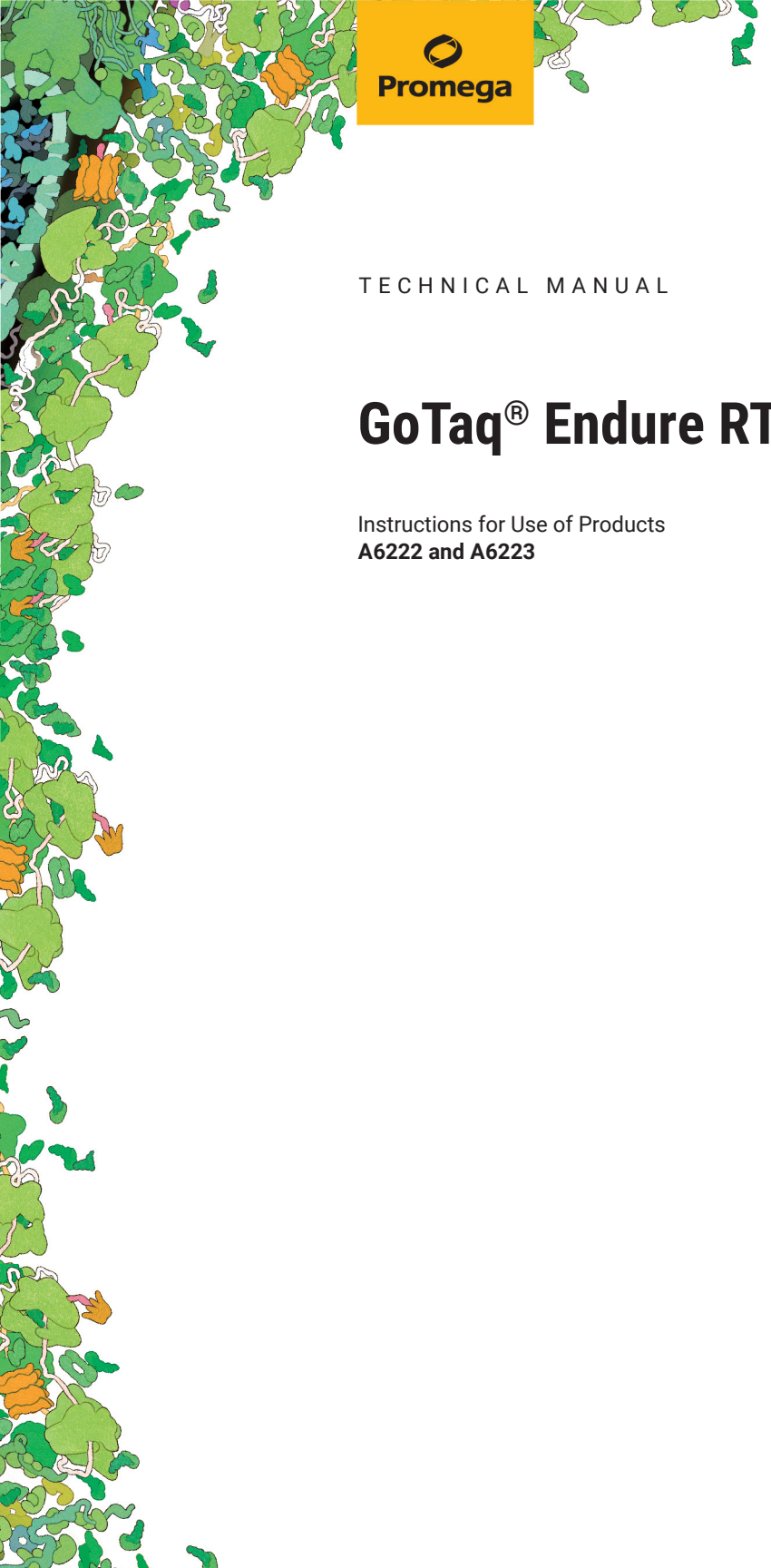

TECHNICAL MANUAL

# GoTaq® Endure RT-qPCR System

Instructions for Use of Products
**A6222 and A6223**

10/24
TM751

# GoTaq® Endure RT-qPCR System

**All technical literature is available at: www.promega.com/protocols/**
**Visit the website to verify that you are using the most current version of this Technical Manual.**
**Email Promega Technical Services if you have questions on use of this system: techserv@promega.com**

