TECHNICAL MANUAL

GoTaq® Probe qPCR Master Mix

Instructions for Use of Products
A6101 and A6102

Revised 6/14
TM378

GoTaq® Probe qPCR Master Mix

All technical literature is available at: www.promega.com/protocols/
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E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

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

The GoTaq® Probe qPCR Master Mix is designed to provide 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.

1. Description (continued)

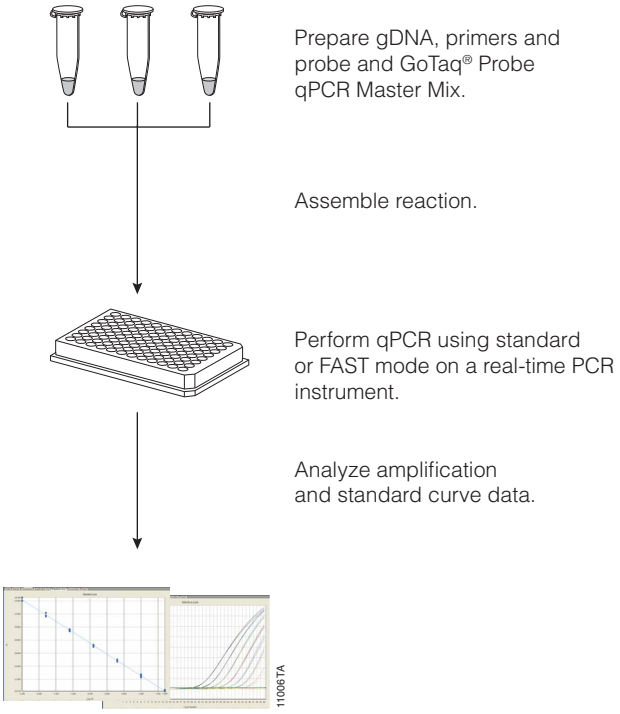

Figure 1. Flow diagram of the GoTaq® Probe qPCR Master Mix protocol.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
GoTaq® Probe qPCR Master Mix	**200 reactions**	**A6101**

For Research Use Only. Not for use in diagnostic procedures. Contains sufficient reagents for 200 × 20µl assays. Includes:

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

PRODUCT	SIZE	CAT.#
GoTaq® Probe qPCR Master Mix	**1,000 reactions**	**A6102**

For Research Use Only. Not for use in diagnostic procedures. Contains sufficient reagents for 1,000 × 20µl assays. Includes:

- 10 × 1ml GoTaq® Probe qPCR Master Mix, dTTP (2X)
- 2 × 200µl CXR Reference Dye, 30µM
- 13ml Nuclease-Free Water

Available Separately

PRODUCT	SIZE	CAT.#
GoTaq 1-Step RT-qPCR System*	**200 reactions**	**A6120**
GoTaq 2-Step RT-qPCR System*	**200 reactions**	**A6110**
Nuclease-Free Water**	**50ml**	**P1193**

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

3. General Considerations

3.A. Prevention of Contamination

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

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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 concentration 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 may range from 200nM to 1µM, while probe concentration may range from 100nM to 300nM; titrations should be performed to ensure optimal results.

We recommend preparing and storing 20X solutions of the PCR primers and hydrolysis probe.

3.C. Genomic DNA Template Quantity

Use ≤250ng of genomic DNA.

3.D. CXR Reference Dye

The GoTaq® Probe qPCR Master Mix formulation does not contain a reference dye; however, a separate tube of carboxy-X-rhodamine (CXR) reference dye is included with this system, allowing users to add reference dye if desired. Addition of the reference dye will help maximize effectiveness of the GoTaq® Probe qPCR Master Mix when used on 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 reaction 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 reaction concentration of 500nM for instruments that recommend a "high" level of ROX™ dye.

Examples of instrument recommendations are listed below. Directions for setting up qPCR amplification reactions for both "low dye" and "high dye" instruments are included in Section 4.

