TECHNICAL MANUAL

GoTaq® Probe qPCR Master Mix

Instructions for Use of Products
A6101 and A6102

Revised 8/18
TM378

GoTaq® Probe 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

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1. Description

The GoTaq® Probe qPCR Master Mix[a] is optimized for quantitative PCR assays in the hydrolysis probe detection format. The master mix is provided as a ready-to-use, stabilized 2X formulation that includes all components for qPCR, including GoTaq® Hot Start Polymerase, $MgCl_2$, dNTPs and a proprietary reaction buffer, but not template, primers and probe. This master mix does not contain a reference dye; a separate tube of carboxy-X-rhodamine (CXR) reference dye is included with this system, allowing you to add reference dye to amplification reactions if desired.

The GoTaq® Probe qPCR Master Mix provides resistance to a wide range of PCR inhibitors. This formulation uses antibody-mediated hot-start chemistry, allowing reaction setup to be performed at room temperature. The master mix also employs rapid hot-start activation and processive enzymes, making it compatible with both standard and fast instrument cycling programs.

An overview of the protocol is shown in Figure 1.

1. Description (continued)

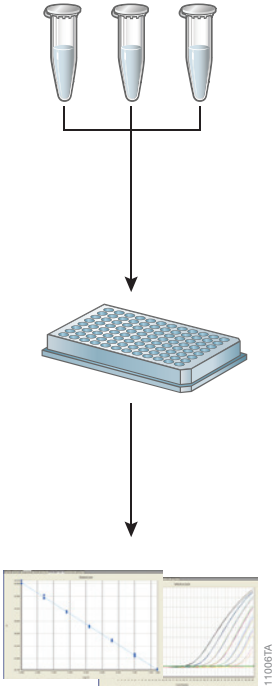

Figure 1. An overview of the GoTaq® Probe qPCR Master Mix protocol.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
GoTaq® Probe qPCR Master Mix	**2ml**	**A6101**

For Research Use Only. Not for use in diagnostic procedures. Each system contains sufficient reagents for 200 × 20µl reactions or 400 × 10µl reactions. Includes:

- 2 × 1ml GoTaq® Probe qPCR Master Mix, 2X
- 100µl CXR Reference Dye, 30µM
- 2 × 1.25ml Nuclease-Free Water

PRODUCT	SIZE	CAT.#
GoTaq® Probe qPCR Master Mix	**10ml**	**A6102**

For Research Use Only. Not for use in diagnostic procedures. Each system contains sufficient reagents for 1,000 × 20µl reactions or 2,000 × 10µl reactions. Includes:

- 10 × 1ml GoTaq® Probe qPCR Master Mix, 2X
- 2 × 200µl CXR Reference Dye, 30µM
- 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. For short-term storage and frequent use, the GoTaq® Probe qPCR Master Mix may be stored at 2–10°C for up to 3 months if protected from light.

Available Separately

PRODUCT	SIZE	CAT.#
GoTaq Probe 1-Step RT-qPCR System*	**2ml**	**A6120**
	12.5ml	**A6121**
GoTaq Probe 2-Step RT-qPCR System*	**2ml**	**A6110**
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 plates or strip wells after amplification is complete. Opening the reaction plates or strip wells increases the risk of contaminating subsequent reactions with the amplified product.
- Use aerosol-resistant pipette tips.

3.B. qPCR Primers and Probes

The concentrations of primers and probes should be optimized for each primer/probe combination. For gene expression assays, primer and probe concentrations may need to be adjusted based on target abundance. As a general rule, a concentration of 900nM for PCR primers and 250nM for the hydrolysis probe is a recommended starting point. Concentrations of PCR primers can range from 200nM to 1µM, while probe concentration can range from 100nM to 300nM; titrations should be performed to ensure optimal results. We recommend preparing and storing the PCR primers and hydrolysis probes as 20X solutions.

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3.C. CXR Reference Dye

The GoTaq® Probe qPCR Master Mix does not contain a reference dye; a separate tube of carboxy-X-rhodamine (CXR) reference dye is included with this system, allowing you to add reference dye if desired. Adding the reference dye will help maximize effectiveness of the GoTaq® Probe qPCR Master Mix when used with real-time PCR instruments that allow normalization. The CXR reference dye has the same spectral properties as ROX™ dye. The dye is provided at a concentration of 30µM.

