TECHNICAL MANUAL

GoTaq® 1-Step RT-qPCR System

Instructions for Use of Product
A6020

Revised 8/23
TM355

GoTaq® 1-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® 1-Step RT-qPCR System[a] combines GoScript™ Reverse Transcriptase and GoTaq® qPCR Master Mix in a single-step real-time amplification reaction. The system, which is optimized for RT-qPCR, contains a proprietary fluorescent DNA-binding dye, BRYT Green® Dye. The system enables detection of RNA expression levels using a one-step RT-qPCR method, combining GoScript™ Reverse Transcriptase and GoTaq® qPCR Master Mix in a single-step real-time amplification reaction. An overview of the protocol is shown in Figure 1.

The GoScript™ RT Mix for 1-Step RT-qPCR (50X) includes optimized amounts of GoScript™ Reverse Transcriptase, RNasin® Plus RNase Inhibitor and additives to enhance single-step reactions.

The GoTaq® qPCR Master Mix is a simple-to-use, stabilized 2X formulation that includes all components for qPCR except 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)

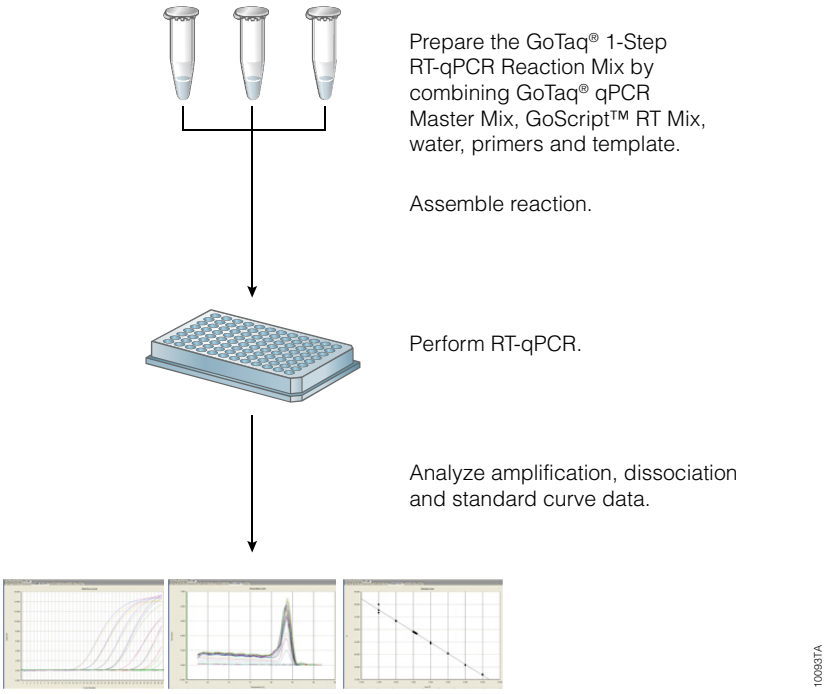

Figure 1. Overview of the GoTaq® 1-Step RT-qPCR protocol.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
GoTaq® 1-Step RT-qPCR Master Mix	**5ml**	**A6020**

For research use only. Not for use in diagnostic procedures. Each system contains sufficient reagents for 500 × 20µl reactions. Includes:

- 5 × 1ml GoTaq® qPCR Master Mix, 2X
- 225µl GoScript™ RT Mix for 1-Step RT-qPCR
- 200µl CXR Reference Dye, 30µM
- 750µl $MgCl_2$, 25mM
- 2 × 13ml Nuclease-Free Water

Storage Conditions: Store all components between –30°C to –10°C. Protect components from light at all times. Thaw the GoScript™ RT Mix for 1-Step RT-qPCR on ice and mix until no visible precipitate 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°C to +10°C for up to 3 months if protected from light.

Available Separately

PRODUCT	SIZE	CAT.#
CXR Reference Dye, 30µM*	**100µl**	**C5411**
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 50nM to 300nM; perform titrations to ensure optimal results. As a general rule, a concentration of 200nM for each PCR primer is a recommended starting point.

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

3.C. 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 may be detected in as little as 500fg, while a low-copy-number RNA transcript may require more than 100ng. Up to 100ng of RNA can be used in each reaction.

For optimal results, the RNA template should be purified to remove genomic DNA contamination. This is particularly important when using amplification targets within a single exon to avoid amplifying any contaminating genomic DNA.

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3.D. 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.E. 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
- 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. GoTaq® 1-Step RT-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
- RNA template
- qPCR primers

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

Some real-time PCR instruments require higher levels of CXR Reference Dye; see Section 3.E. For high reference dye instruments, add CXR Reference Dye to achieve a high dye concentration (500nM), 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 0.33µl per 20µl reaction for a final concentration of 500nM.
4. Vortex for 3–5 seconds to mix.

4.B. Assembling the GoTaq® 1-Step RT-qPCR Reaction Mix

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® qPCR Master Mix and Nuclease-Free Water. Do not thaw the GoTaq® qPCR Master Mix at elevated temperatures (i.e., 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.

4.B. Assembling the GoTaq® 1-Step RT-qPCR Reaction Mix (continued)

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

Component	Volume	Final Concentration
GoTaq® qPCR Master Mix, 2X	10µl	1X
GoScript™ RT Mix for 1-Step RT-qPCR (50X)	0.4µl	1X
Forward Primer (20X)	____µl	50–300nM
Reverse Primer (20X)	____µl	50–300nM
CXR Reference Dye (optional)	0.33µl/20µl reaction	500nM
Nuclease-Free Water	to a final volume of 20µl	

Note: The primer concentrations 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 RNA 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. 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
Reverse transcription	1	≥37°C	15 minutes
Reverse transcriptase inactivation and GoTaq® DNA Polymerase activation	1	95°C	10 minutes
Denaturation		95°C	10 seconds
Annealing and data collection	40	60°C	30 seconds
Extension		72°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

Real-Time PCR

Product	Size	Cat.#
GoTaq® qPCR Master Mix*	5ml	A6001
	25ml	A6002
GoTaq® 2-Step RT-qPCR System*	5ml	A6010
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.

7. 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

8. Summary of Changes

The following changes were made to the 8/23 revision of this document:

1. Updated Sections 2 and 7.
2. Replaced cover image and document font.
3. Updated patent statements.
4. Made minor text edits.

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