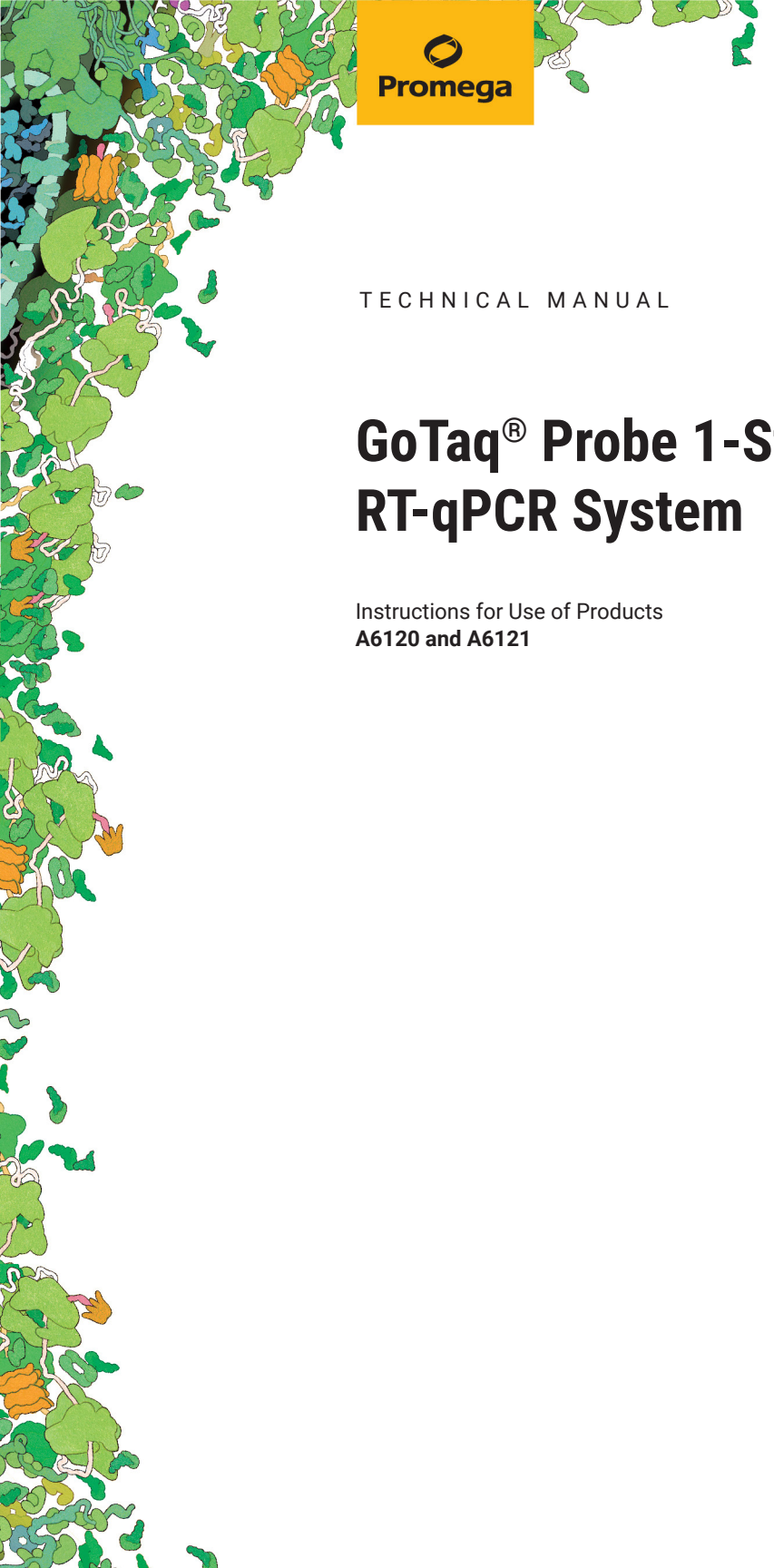

TECHNICAL MANUAL

# GoTaq® Probe 1-Step RT-qPCR System

Instructions for Use of Products
**A6120 and A6121**

Revised 1/24
TM379

Promega

# GoTaq® Probe 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

The GoTaq® Probe 1-Step RT-qPCR System is optimized for quantitative PCR assays in the hydrolysis probe detection format. The system enables detection and relative quantification of RNA expression levels using a one-step RT-qPCR method, combining GoScript™ Reverse Transcriptase and GoTaq® Probe qPCR Master Mix in single-step real-time amplification reactions. An overview of the protocol is shown in Figure 1.