Some instrumentation is designed to normalize with a low concentration of ROX™ reference dye. We recommend that the CXR reference dye be added to a final concentration of 30nM for instruments that recommend a low level of ROX™ dye. Other instruments require ROX™ at a high concentration for normalization. We recommend that the CXR Reference Dye be added to a final concentration of 500nM for instruments that recommend a high level of ROX™ dye.

The recommended dye levels for various instruments are listed below. Directions for supplementing the GoTaq® Probe qPCR Master Mix with CXR Reference Dye are included in Section 4.A.

Instruments That Do Not Require Supplemental Reference Dye

- Bio-Rad CFX96 Real-Time PCR Detection System
- Bio-Rad DNA Engine Opticon® and Opticon® 2 Real-Time PCR Detection Systems
- Bio-Rad/MJ Research Chromo4™ Real-Time Detector
- Bio-Rad iCycler iQ® and iQ®5 Real-Time PCR Detection Systems
- Bio-Rad MyiQ™ Real-Time PCR Detection System
- Roche LightCycler® 480 Real-Time PCR System
- Eppendorf Mastercycler® ep realplex Real-Time PCR System

Instruments That Require Low Levels (30nM) of Reference Dye

- Applied Biosystems 7500 and 7500 FAST Real-Time PCR System
- Applied Biosystems QuantStudio® Real Time PCR Systems
- Applied Biosystems ViiA® 7 Real-Time PCR System
- Stratagene/Agilent Mx3000P® and Mx3005P® Real-Time PCR Systems
- Stratagene/Agilent Mx4000® Multiplex Quantitative PCR System

Instruments That Require High Levels (500nM) of Reference Dye

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

4. GoTaq® Probe 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 and probe

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

Some real-time PCR instruments require addition of the CXR Reference Dye; see Section 3.C. If you wish to add CXR Reference Dye to your amplification reactions, we recommend adding an aliquot of concentrated CXR Reference Dye to the 1ml tube of the GoTaq® Probe qPCR Master Mix. Depending on your instrument, the CXR Reference Dye can be added to either the low dye (30nM) concentration or high dye (500nM) concentration (see Section 3.C).

1. Thaw the GoTaq® Probe qPCR Master Mix. Do not thaw the GoTaq® Probe qPCR Master Mix at elevated temperatures (i.e., above room temperature).
2. Vortex the GoTaq® Probe qPCR Master Mix for 3–5 seconds to mix.
3. When using an instrument designated as a high-dye instrument, add 33.4µl of CXR Reference Dye, 30µM, to the 1ml tube of GoTaq® Probe qPCR Master Mix.

   When using an instrument designated as a low-dye instrument, add 2µl of CXR Reference Dye, 30µM, to the 1ml tube of GoTaq® Probe qPCR Master Mix.
4. Vortex for 3–5 seconds to mix.
5. Mark the tube to indicate that you have performed this step. Store the GoTaq® Probe qPCR Master Mix with CXR at –20°C.

4.B. Assembling the GoTaq® Probe qPCR Reaction Mix

The GoTaq® Probe 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® Probe qPCR Master Mix and Nuclease-Free Water. Do not thaw the GoTaq® Probe qPCR Master Mix at elevated temperatures (i.e., above room temperature).
2. Vortex the GoTaq® Probe Master Mix for 3–5 seconds to mix.
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 have enough reaction mix for all samples.

4.B. Assembling the GoTaq® Probe qPCR Reaction Mix (continued)

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

Component	Volume	Final Concentration
GoTaq® Probe qPCR Master Mix, 2X	10µl	1X
Forward Primer (20X)	1µl	200nM–1µM
Reverse Primer (20X)	1µl	200nM–1µM
Hydrolysis Probe (20X)	1µl	100–300nM
Template DNA	2–5µl	≤250ng
Nuclease-Free Water	to a final volume of 20µl	

Note: The concentrations of primers and hydrolysis probe 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; centrifuge briefly to collect the contents of the wells at the bottom. Protect from extended light exposure or elevated temperatures. The samples are ready for thermal cycling.

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® DNA 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® DNA Polymerase activation	1	95°C	2 minutes
Denaturation	40	95°C	3 seconds
Annealing and extension		60°C	30 seconds

6. General qPCR References

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® qPCR Master Mix	5ml	A6001
GoTaq® 1-Step RT-qPCR System	5ml	A6020
GoTaq® 2-Step RT-qPCR System	5ml	A6010
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
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 8/18 revision of this document:

1. Cat.# A6121 was added to the list of Products Available Separately in Section 2.