TECHNICAL MANUAL

GoTaq® qPCR Master Mix

Instructions for Use of Products
A6001 and A6002

Revised 12/18
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 fluorescent DNA-binding dye, the BRYT Green® Dye, that exhibits greater fluorescence enhancement upon binding to double-stranded DNA (dsDNA) than SYBR® Green I.

GoTaq® qPCR Master Mix is 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.

An overview of the protocol is shown in Figure 1.

1. Description (continued)

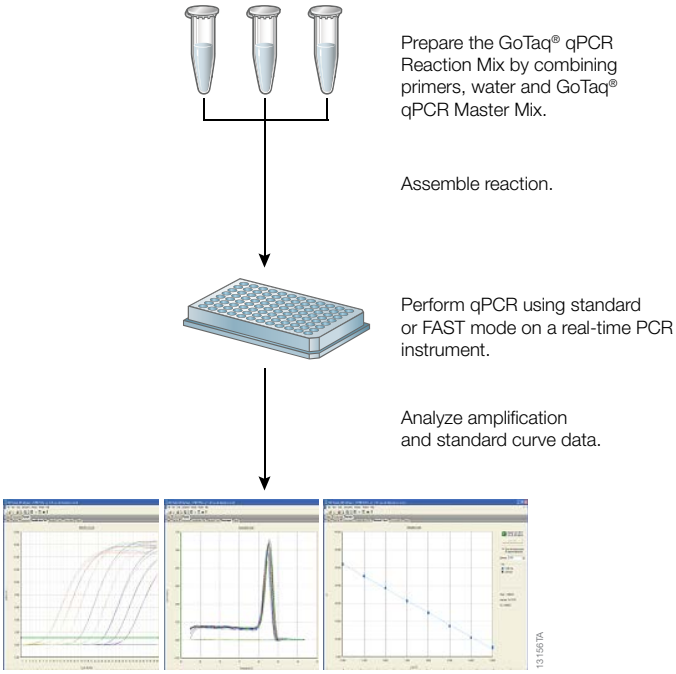

Figure 1. An overview of the GoTaq® qPCR Master Mix protocol.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
GoTaq® qPCR Master Mix	**5ml**	**A6001**

For Research Use Only. Not for use in diagnostic procedures. Each system contains sufficient reagents for 500 × 20µl reactions or 1,000 × 10µl reactions. Includes:

- 5 × 1ml GoTaq® qPCR Master Mix, 2X
- 100µl CXR Reference Dye, 30µM
- 2 × 13ml Nuclease-Free Water

PRODUCT	SIZE	CAT.#
GoTaq® qPCR Master Mix	**25ml**	**A6002**

For Research Use Only. Not for use in diagnostic procedures. Each system contains sufficient reagents for 2,500 × 20µl reactions or 5,000 × 10µl reactions. Includes:

- 25 × 1ml GoTaq® qPCR Master Mix, 2X
- 5 × 100µl CXR Reference Dye, 30µM
- 10 × 13ml Nuclease-Free Water

Storage Conditions: Store all components between –30°C and –10°C. Protect components from light at all times. For best results, mix thawed solutions gently to minimize aeration and foaming, and keep on ice during use. For short-term storage and frequent use, the GoTaq® qPCR Master Mix can be stored at 2–10°C for up to 3 months if protected from light.

Available Separately

Product	Size	Cat.#
GoTaq® 1-Step RT-qPCR System*	5ml	A6020
GoTaq® 2-Step RT-qPCR System*	5ml	A6010
Nuclease-Free Water	50ml	P1193

*For Research Use Only. Not for use in diagnostic procedures.

3. General Considerations

3.A. Preventing Contamination

We recommend the following precautions to prevent contamination:

- Use designated work areas and pipettes for pre- and post-amplification steps to minimize the potential for cross-contamination between samples and prevent carryover of nucleic acids from one experiment to the next.
- Wear gloves and change them often.
- Do not open the reaction plate or strip wells after amplification is complete. Opening the reaction plate or strip wells increases the risk of contaminating subsequent reactions with the amplified product.
- Use aerosol-resistant pipette tips.

3.B. qPCR Primers

Optimize the primer concentrations for each primer combination. Primer concentrations can range from 200nM to 1µM; perform titrations to ensure optimal results. As a general rule, a concentration of 0.2–0.9µM for each PCR primer is a recommended starting point.

We recommend preparing and storing PCR primers as 20X solutions.