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

The GoTaq® Probe qPCR Master Mix with dUTP 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.

## 1. Description (continued)

The GoTaq® Probe qPCR Master Mix with dUTP 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.

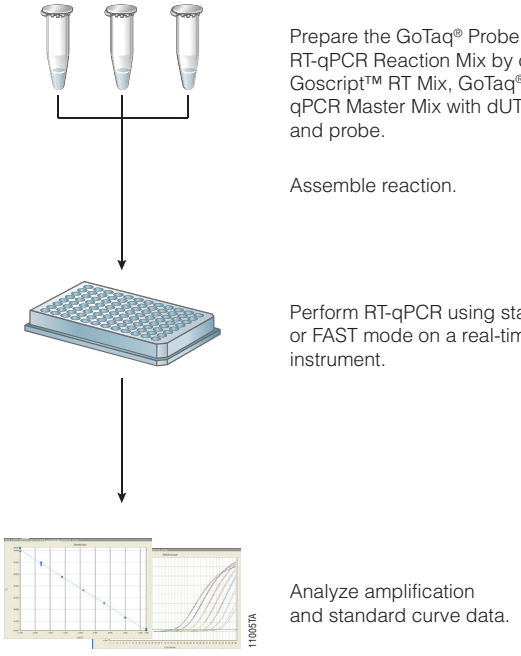


**Figure 1. An overview of the GoTaq® Probe 1-Step RT-qPCR protocol.**

## 2. Product Components and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| **GoTaq® Probe 1-Step RT-qPCR System** | **2ml** | **A6120** |

For Laboratory Use. Each system contains sufficient reagents for 200 × 20µl GoTaq® Probe 1-Step RT-qPCR assays. Includes:

- 225µl GoScript™ RT Mix for 1-Step RT-qPCR
- 2 × 1ml GoTaq® Probe qPCR Master Mix with dUTP (2X)
- 200µl CXR Reference Dye, 30µM
- 2 × 1.25ml Nuclease-Free Water

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| **GoTaq® Probe 1-Step RT-qPCR System** | **12.5ml** | **A6121** |

For Laboratory Use. Each system contains sufficient reagents for 1,250 × 20µl GoTaq® Probe 1-Step RT-qPCR assays. Includes:

- 500µl GoScript™ RT Mix for 1-Step RT-qPCR
- 12.5ml GoTaq® Probe qPCR Master Mix with dUTP (2X)
- 500µl CXR Reference Dye, 30µM
- 13ml Nuclease-Free Water

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

## 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 reactions plate 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–1µM, while probe concentration can range from 100–300nM; titrations should be performed to ensure optimal results.

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

### 3.C. RNA Template

The amount of RNA required to detect the target of interest depends on 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 10pg, while a low-copy-number RNA transcript may require more than 100ng. Up to 1µg of RNA may be used in each reaction.

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

### 3.D. dUTP Formulation

The GoTaq® Probe qPCR Master Mix included in the GoTaq® Probe 1-Step RT-qPCR System is formulated with dUTP. When dUTP is incorporated into the amplification products, the amplicons are susceptible to degradation by uracil-DNA glycosylase (UNG); this allows you to incorporate UNG into subsequent reactions to control possible carryover contamination.