Promega

## 1. Description

The GoTaq® Endure RT-qPCR System was developed for performing quantitative PCR assays using hydrolysis probes for real-time amplicon detection. The RT-qPCR System is optimized for amplifying RNA targets from challenging sample types that may contain PCR and RT-qPCR inhibitors, such as humic acid, heparin, hematin and other compounds found in clinical, surveillance and agricultural research samples. The GoTaq® Endure RT-qPCR System detects and quantifies relative RNA levels using a one-step RT-qPCR method, combining GoScript™ Reverse Transcriptase and GoTaq® Endure Master Mix in a real-time amplification reaction.

The GoScript™ Enzyme Mix for 1-Step RT-qPCR (50X) combines optimized amounts of GoScript™ Reverse Transcriptase, RNasin® Plus RNase Inhibitor and additives to enhance reverse transcription combined with qPCR.

The GoTaq® Endure 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. The master mix does not contain a reference dye. Carboxy-X-rhodamine (CXR) reference dye is included with this system for adding reference dye to amplification reactions if desired. Section 3.D includes details on instruments and requirements for adding CXR reference dye.

The GoTaq® Endure RT-qPCR System provides resistance to a wide range of PCR inhibitors and is optimized for multiplexing, allowing detection of at least four targets in the same reaction. This formulation uses antibody-mediated hot-start chemistry for reaction setup 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.

## 2. Product Components and Storage Conditions

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| **GoTaq® Endure RT-qPCR System** | **200 reactions** | **A6222** |

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

- 2 × 1ml GoTaq® Endure Master Mix, 2X
- 1 × 100µl GoScript™ Enzyme Mix, 50X
- 1 × 100µl CXR Reference Dye, 30µM
- 2 × 1.25ml Nuclease-Free Water

| PRODUCT | SIZE | CAT.# |
|---|---|---|
| **GoTaq® Endure RT-qPCR System** | **1,000 reactions** | **A6223** |

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

- 10 × 1ml GoTaq® Endure Master Mix, 2X
- 5 × 100µl GoScript™ Enzyme Mix, 50X
- 2 × 200µl CXR Reference Dye, 30µM
- 1 × 13ml Nuclease-Free Water

**Storage Conditions:** Store all components at –30°C to –10°C. Protect CXR Reference Dye, 30µM, from light at all times. For best results, mix thawed solutions gently to minimize aeration and foaming. For short-term storage and frequent use, store GoTaq® Endure Master Mix, 2X, at +2°C to +10°C for up to 3 months, protected from light. GoTaq® Endure Master Mix can also be stored for up to 4 hours at room temperature (+15°C to +30°C). Do not freeze-thaw the GoTaq® Endure Master Mix, 2X, more than five times.

## 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 barrier 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 and/or primer specificity. We recommend a concentration of 900nM for PCR primers and 250nM for the hydrolysis probe as a starting point.

Concentrations of PCR primers can range from 200nM–1µM, while probe concentration can range from 100nM–300nM; titrations should be performed to ensure optimal results.

We recommend preparing and storing the PCR primers and hydrolysis probes 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. CXR Reference Dye

The GoTaq® Endure Master Mix, 2X, does not contain a reference dye; however, a separate tube of carboxy-X-rhodamine (CXR) Reference Dye is included with this system, allowing the addition of reference dye if desired. Adding the reference dye will help maximize effectiveness of the GoTaq® Endure Master Mix, 2X, when used with real-time PCR instruments that allow normalization. The CXR Reference Dye, 30µM, has the same spectral properties as ROX™ dye.