3.C. BRYT Green® Dye

The BRYT Green® Dye in the GoTaq® qPCR Master Mix has spectral properties similar to those of SYBR® Green I: excitation at 493nm and emission at 530nm. Use the instrument optical settings established for SYBR® Green I assays with GoTaq® qPCR Master Mix.

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3.D. CXR Reference Dye and Instrument Considerations

The GoTaq® qPCR Master Mix contains a reference dye, carboxy-X-rhodamine (CXR), which is identical to ROX™ and allows GoTaq® qPCR Master Mix to be used directly on most instruments that perform passive reference normalization, e.g., from Applied Biosystems. A separate tube of CXR Reference Dye is included with the GoTaq® qPCR Master Mix for users of instruments requiring a high concentration of reference dye (e.g., ABI 7900). The supplemental CXR Reference Dye is is provided at a concentration of 30µM.

If you are unsure if your instrument was designed to use no, low or high amounts of ROX™ reference dye for normalization, contact your instrument vendor.

Recommendations for common instruments are listed below. Directions for setting up qPCRs with supplemental CXR Reference Dye are included in Section 4.

Instruments That Do Not Require Supplemental Reference Dye

- Applied Biosystems 7500 and 7500 FAST Real-Time PCR System
- Bio-Rad CFX96 Real-Time PCR Detection System
- Bio-Rad/MJ Research Chromo4™ Real-Time Detector
- Eppendorf Mastercycler® ep realplex Real-Time PCR System
- Roche LightCycler® 480 Real-Time PCR System
- Stratagene Mx3000P® and Mx3005P® Real-Time PCR Systems
- Stratagene Mx4000® Multiplex Quantitative PCR System
- Bio-Rad iCycler iQ® and iQ®5 Real-Time PCR Detection Systems
- ViiA® 7 Real-Time PCR System
- QuantStudio® Real Time PCR Systems

Instruments That Require High Levels (300nM) of Reference Dye

- Applied Biosystems 7300 and 7900HT Real-Time PCR System
- Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems

4. GoTaq® qPCR Master Mix Protocol

Materials to be Supplied by the User

- real-time PCR instrument and related equipment (i.e., optical-grade PCR plates and appropriate plate covers)
- sterile, aerosol-resistant pipette tips
- nuclease-free pipettors dedicated to pre-amplification work
- DNA template
- qPCR primers

4.A. Adding CXR Reference Dye to the GoTaq® qPCR Master Mix (Optional)

Some real-time PCR instruments require higher levels of CXR Reference Dye; see Section 3.D. For high reference dye instruments, add CXR Reference Dye to achieve a high dye concentration (300nM), as follows:

1. Thaw the GoTaq® qPCR Master Mix. Do not thaw the master mix at temperatures above room temperature.
2. Vortex the GoTaq® qPCR Master Mix for 3–5 seconds to mix.
3. When using an instrument designated as a high reference dye instrument, add 0.2µl per 20µl reaction for a final concentration of 300nM.
4. Vortex for 3–5 seconds to mix.

4.B. Assembling the Reaction Mix

The GoTaq® qPCR Master Mix uses a hot-start chemistry, allowing reaction setup to be performed at room temperature.

The final reaction volume in this protocol is 20µl. The volumes given here may be scaled for larger or smaller reaction volumes.

1. Thaw the GoTaq® Master Mix and Nuclease-Free Water. Do not thaw the GoTaq® Master Mix at temperatures above room temperature.
2. Vortex the GoTaq® Master Mix for 3–5 seconds to mix. Vortex at low speed to avoid aeration.
3. Determine the number of reactions to be set up, including negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does require using a small amount of extra reagent, it ensures that you will have enough reaction mix for all samples.

4.B. Assembling the Reaction Mix (continued)

4. Prepare the reaction mix (minus DNA template) by combining the GoTaq® qPCR Master Mix, PCR primers and Nuclease-Free Water as described below. The DNA template is added in Step 6. Vortex briefly to mix.

Component	Volume	Final Concentration
GoTaq® qPCR Master Mix (2X)	10µl	1X
Forward Primer (20X)	_____µl	200nM–1µM
Reverse Primer (20X)	_____µl	200nM–1µM
Supplemental CXR Reference Dye (if required)	0.2µl per reaction	300nM
Nuclease-Free Water	to a final volume of 20µl	

Note: The primer concentration should be optimized for each primer combination.