### 3.E. CXR Reference Dye

The GoTaq® Probe qPCR Master Mix with dUTP does not contain a reference dye; however, 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 with dUTP 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 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
- pipettors dedicated to pre-amplification work
- RNA template
- qPCR primers and probe

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

Some real-time PCR instruments require addition of the CXR Reference Dye; see Section 3.E. 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 (Cat.# A6120) or the 12.5ml bottle (Cat.# A6121) of the GoTaq® Probe qPCR Master Mix with dUTP. Depending on your instrument, the CXR Reference Dye should be added to either the low dye (30nM) concentration or high dye (500nM) concentration (see Section 3.E).

1. Thaw the GoTaq® Probe qPCR Master Mix with dUTP. Do not thaw the master mix at elevated temperatures (i.e., above room temperature).
2. Vortex the GoTaq® Probe qPCR Master Mix with dUTP for 3–5 seconds to mix.
3. Add CXR Reference Dye (supplied at a concentration of 30µM) to the 1ml tube (Cat.# A6120) or the 12.5ml bottle (Cat.# A6121) of GoTaq® Probe qPCR Master Mix with dUTP as follows:

| Instrument Designation | CXR Volume for 1ml tube (Cat.# A6120) | CXR Volume for 12.5ml bottle (Cat.# A6121) |
|---|---|---|
| Low-dye instrument | 2µl | 25µl |
| High-dye instrument | 33.4µl | 420µl |

4. Vortex for 3–5 seconds to mix.
5. Mark the tube or bottle to indicate that you have performed this step. Store the GoTaq® Probe qPCR Master Mix with dUTP and CXR at –20°C and protect from light at all times.

   Note: Aliquot the GoTaq® Probe qPCR Master Mix with dUTP and CXR if more than 10 freeze/thaw cycles will occur before used completely.

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

The GoTaq® Probe qPCR Master Mix with dUTP 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 with dUTP and Nuclease-Free Water. Do not thaw the master mix at elevated temperatures (i.e., above room temperature).
2. Vortex the GoTaq® Probe qPCR Master Mix with dUTP 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 requires using a small amount of extra reagent, it ensures that you have enough reaction mix for all samples.
4. Prepare the reaction mix (minus the RNA template) by combining the GoTaq® Probe qPCR Master Mix with dUTP, GoScript™ RT Mix for 1-Step RT-qPCR, primers, hydrolysis probe and Nuclease-Free Water as described below. The RNA template is added in Step 6. Vortex briefly to mix.

| Component | Volume | Final Concentration |
|---|---|---|
| GoTaq® Probe qPCR Master Mix with dUTP | 10µl | 1X |
| GoScript™ RT Mix for 1-Step RT-qPCR | 0.4µl | 1X |
| Forward primer (20X) | 1µl | 200nM–1µM |
| Reverse primer (20X) | 1µl | 200nM–1µM |
| Hydrolysis probe (20X) | 1µl | 100–300nM |
| RNA Template | 2–5µl | 10pg–1µg |
| 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 (without the RNA template) 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; centrifuge briefly to collect the contents of the wells at the bottom. Protect from extended light exposure or elevated temperatures before cycling. 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 | 45°C | 15 minutes |
| Reverse transcriptase inactivation and GoTaq® DNA Polymerase activation | 1 | 95°C | 2 minutes |
| Denaturation | 40 | 95°C | 15 seconds |
| Annealing and extension | | 60°C | 1 minute |

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## 5. Thermal Cycling (continued)

### FAST Cycling Conditions

| Step | Cycles | Temperature | Time |
|---|---|---|---|
| Reverse transcription | 1 | 45°C | 5 minutes |
| Reverse transcriptase inactivation and 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

### Real-Time PCR

| 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 |
| GoTaq Probe 2-Step RT-qPCR System | 2ml | A6110 |
| Nuclease-Free Water | 50ml | P11930 |

### 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® RSC simplyRNA Blood Kit | 48 preps | AS1380 |
| | 144 preps | ASB1380 |
| Maxwell® RSC simplyRNA Cells Kit | 48 preps | AS1390 |
| 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 |

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## 8. Summary of Changes

The following change was made to the 1/24 revision of this document:

1. Updated patent statements.
2. Changed font and cover image.
3. Made minor text edits.