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

Recommended CXR Reference Dye, 30µM, levels for various qPCR instruments are listed below. Directions for supplementing the GoTaq® Endure Master Mix, 2X, with CXR Reference Dye, 30µM, 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® Endure RT-qPCR System Protocol

**Materials to Be Supplied by the User**

- real-time PCR instrument and related consumables (e.g., optical-grade PCR plates and appropriate well caps or sealing film)
- sterile, aerosol-resistant barrier pipette tips
- nuclease-free pipettors
- RNA
- primers and probe

### 4.A. Optional: Adding CXR Reference Dye, 30µM

Some real-time PCR instruments require addition of 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, 30µM, to the 1ml tube of the GoTaq® Endure Master Mix, 2X. Depending on your instrument, the CXR Reference Dye, 30µM, can be added to either the low dye (30nM) or high dye (500nM) concentration (Section 3.D).

1. Thaw the GoTaq® Endure Master Mix, 2X and CXR Reference Dye, 30µM, at ambient temperature or on ice.
2. Vigorously vortex the GoTaq® Endure Master Mix, 2X, for 30–60 seconds to ensure homogeneity before use. Briefly centrifuge to collect contents at the bottom of the tube.
3. Add CXR Reference Dye, 30µM, to the GoTaq® Endure Master Mix, 2X:
    a. **For high-dye instruments:** Add 33.4µl of CXR Reference Dye, 30µM, to the 1ml tube of GoTaq® Endure Master Mix, 2X.
    b. **For low-dye instruments:** Add 2µl of CXR Reference Dye, 30µM, to the 1ml tube of GoTaq® Endure Master Mix, 2X.
4. Vortex for 3–5 seconds to mix.
5. Mark the tube to indicate that you have performed this step. Store the GoTaq® Endure Master Mix, 2X, with CXR Reference Dye, at –30°C to –10°C, protected from light.

    **Note:** Aliquot the GoTaq® Endure Master Mix, 2X, combined with CXR Reference Dye if more than five freeze-thaw cycles will occur before used completely.

Promega

### 4.B. Assembling the GoTaq® Endure RT-qPCR Amplification Mix

GoTaq® Endure Master Mix, 2X, uses a hot-start chemistry for performing reaction setup at room temperature. The final reaction volume in this protocol is 20µl.

1. Thaw GoTaq® Endure Master Mix, 2X, and Nuclease-Free Water and vigorously vortex the master mix for 30–60 seconds to ensure homogeneity before use. Briefly centrifuge to collect contents at the bottom of the tube.
2. Determine the number of reactions to be set up, including negative control reactions. Add one or two reactions to this number to compensate for pipetting error. While this approach uses a small additional amount of reagent, it ensures enough reaction mix for all samples.
3. Prepare the amplification mix (minus the RNA template) by combining the components as shown in the table below. The RNA template is added in Step 5. Vortex briefly to mix.

| Component | Volume | Final Concentration |
|---|---|---|
| GoTaq® Endure Master Mix, 2X | 10µl | 1X |
| GoScript™ Enzyme Mix, 50X | 0.4µl | 1X |
| forward primer (20X) | 1µl | 200nM–1µM |
| reverse primer (20X) | 1µl | 200nM–1µM |
| hydrolysis probe (20X) | 1µl | 100–300nM |
| template RNA | 2–5µl | 1pg–1µg |
| **Nuclease-Free Water to a final volume of** | **20µl** | — |

**Note:** Optimize the concentrations of primers and hydrolysis probe for each primer combination.

4. Add the appropriate volume of amplification mix (without the template) to each PCR tube or well of an optical-grade PCR plate.
5. Add the RNA template (or water for the no-template control [NTC] reactions) to the appropriate wells of the reaction plate.
6. 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 now ready for thermal cycling.

   **Note:** Assembled reaction plates can be stored protected from light at ambient temperatures for up to 4 hours.

## 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 | Temperature | Time | Number of Cycles |
|---|---|---|---|
| Reverse transcription | 45°C | 15 minutes | 1 |
| Reverse transcriptase inactivation/ GoTaq® DNA Polymerase activation | 95°C | 2 minutes | 1 |
| Denaturation | 95°C | 15 seconds | 40 |
| Annealing and extension | 60°C | 1 minute | |

### FAST Cycling Conditions

| Step | Temperature | Time | Number of Cycles |
|---|---|---|---|
| Reverse transcription | 45°C | 15 minutes | 1 |
| Reverse transcriptase inactivation/ GoTaq® DNA Polymerase activation | 95°C | 2 minutes | 1 |
| Denaturation | 95°C | 3 seconds | 40 |
| Annealing and extension | 60°C | 30 seconds | |

