green I or FAM™ dye. GoTaq® qPCR Master Mix contains a low level of CXR reference dye.

If you are using any of the following instruments, supplement the GoTaq® qPCR reaction mix with 0.5µl of CXR Reference Dye per 50µl reaction.

Applied Biosystems 7000 Sequence Detection System

Applied Biosystems 7300 Real-Time PCR System

Applied Biosystems 7700 Sequence Detection System

Applied Biosystems 7900HT Real-Time PCR System

4. GoTaq® qPCR Master Mix Protocol

If you are currently performing dye-based qPCR, the GoTaq® qPCR Master Mix can simply be substituted for your current master mix. For consistency within an experimental set, prepare a sufficient volume of reaction mix without template DNA for the DNA standard reactions and experimental sample reactions.

The protocol for a 50µl reaction is outlined below. Component volumes may be scaled as appropriate. This protocol assumes that 20% of the reaction volume is DNA template (e.g., 10µl of DNA template added to 40µl of reaction mix). If the volume of DNA template is more or less than 10µl, adjust the volume of Nuclease-Free Water accordingly so that the final reaction volume is 50µl.

4. GoTaq® qPCR Master Mix Protocol (continued)

Materials to Be Supplied by the User

- qPCR primers
- DNA template, positive control template standards
- barrier pipette tips
- sterile, nuclease-free, DNA-free tubes for reaction mix setup
- optical multiwell reaction plates and adhesive film covers
- real-time thermal cycler
- optional: sterile $MgCl_2$ stock solution
- alternative normalization dye, if required (e.g., fluorescein for BioRad instruments)

1. Prepare the standard DNA dilution series and experimental samples in nuclease-free water. Store on ice until use. Carefully add 10µl of template (or water for no-template control reactions) to the appropriate wells of the reaction plate. Store plate at room temperature or on ice.
2. Thaw the GoTaq® qPCR Master Mix at room temperature. Gently vortex to ensure it is adequately mixed. Take care to avoid foaming or extended exposure to light. Store on ice until use.
3. Prepare the reaction mix, without template DNA, by combining the reagents in the order listed in Table 1. See Notes 1 and 2. Gently vortex to mix. Take care to avoid foaming.

Table 1. Preparation of the Reaction Mix.

Component	Volume per 50µl Reaction	Final Concentration
GoTaq® qPCR Master Mix, 2X	25µl	1X
Nuclease-Free Water	to a final volume of 40µl	
upstream and downstream PCR primers	_____µl	0.2µM or 0.05–0.9µM each

Notes:

a. See Section 3.C for a list of instruments that require addition of the CXR Reference Dye.

b. Some instruments such as the BioRad instruments require addition of a normalization dye (e.g., fluorescein).

4. Carefully add the appropriate volume of reaction mix prepared in Step 3 (e.g., 40µl of reaction mix for a 50µl reaction) to the appropriate wells of the reaction plate prepared in Step 1. Take care to avoid cross-contamination.
5. Seal the reaction plate, and centrifuge at low speed for 1 minute to bring all reaction components together and eliminate air bubbles.

6. Program the thermal cycler as per the manufacturer's instructions using the following guidelines:

   a. Select SYBR® or FAM™ as the detection dye for the entire plate.

   b. Select the ROX™ channel to detect CXR as the reference dye for the entire plate.

   c. Select a standard or fast, two-step, 40-cycle qPCR and dissociation program. Please note that the cycling parameters given below are offered as a guideline and may be modified as necessary.

	# Cycles	Standard Cycling Program	Fast Cycling Program
Hot-Start Activation	1	95°C for 2 minutes	95°C for 2 minutes
Denaturation	40	95°C for 15 seconds	95°C for 3 seconds
Annealing/Extension		60°C for 60 seconds	60°C for 30 seconds
Dissociation	1	60–95°C	60–95°C

   d. Designate that data will be collected during the annealing step of each cycle.

7. Place the plate into the instrument, and press "Start".

   When the run is complete, analyze the data using your usual procedures.

5. Related Products

DNA Purification

Product	Size	Cat.#
Wizard® Genomic DNA Purification Kit	100 isolations × 300µl	A1120
	500 isolations × 300µl	A1125
	100 isolations × 10ml	A1620
Wizard® SV Genomic DNA Purification System	50 preps	A2360
	250 preps	A2361
MagneSil® Blood Genomic, Max Yield System	1 × 96 preps	MD1360
MagneSil® ONE, Fixed Yield Blood Genomic System	1 × 96 preps	MD1370
MagneSil® Genomic, Fixed Tissue System	100 samples	MD1490
MagneSil® Genomic, Large Volume System	48 preps	A4082
Maxwell® 16 Blood DNA Purification Kit	48 preps	AS1010
Maxwell® 16 Cell DNA Purification Kit	48 preps	AS1020
Maxwell® 16 Tissue DNA Purification Kit	48 preps	AS1030
PureYield™ Plasmid Miniprep System	100 preps	A1223
	250 preps	A1222
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495
	300 preps	A2496
PureYield™ Plasmid Maxiprep System	10 preps	A2392
	25 preps	A2393

Accessory Products

Product	Size	Cat.#
Maxwell® 16 Instrument	1 each	AS2000
Maxwell® 16 SEV Hardware Kit	1 each	AS1200
Maxwell® 16 LEV Hardware Kit	1 each	AS1250

RNA Purification

Product	Size	Cat.#
PureYield™ RNA Midiprep System	10 preps	Z3740
	50 preps	Z3741
SV Total RNA Isolation System	10 preps	Z3101
	50 preps	Z3100
	250 preps	Z3105
Maxwell® 16 Total RNA Purification Kit	48 preps	AS1050
Maxwell® 16 Tissue LEV Total RNA Purification Kit	48 preps	AS1220
Maxwell® 16 Cell LEV Total RNA Purification Kit	48 preps	AS1225

For Laboratory Use.

Product	Size	Cat.#
Reverse Transcription System	100 reactions	A3500
AMV Reverse Transcriptase	300u	M5101
M-MLV Reverse Transcriptase	10,000u	M1701
	50,000u	M1705
M-MLV Reverse Transcriptase, RNase H Minus	10,000u	M5301
M-MLV Reverse Transcriptase, RNase H Minus, Point Mutation	2,500u	M3681
	10,000u	M3682
	50,000u	M3683
ImProm-II™ Reverse Transcription System	100 reactions	A3800
ImProm-II™ Reverse Transcriptase	10 reactions	A3801
	100 reactions	A3802
	500 reactions	A3803

6. Summary of Changes

The following changes were made to the 6/14 revision of this document:

1. Expired legal disclaimers were removed.
2. Related products section (Section 5) was updated.
3. Document design was updated.

[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.

[b] **NOTICE TO PURCHASER: LIMITED LICENSE**

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