TECHNICAL MANUAL

GoTaq® 2-Step RT-qPCR System

Instructions for Use of Product
A6010

Revised 12/18
TM337

GoTaq® 2-Step RT-qPCR System

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® 2-Step RT-qPCR System[a,b] combines the components of GoScript™ Reverse Transcriptase and GoTaq® qPCR Master Mix into a sensitive, robust kit for detection of RNA expression. The system, which is optimized for RT-qPCR, contains a proprietary fluorescent DNA-binding dye, BRYT Green® Dye. The system protocol facilitates detection and quantification of RNA expression levels via a two-step RT-qPCR method using GoScript™ Reverse Transcriptase and GoTaq® qPCR Master Mix.

GoScript™ Reverse Transcriptase includes an optimized reaction buffer and reverse transcriptase that enable efficient synthesis of first-strand cDNA in preparation for qPCR amplification. The cDNA product can be added directly to downstream qPCR amplification reactions.

GoTaq® qPCR Master Mix is provided as a simple-to-use, stabilized 2X formulation that includes all components for qPCR except DNA template, 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.

1. Description (continued)

An overview of the protocol is shown in Figure 1.

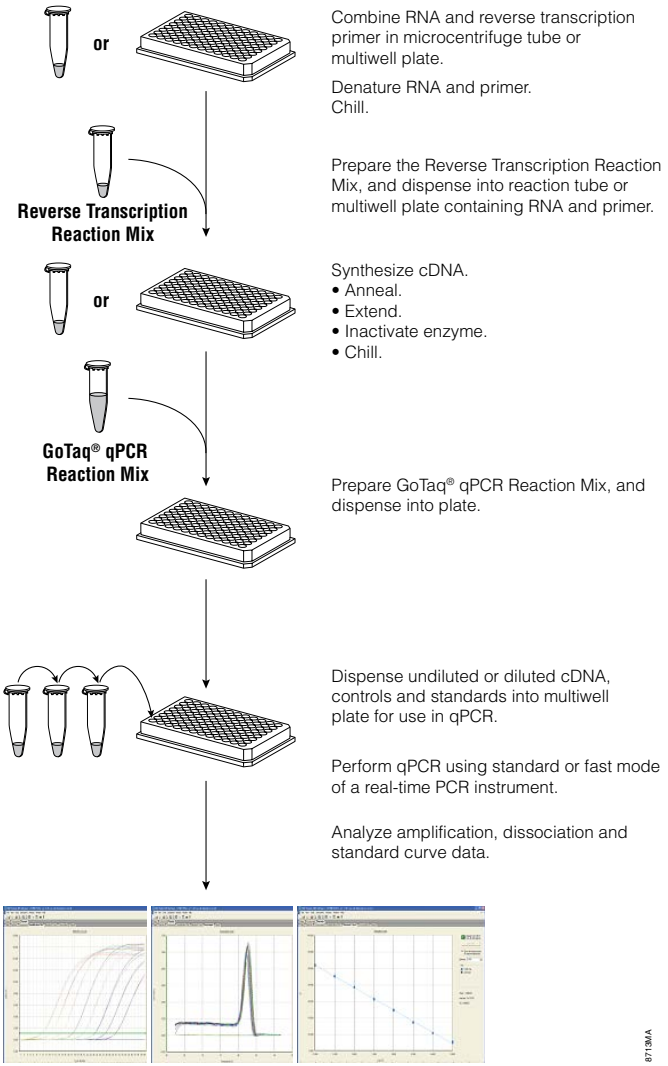

Figure 1. An overview of the GoTaq® 2-Step RT-qPCR Master Mix protocol.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
GoTaq® 2-Step RT-qPCR System	**5ml**	**A6010**

For Research Use Only. Not for use in diagnostic procedures. Each system contains sufficient reagents for 500 × 20µl qPCR assays and 50 × 20µl RT assays. Includes:

- 5 × 1ml GoTaq® qPCR Master Mix, 2X
- 750µl $MgCl_2$, 25mM
- 100µl CXR Reference Dye, 30µM
- 300µl GoScript™ 5X Reaction Buffer
- 50µl GoScript™ Reverse Transcriptase
- 50µg Oligo$(dT)_{15}$ Primer
- 200µl PCR Nucleotide Mix, 10mM
- 50µg Random Primers
- 2,500u Recombinant RNasin® Ribonuclease Inhibitor
- 2 × 13ml Nuclease-Free Water

Storage Conditions: Store all components between –30°C and –10°C. Protect components from light at all times. Thaw the GoScript™ 5X Reaction Buffer on ice and mix until no visible precipiate is present. Store the buffer on ice after thawing. For best results, mix thawed solution 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® qPCR Master Mix*	**5ml**	**A6001**
	25ml	**A6002**
GoTaq® 1-Step RT-qPCR System*	**5ml**	**A6020**
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.

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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.

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 5.

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
- Applied Biosystems ViiA® 7 Real-Time PCR System
- Applied Biosystems 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. Preparing cDNA using the GoScript™ Reverse Transcriptase

Materials to be Supplied by the User

- nuclease-free reaction tubes
- sterile, aerosol-resistant tips and pipettors
- RNA template
- 25°C, 45°C and 70°C controlled-temperature heat blocks
- ice-water bath

GoScript™ Reverse Transcriptase includes an optimized reaction buffer and reverse transcriptase that enable efficient synthesis of first-strand cDNA in preparation for qPCR amplification. The cDNA product can be added directly to downstream qPCR amplification reactions.