Promega

## 6. Appendix

### 6.A. Inhibitor Tolerance Data with GoTaq® Endure Master Mix, 2X

The GoTaq® Endure Master Mix, 2X, is designed to tolerate PCR inhibitors that may be present in challenging sample types. A series of titrations was performed to determine the maximum concentration of common inhibitors that could be present without significant amplification inhibition. The table below shows the highest concentration of inhibitors tolerated.

**Table 1. GoTaq® Endure Master Mix Tolerates qPCR Inhibitors.** Inhibitors were individually added to reactions containing control template at increasing concentrations to determine their effect on $C_q$ values. The maximum inhibitor amount that produced a less than 2 $C_q$ shift is shown.

| Inhibitor | Amount Tolerated with Minimal Inhibition |
|---|---|
| Hematin | 50µM |
| Ethanol | 9% |
| Sodium citrate | >14mM* |
| Heparin | 0.01U/µl (0.2U/reaction) |
| EDTA | 4.5mM |
| Humic acid | 20ng/reaction |

*Maximal amount tested, no inhibition observed.

### 6.B. Using the GoTaq® Endure Master Mix with an RT-qPCR Exogenous Internal Amplification Control (IAC) Assay

The GoTaq® Endure RT-qPCR System is designed to tolerate PCR inhibitors that may be present in challenging sample types. Including an exogenous amplification positive control provides additional confidence in qPCR results and data interpretation. The IAC RT-qPCR Inhibition Control Assay, CAL Fluor® 560 (Cat.# AM2040) contains primers, probe and an exogenous template for a complete amplification control in one step. CAL Fluor® 560 is compatible with HEX™/JOE/VIC® dye channels. For more information, see the *IAC RT-qPCR Inhibition Control Assay, CAL Fluor® 560 and IPC qPCR Inhibition Control Assay, CAL Fluor® 560 Technical Manual,* #TM657.

#### Assembling a Reaction Mix

**Note:** The final reaction volume for this protocol is 20µl.

1. Thaw the IAC Inhibition Control, GoTaq® Endure Master Mix, 2X, and Nuclease-Free Water at ambient temperature or on ice. Add CXR dye to the master mix according to instructions in Section 4.A if desired.
2. Vortex the GoTaq® Endure Master Mix, 2X, and IAC RT-qPCR Inhibition Control for 3–5 seconds to mix.
3. Determine the number of reactions to be set up, including negative control reactions. Add one or two reactions to this number to compensate for pipetting error. While this approach requires an additional small reagent amount, it ensures enough reaction mix for all samples.

4. Prepare the reaction mix (minus the RNA template) by combining the reagents as described below. Add 1µl of IAC RT-qPCR Inhibition Control Assay and template per 20µl reaction. Vortex briefly to mix.

| Component | Volume per 20µl | Final Concentration |
|---|---|---|
| GoTaq® Endure Master Mix, 2X | 10µl | 1X |
| GoScript™ Enzyme Mix, 50X | 0.4µl | 1X |
| forward primer (20X) | 1µl | 200nM–1µM |
| reverse primer (20X) | 1µl | 200nM–1µM |
| hydrolysis probe (20X) | 1µl | 100nM–300nM |
| IAC RT-qPCR Inhibition Control, CAL Fluor® 560, 20X* | 1µl | 1X |
| RNA template | 2–4µl | 1pg–1µg |
| **Nuclease-Free Water to a final volume of** | **20µl** | **–** |

***Note:** The IAC RT-qPCR Inhibition Control, CAL Fluor® 560, 20X, contains primers, probes and exogenous RNA template.

5. Add the appropriate volume of reaction mix to each PCR tube or to each well of an optical-grade PCR plate.
6. Add the RNA template (or Nuclease-Free Water for the no-template control reactions) to the appropriate reaction plate wells.
7. Seal the tubes or optical plate. Centrifuge briefly to collect the contents at the bottom of the tube or wells. Protect from extended light exposure and elevated temperatures. The samples are now ready for thermal cycling.
8. Analyze the inhibitor control in a CAL Fluor® 560-compatible channel (HEX/JOE/VIC).