3.E. Instruments for Low-Level (30nM) Reference Dye

- Applied Biosystems 7500 and 7500 FAST Real-Time PCR System
- 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
- Cepheid SmartCycler® system
- Corbett Rotor-Gene™ 3000 and 6000 Real-Time Rotary Analyzer
- 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

3.F. Instruments for High-Level (500nM) Reference Dye

- Applied Biosystems ABI PRISM® 7000 and 7700 Sequence Detection System
- Applied Biosystems 7300 and 7900HT Real-Time PCR System
- Applied Biosystems GeneAmp® 5700 Thermal Cycler
- Applied Biosystems StepOne™ and StepOnePlus™ Real-Time PCR Systems

4. GoTaq® Probe qPCR Protocol

Materials to Be Supplied by the User

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

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

For users who wish to include CXR Reference Dye in their amplification reactions, we recommend adding an aliquot of concentrated CXR Reference Dye to the 1ml tube of GoTaq® Probe qPCR Master Mix. Depending on your instrumentation, the CXR Reference Dye should be added at either "low dye" concentration or "high dye" concentration, as follows (refer to the list in Section 3 to determine if your instrument requires low CXR or high CXR):

1. Thaw the GoTaq® Probe qPCR Master Mix and the Nuclease-Free Water. Do not thaw the Master Mix at elevated temperatures (i.e., above room temperature).
2. Briefly vortex the GoTaq® Probe qPCR Master Mix for 3–5 seconds to mix.
3. Add CXR Reference Dye to the 1ml tube of GoTaq® Probe qPCR Master Mix:

   For "Low Dye" Instruments: Add 2µl of CXR Reference Dye (at 30µM) to the 1ml tube of GoTaq® Probe qPCR Master Mix.

   For "High Dye" Instruments: Add 17µl of CXR Reference Dye (at 30µM) to the 1ml tube of GoTaq® Probe qPCR Master Mix.
4. Briefly vortex the GoTaq® Probe qPCR Master Mix with CXR added for 3–5 seconds to mix.
5. After adding the CXR to the GoTaq® Probe qPCR Master Mix, mark the tube to indicate that you have performed this step. The GoTaq® Probe qPCR Master Mix with CXR added should be stored at –20°C.

4.B. Preparation of GoTaq® Probe qPCR Amplifications

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

1. Thaw the GoTaq® Probe qPCR Master Mix and the Nuclease-Free Water. Do not thaw the Master Mix at elevated temperatures (i.e., above room temperature).
2. Briefly vortex the GoTaq® Probe qPCR Master Mix for 3–5 seconds to mix.
3. Determine the number of reactions to be set up. This should include 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 PCR master mix for all samples.

Notes:

- The reagent composition for a 20µl reaction volume is shown. Component volumes may be scaled for larger or smaller reaction volumes.
- The reaction concentrations of primers and hydrolysis probe should be optimized for each primer/probe combination.

Component	Volume	Final Reaction Concentration
GoTaq® Probe qPCR Master Mix (2X)	10µl	1X
Forward primer (20X)	1µl	900nM
Reverse primer (20X)	1µl	900nM
Hydrolysis probe (20X)	1µl	250nM
Template DNA	2–5µl	≤250ng
Nuclease-Free Water	To a 20µl total reaction volume	

4. Prepare the reaction mix (without the template DNA) by combining the GoTaq® Probe qPCR Master Mix, the PCR primers, hydrolysis probe, and Nuclease-Free Water. Vortex briefly to mix.
5. Add the appropriate volume of reaction mix (without the template DNA) to each PCR tube or to each well of an optical grade PCR plate.
6. Add DNA template to the sample reactions.
7. Seal the tubes or optical plates; 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 before 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® activation	1	95°C	2 minutes
Denaturation	40	95°C	15 seconds
Annealing/Extension		60°C	1 minute

FAST Cycling Conditions

Step	Cycles	Temperature	Time
GoTaq® activation	1	95°C	2 minutes
Denaturation	40	95°C	3 seconds
Annealing/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. Summary of Changes

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

1. Expired patent and license statements were removed.
2. 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: DISCLAIMER OF LICENSE**

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