The final reaction volume in this protocol is 20µl. The volumes given here may be scaled for larger or smaller reaction volumes. Reverse transcription reactions can be performed in tubes or wells of a multiwell plate.

RNA Template

The amount of RNA required to detect the target of interest depends on several factors, primarily the abundance of that RNA target in each sample. As a starting point to detect RNA at unknown expression levels, we recommend using 100ng of total RNA template per reaction. A high-copy-number RNA transcript can be detected in as little as 10pg, while a low-copy-number RNA transcript may require more than 100ng. Up to 5µg of RNA can be used in each reaction.

For optimal results, the RNA template should be purified to remove genomic DNA contamination. This is of particular importance when amplifying a target within a single exon to avoid amplifying any contaminating genomic DNA.

cDNA Synthesis Negative Control

To test for the presence of contaminating genomic DNA in the RNA template, we recommend performing a no-reverse transcriptase control reaction.

Note: RQ1 RNase-Free DNase (Cat.# M6101) can be used to remove DNA from RNA samples prior to RT-qPCR.

Protocol

1. Denature RNA and primers.
   a. On ice, combine the RNA template and primers as described below.

Component	Volume
RNA template (up to 5µg per reaction)	1–5µl
Oligo(dT)$_{15}$ Primer (0.5µg/µl)[1]	1µl
Random Primers (0.5µg/µl) [1]	1µl
Nuclease-Free Water	to a final volume of 10µl

[1]Alternatively, you can use a gene-specific reverse transcription primer.

   b. Close each tube tightly. Place tubes in a 70°C heat block for 5 minutes. Immediately chill in an ice-water bath for at least 5 minutes. Centrifuge each tube for 10 seconds in a microcentrifuge to bring contents to the bottom and maintain the original volume. Keep the tubes closed and on ice until the reverse transcription reaction mix is added in Step 4.

4. Preparing cDNA using the GoScript™ Reverse Transcriptase (continued)

2. 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.

3. Prepare the Reverse Transcription Reaction Mix by combining the following components in the order listed below in a sterile 1.5ml microcentrifuge tube on ice. Vortex gently to mix, and store on ice.

Component	Reverse Transcription Reaction Mix (Volume per Reaction)	Minus-Reverse Transcriptase Reaction Mix (Volume per Reaction)
Nuclease-Free Water	1.5µl	2.5µl
GoScript™ 5X Reaction Buffer	4µl	4µl
$MgCl_2$	2µl	2µl
PCR Nucleotide Mix	1µl	1µl
Recombinant RNasin® Ribonuclease Inhibitor	0.5µl	0.5µl
GoScript™ Reverse Transcriptase	1µl	0µl
Final volume	10µl	10µl

4. Add 10µl of Reverse Transcription Reaction Mix to each RNA/primer tube for a final reaction volume of 20µl. The volume of RNA + primers from Step 1 should not exceed 50% (v/v) of the final reaction volume.

5. Place tubes in a 25°C heat block, and incubate for 5 minutes to allow the primers and RNA to anneal.

6. Incubate tubes in a 42°C heat block for 1 hour.

7. Inactivate the reverse transcriptase by incubating tubes in a 70°C heat block for 15 minutes.

   Note: The reverse transcriptase must be thermally inactivated prior to amplification.

8. Store cDNA at 4°C or on ice for immediate analysis. Alternatively, store the cDNA at –20°C until ready to use.

5. qPCR 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
- cDNA template
- qPCR primers

5.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.

5.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. cDNA template added should not exceed 20% (v/v) of the qPCR reaction volume, unless diluted.

1. Thaw the GoTaq® qPCR Master Mix and Nuclease-Free Water. Do not thaw the GoTaq® qPCR Master Mix at temperatures above room temperature.
2. Vortex the GoTaq® qPCR 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.

5.B. Assembling the Reaction Mix (continued)

4. Prepare the reaction mix (minus the cDNA template) by combining the GoTaq® qPCR Master Mix, PCR primers and Nuclease-Free Water as described below. The cDNA 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
cDNA template	2–4µl	
Supplemental CXR Reference Dye (if required)	0.2µl per reaction	300nM
Nuclease-Free Water	to a final volume of 20µl	

Notes: The primer concentrations should be optimized for each primer combination. The cDNA volume should not exceed 20% of the qPCR volume unless the cDNA is diluted prior to qPCR.

5. Add the appropriate volume of reaction mix to each PCR tube or well of an optical-grade PCR plate.
6. Add the cDNA 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. The samples are ready for thermal cycling. Protect from extended light exposure or elevated temperatures.

6. 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

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

7. 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.

8. Related Products

Real-Time PCR

Product	Size	Cat.#
GoTaq® Probe qPCR Master Mix	2ml	A6101
	10ml	A6102
GoTaq® Probe 1-Step RT-qPCR System	2ml	A6120
	12.5ml	A6121
GoTaq® Probe 2-Step RT-qPCR System	2ml	A6110

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.

8. Related Products (continued)

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

Accessories

Product	Size	Cat.#
GoScript™ Reverse Transcription System	50 reactions	A5000
	100 reactions	A5001
GoScript™ Reverse Transcriptase	100 reactions	A5003
	500 reactions	A5004
RNasin® Plus RNase Inhibitor	2,500u	N2611
	10,000u	N2615
Recombinant RNasin® Ribonuclease Inhibitor	2,500u	N2511
Nuclease-Free Water	50ml	P1193

9. 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.

[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]U.S. Pat. Nos. 8,598,198 and 9,206,474 and other patents and patents pending.

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