**Table 2. GoTaq® Endure RT-qPCR System Tolerates qPCR Inhibitors.** RT-qPCR reactions were performed with the IAC RT-qPCR Inhibition Control Assay using either GoTaq® Endure Master Mix or a traditional master mix with varying amounts of humic acid, a known PCR inhibitor. Nuclease-Free Water was used as a no-inhibitor control. No $C_q$ indicates that PCR was completely inhibited by humic acid, while a $\Delta C_q$ of 0 indicates no inhibition by humic acid.

| | Humic Acid (ng/reaction) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Assay** | **125** | **62.5** | **31.25** | **15.63** | **7.81** | **3.91** | **1.95** | **0** |
| GoTaq® Endure RT-qPCR System ($\Delta C_q$) | 10.58 | 5.81 | 2.42 | 1 | 0.42 | 0.2 | 0.02 | 0 |
| Traditional RT-qPCR Master Mix ($\Delta C_q$) | No $C_q$ | No $C_q$ | 7.54 | 3.75 | 1.83 | 1.15 | 0.1 | 0 |

A shift in $C_q$ value from the no-inhibitor control reflects the level of RT-qPCR inhibition.

$\Delta C_q = C_q$ [with inhibitor] – $C_q$ [no inhibitor]

$\Delta C_q$ >2 represents significant inhibition of the reaction.

### 6.C. Using XpressAmp™ Direct Amplification Reagent with the GoTaq® Endure RT-qPCR System

The GoTaq® Endure RT-qPCR System is designed to be a robust mix that can handle the most challenging sample types. This system can be paired with the XpressAmp™ Direct Amplification Reagent (Cat.# A8880) to enable RT-qPCR directly from sample lysates. For more information, see the *XpressAmp™ Direct Amplification Reagents Technical Manual* #TM647.

#### Preparing XpressAmp™ Solutions

1. Ensure the XpressAmp™ Lysis Buffer is at room temperature (+15°C to +30°C) before use. If the XpressAmp™ Lysis Buffer has been stored below room temperature, you may see a precipitate. Warm the XpressAmp™ Lysis Buffer to room temperature, with gentle mixing, for at least 30 minutes prior to use to ensure the precipitate is back in solution.
2. Add 1-Thioglycerol to a concentration of 1% (v/v) to an aliquot of XpressAmp™ Lysis Buffer before use.

   **Note:** For best results, use XpressAmp™ Lysis Buffer containing 1% 1-Thioglycerol within 1 hour of preparation.

| Component | Volume for 1 Amplification Reaction | Volume for 96 Amplification Reactions |
|---|---|---|
| 1-Thioglycerol | 0.025µl | 2.5µl |
| XpressAmp™ Lysis Buffer | 2.475µl | 247.5µl |
| **final volume** | **2.5µl** | **250µl** |

#### Preparing XpressAmp™ Sample Lysates

1. Combine sample 1:1 with freshly prepared XpressAmp™ Lysis Buffer containing 1% 1-Thioglycerol (e.g., 2.5µl of sample with 2.5µl of XpressAmp™ Lysis Buffer containing 1% 1-Thioglycerol, as shown in the table below).

Samples may be infectious. Use appropriate protection equipment when handling samples. Adhere to your institutional guidelines for the handling and disposal of potentially infectious substances when using these reagents.

| Component | Volume for One Amplification | Volume for Multiple (X) Amplifications |
|---|---|---|
| Sample | 2.5µl | 2.5µl × (X + 1) reactions |
| Prepared XpressAmp™ Lysis Buffer containing 1-Thioglycerol | 2.5µl | 2.5µl × (X + 1) reactions |
| **final volume** | **5µl** | **5µl × (X + 1) reactions** |

2. Mix the sample by pipetting.
3. Incubate at room temperature for 10 minutes.
4. Proceed with PCR amplification of the XpressAmp™ sample lysate. Holding XpressAmp™ sample lysates for greater than 24 hours before amplification reduces amplification sensitivity.