5. Add the appropriate volume of reaction mix to each PCR tube or well of an optical-grade PCR plate.
6. Add the DNA template (or water for the no-template control reactions) to the appropriate wells of the reaction plate.
7. Seal the tubes or optical plate, and centrifuge briefly to collect the contents of the wells at the bottom. The samples are ready for thermal cycling. Protect from extended light exposure or elevated temperatures.

5. Thermal Cycling

The cycling parameters below are offered as a guideline and may be modified as necessary for optimal results.

Standard Cycling Conditions

Step	Cycles	Temperature	Time
GoTaq® Hot Start Polymerase activation	1	95°C	2 minutes
Denaturation	40	95°C	15 seconds
Annealing and extension		60°C	1 minute

FAST Cycling Conditions

Step	Cycles	Temperature	Time
GoTaq® Hot Start Polymerase activation	1	95°C	2 minutes
Denaturation	40	95°C	3 seconds
Annealing and extension		60°C	30 seconds

Use the instrument optical settings established for SYBR® Green I assays with GoTaq® qPCR Master Mix.

6. General References for qPCR

1. Bustin, S.A. *et al*. (2009) The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem*. **55**, 611–22.
2. Dorak, M.T (2009) Glossary of real-time PCR terms. This can be viewed online at: **www.dorak.info/genetics/glosrt.html**
3. Fleige, S. and Pfaffl, M.W. (2006) RNA integrity and the effect on the real-time qRT-PCR performance. *Mol. Aspects Med*. **27**, 126–39.
4. Lefever, S. *et al*. (2009) RDML: Structured language and reporting guidelines for real-time quantitative PCR data. *Nucleic Acids Res*. **37**, 2065–9.
5. Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C_T}$ Method. *Methods* **25**, 402–8.

7. Related Products

Product	Size	Cat.#
GoTaq® Probe qPCR Master Mix	2ml	A6101
	10ml	A6102
GoTaq® Probe 1-Step RT-qPCR System	2ml	A6120
GoTaq® Probe 2-Step RT-qPCR System	2ml	A6110
Nuclease-Free Water	50ml	P1193

DNA Purification

Product	Size	Cat.#
ReliaPrep™ gDNA Tissue Miniprep System*	100 preps	A2051
ReliaPrep™ 96 gDNA Miniprep HT System*	1 × 96 preps	A2670
ReliaPrep™ Large Volume HT gDNA Isolation System	96 × 10ml preps	A2751
ReliaPrep™ Blood gDNA Miniprep System*	100 preps	A5081
ReliaPrep™ FFPE gDNA Miniprep System*	10 reactions	A2351
Wizard® Genomic DNA Purification Kit*	100 isolations × 300µl	A1120
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
PureYield™ Plasmid Midiprep System*	25 preps	A2492
PureYield™ Plasmid Maxiprep System*	10 preps	A2392

*Additional sizes are available.

7. Related Products (continued)

RNA Purification, Manual Systems

Product	Size	Cat.#
ReliaPrep™ RNA Cell Miniprep System	10 preps	Z6010
ReliaPrep™ RNA Tissue Miniprep System	10 preps	Z6110
ReliaPrep™ FFPE Total RNA Miniprep System	10 reactions	Z1001
SV Total RNA Isolation System	10 preps	Z3101
PureYield™ RNA Midiprep System	10 preps	Z3740

Additional sizes are available.

Manual or Automated RNA Purification

Product	Size	Cat.#
SV 96 Total RNA Isolation System	1 × 96 each	Z3500
	5 × 96 each	Z3505
Vac-Man® 96 Vacuum Manifold	1 each	A2291

Automated RNA Purification

Product	Size	Cat.#
Maxwell® 16 LEV simplyRNA Cells Kit	48 preps	AS1270
Maxwell® 16 LEV simplyRNA Tissue Kit	48 preps	AS1280
Maxwell® 16 Total RNA Purification Kit	48 preps	AS1050
MagneSil® Total RNA mini-Isolation System	4 plate	Z3351

Reverse Transcription Enzymes and Systems

Product	Size	Cat.#
GoScript™ Reverse Transcription System	50 reactions	A5000
	100 reactions	A5001
GoScript™ Reverse Transcriptase	100 reactions	A5003
	500 reactions	A5004
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 Mutant	2,500u	M3681
	10,000u	M3682
	50,000u	M3683

8. Summary of Changes

The following change was made to the 12/18 revision of this document:

1. The product size was updated to reflect volume provided.
2. Incorporated other general updates.