**Amplifying XpressAmp™ Sample Lysates with the GoTaq® Endure RT-qPCR System**

Determine the number of reactions to be set up, including control reactions.

Prepare enough amplification reaction mix for the number of reactions needed plus 10% additional reaction volume to compensate for pipetting error. This ensures that you have enough reaction mix for all samples. Add CXR dye to the Master Mix according to instructions in Section 4.A if desired. If adapting a previously optimized PCR assay to direct amplification by adding XpressAmp™ Solution to your reaction, you may need to re-optimize your PCR amplification conditions (e.g., annealing temperature and primer/probe concentration). Optimizing the amount of sample lysate added (e.g., dilution of 1:50 or 1:100) may be necessary to achieve efficient amplification.

1. Prepare the amplification reaction mix (minus the sample lysate) by combining qPCR master mix, reverse transcription (RT) mix, primer/probe mix, XpressAmp™ Solution and Nuclease-Free Water. See the following example for setup when using the GoTaq® Endure RT-qPCR System. Vortex briefly to mix. The prepared XpressAmp™ sample lysate is added in Step 3.

| Component | Starting Concentration | Final Concentration | Single-Well Reaction Volume | | Number of Reactions (n × 1.1) | | Master Mix Volume |
|---|---|---|---|---|---|---|---|
| GoTaq® Endure Master Mix | 2X | 1X | 12.5µl | × | | = | |
| GoScript™ RT Mix for 1-Step RT-qPCR | 50X | 1X | 0.5µl | × | | = | |
| Primer/Probe Mix[1] | 25X | Primers: 0.1–1µM Probe: 0.1–0.5µM | 1µl | × | __ | = | |
| XpressAmp™ Solution[2] | 5X | 1X | 5µl | × | | = | |
| Nuclease-Free Water | | | 1µl | × | | = | |
| Prepared XpressAmp™ sample lysate | | | 5µl | | | | |
| **final reaction volume** | **20µl** | **—** | **25µl** | | | | |

[1]If adapting a previously optimized PCR assay to direct amplification by adding XpressAmp™ Solution to your reaction, you may need to reoptimize your PCR amplification conditions (e.g., annealing temperature and primer/probe concentration).
[2]You can change the final volume of the PCR amplification to accommodate other volumes of prepared XpressAmp™ sample lysate. If you change the final PCR volume, adjust the volume of XpressAmp™ Solution to ensure a 1X final reaction concentration. For samples containing strong PCR inhibitors or very high levels of transcript, such as whole blood, a dilution of 1:50 or 1:100 of the sample lysate may be necessary.

2. Add 20µl of reaction mix (without XpressAmp™ sample lysate) to each PCR tube or well of an optical-grade PCR plate.

3. Add 5µl of XpressAmp™ sample lysate to the appropriate tubes or wells of the reaction plate.

Promega

### 6.C. Using XpressAmp™ Direct Amplification Reagent with the GoTaq® Endure RT-qPCR System (continued)

4. Seal the tubes or optical plate. Centrifuge briefly to collect the contents at the bottom of tubes or wells. The samples are ready for thermal cycling. Protect tubes or plate from extended light exposure or elevated temperatures before cycling.

5. Begin thermal cycling. See Section 5 for example cycling parameters. The optimal annealing and extension temperatures and times are dependent on the sequence of your primers/probes, the length of your amplicon and the amplification reagents used in the RT-qPCR. We recommend optimizing the annealing/extension temperature and time for your assay in the presence of XpressAmp™ Direct Amplification Reagents containing known amounts of control DNA before testing samples.

### 6.D. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. Email: **techserv@promega.com**

| Symptoms | Causes and Comments |
|---|---|
| Failure to detect qPCR signal | Improper nucleic acid extraction from samples, resulting in loss of RNA, RNA degradation or both. |
| | Inhibition of reverse transcriptase and/or DNA polymerase by inhibitors in the sample. Absence of sufficient nucleic acid due to poor collection or pasteurization of sample. |
| | Improper assay set up or execution. Reagent or equipment malfunction. |
| Low yield of RT-qPCR product | RNA degradation. Always use nuclease-free, commercially autoclaved reaction tubes, sterile aerosol resistant tips and gloves. Ensure that reagents, tubes and tips are kept RNase-free by using sterile technique. |
| | Isolate RNA in the presence of RNasin® Ribonuclease Inhibitor. Use RNasin® Ribonuclease Inhibitor to inhibit degradation of target during cDNA synthesis (20u/20µl reaction). |
| | Poor primer design. If the reaction products appear to be entirely primer artifacts, the reaction may not have amplified the desired RT-PCR product because of primer-primer interactions. Make sure the primers are not self-complementary. Check the length and melting temperature of the PCR primers. |
| | Extension time was too brief for amplicon length. To minimize interactive effects of reverse transcriptase and thermophilic DNA polymerase, design the thermal cycling program with a longer extension time in each cycle. Begin with 1 minute per kilobase per cycle and increase to 2 minutes or more if necessary. |

| Symptoms | Causes and Comments |
|---|---|
| Low yield of RT-qPCR product | Too few PCR cycles. To detect rare or challenging RNA targets by RT-PCR, increase the cycle number to 40 to maximize sensitivity. |
| | Wrong reaction tubes were used. Make sure to use thin-walled reaction tubes for optimal heat transfer during PCR. Use only sterile, nuclease-free commercially autoclaved tubes, strip tubes or plates for PCR. Autoclaving eliminates volatile contaminants that inhibit amplification. |
| | Reverse transcriptase effect on primer-dimer artifact synthesis in RT-PCR. Make sure to thoroughly heat-inactivate the reverse transcription reactions prior to use (6,7). |

### 6.E. 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.
6. Chumakov, K.M. (1994) Reverse transcriptase can inhibit PCR and stimulate primer-dimer formation. *PCR Methods Appl.* 4, 62–4.
7. Sellner, L.N., Coelen, R.J. and Mackenzie, J.S. (1992) Reverse transcriptase inhibits Taq polymerase activity. *Nucleic Acids Res.* 20, 1487–90.

Promega

### 6.F. Related Products

| Product | Size | Cat.# |
|---|---|---|
| GoTaq® qPCR Master Mix* | 5ml | A6001 |
| IPC qPCR Inhibition Control Assay, CAL Fluor® 560* | 100 reactions | AM2030 |
| GoTaq® Endure qPCR System* | 200 reactions | A6220 |
| | 1,000 reactions | A6221 |
| GoTaq® 1-Step RT-qPCR Master Mix* | 5ml | A6020 |
| GoTaq® Endure qPCR Direct Amp Bundle* | 200 reaction | A6224 |
| GoTaq® Endure RT-qPCR Direct Amp Bundle* | 200 reaction | A6225 |
| IAC RT-qPCR Inhibition Control Assay, CAL Fluor® 560* | 100 reactions | AM2040 |
| Nuclease-Free Water | 50ml | P1193 |
| Set of dATP, dCTP, dGTP, dUTP | 10µmol each | U1335 |
| | 40µmol each | U1245 |
| CXR Reference Dye | 100µl | C5411 |
| XpressAmp™ Direct Amplification Reagent | 250 reactions | A8882 |
| | 3,000 reactions | A8880 |

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

**Nucleic Acid Purification Kits and Accessories**

| Product | Size | Cat.# |
|---|---|---|
| Maxwell® RSC PureFood GMO and Authentication Kit** | 48 preps | AS1600 |
| Maxwell® RSC PureFood Pathogen Kit** | 48 preps | AS1660 |
| Maxwell® RSC Plant DNA Kit | 48 preps | AS1490 |
| Maxwell® RSC Whole Blood DNA Kit* | 48 preps | AS1520 |
| Wizard® Genomic DNA Purification Kit* | 100 isolations × 300µl | A1120 |
| Maxwell® RSC simplyRNA Cells Kit** | 48 preps | AS1390 |
| Maxwell® RSC simplyRNA Blood Kit** | 48 preps | AS1380 |
| MagneSil® Total RNA Mini-Isolation System | 4 plates | Z3351 |

*Additional sizes are available.

**Not for Medical Diagnostic Use.